Viorel PRISACARU

SPECIAL EPIDEMIOLOGY

Textbook

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Nicolae Testemitanu State University
of Medicine and Pharmacy
of the Republic of Moldova

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Translated by Angela PARASCHIV

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Viorel Prisacaru
Special epidemiology

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Author – Viorel Prisacaru

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Translated by Angela Paraschiv

The textbook deals with epidemiological features of 80 communicable and noncommunicable diseases, which are of interest for public health. The description of diseases includes a brief characteristic of the pathogen, reservoir and source of pathogens, modes, ways and factors of transmission, clinical and epidemiological manifestations, laboratory diagnosis, epidemiological surveillance, preventive and control measures.

The textbook is written in compliance with the syllabus in epidemiology for students and residents, but it can be useful for practicing physicians as well.

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SUMMARY

ABBREVIATIONS

ALAT – alanine aminotransferase
RNA – ribonucleic acid
ASAT – aspartate aminotransferas
ADD – acute diarrheal disease
CDC – Center for Diseases Control
Cell tox$^+$ – cells that produce exotoxin
NCPH – National Center for Public Health
CPH – Center for Public Health
DALY – Disability-Adjusted Life Year
ELISA – Enzyme-Linked Immunoabsorbent Assay
HA – hemagglutinin
IHAR – Indirect Hemagglutination Test
HIV – virus of human imunodeficiency
HAV – hepatitis A virus
HEV – hepatitis E virus
HBV – hepatitis B virus
HCV – hepatitis C virus
HDV – hepatitis D virus
HGV – hepatitis G virus
URTI – Upper respiratory tract infection
EF – Electrophoretic test
STDs – sexual transmitted diaseses
LAV – Lymphadenopathy-Associated Virus
NA – neuraminidase
NP – nucleoprotein
WHO – World Health Organization
PCR – Polymerase chain reaction
PCV-7 – Pneumococcal polysaccharide vaccine
AR – agglutination reaction
MAR – microscopic agglutination reaction
CFR – complement fixation reaction
RIA – radioimmunoassays
IFR – immunofluorescence reaction
IIFR – indirect immunofluorescence reaction
NMR – nuclear magnetic resonance

SARS – sever acute respiratory syndrom
AIDS – Acquired immune deficiency syndrome
CNS – central nervous system
Tox$^+$gen – gen responsable of toxin production
PCV-13 – Pneumococcal vaccine 13-valent Prevenar^{TM}
IPV – Inactivated poliomyelitis vaccine
OPV – Oral live poliomyelitis vaccine
VPP-23 – Pneumococcal polysaccharide polyvalent vaccine
WB – Western Blot

"Writting a book is always a high gain, success, satisfaction of fulfillment, a debt to the predecessors and to those who will be formed, to improve their in a field of science and technology".

Aurel Ivan

INTRODUCTION

Although epidemiology originates from the Antiquity, its rapid development at present determines new knowledge about the content and structure of human pathology. Knowing the bases of general epidemiology (the mechanisms of formation and spread of diseases in human populations) and special epidemiology is absolutely necessary (essential) for the implimentation of certain prevention and control measures by doctors.

Currently such diseases as smallpox, glanders, recurrent typhus, malaria, native Shiga dysentery, trachoma, brucellosis and polio, are no longer present in the country. These serious diseases have been eradicated, largely due to better knowledge about the epidemic features and proper organization and management of control measures. The decrease of infectious morbidity in other diseases was recorded as well. Furthermore, at the background of success in science and epidemiological practice, the modern society is facing new epidemiological problems, primarily related to the emergence of numerous infectious diseases and re-emerging diseases, and high increase of noninfectiuos morbidity. In this context, the essential knowledge of etiology and epidemiology of many unknown pathological nosologies in the past, such as legionellosis, haemorrhagic fevers (Ebola, Marburg, and Lassa), HIV infection, severe acute respiratory syndrome (SARS) and Lyme disease has increased. Streptococcal infections due to their ubiquitous spread and multiple serious pathologies caused by them are of particular interest.

The book contains a compact description of infectious (infectious and invasive) and noncontagious diseases.

The list of nosological forms described in the book is determined by both undergraduate and postgraduate training program and the actuality of pathological problem facing our society today.

Description of all the pathologies is based on a unique form and includes a brief history, features of pathogen, reservoir and sources of pathogen, the way, factors and ways of transmission, factors that favoring the manifestations of epidemic and infectious process, the main directions of supervision, control measures, preventive and anti-epidemic measures.

The book will prove to be useful and be a mentor for both students and residents, as well as practicing physicians - epidemiologists, microbiologists, infectious disease specialists, internists, family doctors etc.

I would like to thank the teaching staff of Primary Care Discipline of Health and Epidemiology of Grigore T. Popa University, namely Professor MD Doina Azoicai, lectures Mioara Matei and Alina Manole for their participation in writing this textbook. I am also grateful to the respectable spouses Elvira-Sînziana and Constantin Ciufecu the greatists scientists in epidemiology and virology (Bucharest).

A. EPIDEMIOLOGY OF COMMUNICABLE DISEASES

1. Epidemiology of infectious diseases

1.1. Anthroponosis

1.1.1. Gastrointestinal infections

1.1.1.1. Typhoid fever

Short history

Typhoid fever has been known since ancient times, being confused over time with other illnesses with pronounced fever, such as typhus, set far into the nineteenth century as a disease tified because of his severe fever with loss of consciousness (in Greek : typhi - fog, confusion). Typhoid fever was first described and named by Charles Louis (1829). The causative agent was identified by N. Sokolov (1876) and R. Eberth (1880) in the lymph nodes and spleen deseased of person who died of typhoid, being cultivated by G. Gaffky in 1884. Typhoid fever epidemic has highlighted the significant effects in particular social calamity.

The pathogen is Salmonella typhi, Gram-negative from genus Salmonella, Enterobacteriaceae family. It grows on simple or selective culture media containing bile. It is aerobic or anaerobic optional mobile, does not form spores and capsule. Its antigenic structure is complex, consisting of 3 types of antigens: antigen "O" - somatic of lipopolysaccharide nature, thermostable; antigen "H" - flagellar of protein, heat-labile, and type-specific antigen; "Vi"- the surface (capsular) glucido-lipid or virulent by interfering in virulence, preventing phagocytosis. Currently, over 100 known fagovariante (phagotypes, lysotypes) of S.typhi circulating in nature have epidemiological importance, depending on the sensitivity of different types to bacteriophages. Lysotypes are significant markers in the epidemiological investigation of typhoid (determining the source of infection transmission factors; find the link between cases of disease in outbreaks etc.). S. typhi resistance is great in the environment. Thus, it resists in running water up to 2 weeks, in lakes - up to 4 weeks, wells - up to 3 months, in ice - up to 6 months, on vegetables and greens - up to 10-12 days, butter and cheese - up to 3 months, on contaminated hands -

48 hours. It is sensitive to UV rays, high temperatures (at 60°C it persists up to 30 min., at 100°C destroyed instantly), disinfectants, in particular chlorine.

Sources of pathogens are human, the sick or carriers

Patient as a source of pathogens. After oral contamination, the pathogen enters into the lymph follicles of the small intestine (Peyer's patches), where it multiplies. After the multiplication (incubation period) which is from 3 to 25 days (depending on the virulence of the pathogen and the dose of contaminating microorganisms), the pathogen from the follicles nodes enters into the bloodstream, causing bacteraemia and endotoxinemia. The pathogen occurs in parenchymal organs (liver, spleen, lymph nodes, bone marrow) by bloodstream dissemination. Then, bacteria enter the intestine from the liver with bile fluid, where, it is excreted with the feces in the environment. It may be eliminated from the body by urine as well. Thus, the diseased, practically, is not contagious in the incubation period and first week of the disaese. Contagiousness starts on the 8^{th} – 10^{th} day of the disease, with the elimination of the causative agent in the environment through feces or urine (in 30-50% of cases). The patient is most contagious during the second and third weeks of illness, after which the causative agent concentration in stool decreases, but the contagiousness persists throughout the convalscence period. Most of patients do not eliminate pathogen after the recovery or can eliminate it in the fellowing 2-3 months. Approximately 3-5% of S. typhi continues to eliminate longer period of time. In these cases, S. typhi agents locate in the gallbladder, where they multiply and eliminate for years, decades or a lifetime.

Carriers of germs can be of two categories: postinfectious and healthy

Postinfectious carriers are pathogen carriers after the disease, which may be temporary - people continue to eliminate bacteria up to 3 months after recovery, and chronic - people continue to eliminate S. typhi more than 3 months. Carrier state is more common in middle-aged females with gallstones. The presence of chronic diseases and somatic parasitic diseases those of bile ducts and liver damage, in particular, contributes to the formation and persistance of the carriage.

Healthy carriers are persons who have contacts with patients, and who do not catch the disease, but eliminate temporaly S. typhi – within - 14 days.

The most contagious are chronic S. typhi carriers. It is determined by the profession of the person, hygiene compliance, etc. Carriers of germs present a higher risk if they participate in the preparation and sale of food.

Receptivity of a human to typhoid fever is high from the age of 3-4 years, with the formation of lymph follicles of the intestine. Postinfectious immunity is longlife. Reinfection occurs rarely. Antibodies production take place already in the first week of the disease, reaching a maximum titer on the 3rd week of the disease. This contributes to neutralization of the pathogen and has a diagnostic significance. The use of antibiotics to treat patients leads to the decrease of antibody production and irrational use of antibiotics lead to the recurrence and chronic carriage.

The mechanism, factors and ways of transmission

The mode of transmission of the causative agent from the body to the receptive host is fecal-oral, and it is achieved by different factors: water, food, soil, household items, house flies and contaminated hands.

Epidemiological studies and practice showed that water is the main factor in contamination with S. typhi (≈60%). Natural river waters, wells, water tanks and kept in pots, and even centralized water distribution have a asignificant epidemiological importance in this regard. Centralized water distribution can be as a rik in case of damage of water supply, water supply pressure fluctuation, irregular supplyof an aqueduct, accompanied by absorption of water contaminated by leaking segments of the system.

Important factors in typhoid fever have transmission are food products of plant origin (vegetables, fruits), eaten raw salad or food products of animal origin (milk, cheese, meat, eggs, molluscs).

Infection can be transmitted by a casual contact and through contaminated household items of a sick person or carrier.

Therefore, in typhoid transmission of the pathogen can be via water, food and common household itemes (habitually), the main being the waterborne and foodborne ways. The habitually contamination occurs usually in case of non-compliance of personal hygiene and anti-epidemic regime.

Contributing factors

The level of the development of epidemic, and morbidity due to typhoid fever respectively, depends largely on socio - economic factors, low level of hygiene, providing the population with qualitative drinking water and food, sanitation and hygiene of territory, migration of the population, and also on natural factors such as high temperatures, low precipitation (rainfall, floods), increase in the number of flies.

Manifestations of epidemic process

Typhoid fever is spread worlwide. According to the WHO, there is no country where cases of typhoid fever have not been registered. Annually, about 20 million people with typhoid fever and about 800 000 deaths of it are registered. Nowdays, it is characterized by sporadic morbidity. Typhoid fever morbidity has decreased significantly in Moldova, where for the last two decades the incidence is below 0.2 cases per 100 thousand population, which constitutes a real progress compared to the situation before (29.13 to 100,000 population in the period 1944-1953) *(Figure 1)*.

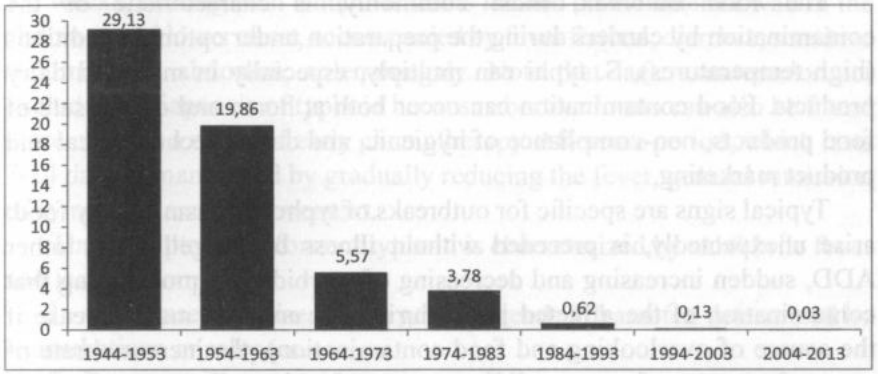

Figure 1. *Incidence of typhoid fever per 100,000 of the population in the Republic of Moldova, the avarage anual rate of the periods.*

However, in different continents, regions or countries the epidemic has a variable intensity and a direct correlation with the socioe-conomic level of the country. Sporadic morbidity is specific for economically developed countries, and endemic morbidity is characteristic of underdeveloped ones. Currently, countries located in Asia, Africa, South America and certain territories of the Russian Federation are considered to be endemic. The availability of S. typhi carriers and high population migration make possible the appearance of disease in any territory and at any time of the year. Besides socio-economic factors and health culture, an important role in maintaining the epidemic and endemic manifestatios belongs to chronic carriers. Epidemics are frequently associated with social and natural cataclysms. Pathogen transmission occurs more frequently by water. Water outbreaks can be distinguished both by the source of water and mechanism of pollution of water sources.

Water outbreaks are characterized by the following signs: common source of water supply; suddenly increase of the number of patients, cases of Shigellosis and other acute diarreal deases (ADD) preceded by a short incubation period (when water is contaminated with other intestinal bacteria as well); relatively mild clinical forms; non-significant rate of children, and on the contrary, the high rate of adolescents; various types of pathogen (the eruptions by water from wells – monofagotypes); the link between outbreak and accidents water supply or sewerage systems (removal of the cause leads to reduction of desease manifestions).

The food outbreak, most commonly, is characteristic of the contamination by carriers during the preparation under optimal conditions (high temperatures), S. typhi can multiply, especially in milk and dairy products. Food contamination can occur both at home and on the sale of food products, non-compliance of hygienic, and during technological and product marketing.

Typical signs are specific for outbreaks of typhoid transmitted by food: arise unexpectedly, is preceded without illness by Shigellosis or other ADD, sudden increasing and decreasing of morbidity, demonstrating that contamination of the affected joint (during the eruption may increase if the source of overlooking and food contamination), the increased rate of severe form, monofagotype, different ages of patients, but more frequent preschool age children are affected.

Sporadic morbidity occurs more frequently, if the pathogen contamination is produced by vegetables and greens.

The seasonal distribution of morbidity is characterized by increased incidence during the summer and autumn. Seasonal increases of morbidity are more pronounced in endemic areas, as determined by increased water consumption, often from unknown sources, swiming in pools, increasing the consumption of vegetables and fruits, high temperature air during the summer, which contributes to multiplying pathogen, activation flies, increasing population migration.

The incidence of typhoid according to the age structure of the population is varied. In infants and children up to 3-4 years is very rare typhoid, after which the incidence is increasing, the highest in the age groups 20-29 and 30-39 years, with decreases intensity in the oldest age groups. The incidence varyies in different group of the population. More commonly at risk of contamination: the people of sanitation services and maintenance of

sewerage, persons employed in bacteriological laboratories working with live cultures of S. typhi, medical and auxiliary staff in infectious disease hospitals providing care for patients with intestinal infections.

The manifestations of the infectious process

Period of invasion of the pathogen into the bloodstream, following the incubation period, is characterized by fever gradually ascending, progressive accompanied by fatigue, headache, loose stools with greenish, discreet sensitive abdomen, splenomegaly. Fever reaches a maximum of 39°C - 40°C in a 3 - 4 day of the disease. During the state, which lasts about 2-3 weeks, starting with lenticular stains located on the anterior wall of the abdomen and lower chest, accompanied by fever flatbed, diarrhea, sensitive and meteoric abdomen, splenomegaly. Note that lack exantemelor not exclude the diagnosis of typhoid because abortive forms can also be found more often as a result of early chemotherapy. Aftercare period, which lasts 7-14 days is manifested by gradually reducing the fever, general remission of symptoms and improvement.

The asymptomatic form of typhoid is characterized by subfebrile fever and lack of symptoms.

In some cases the disease can get an extended character, determined by exacerbations or relapses.

Laboratory diagnosis

The most reliable method for diagnosing typhoid is considered bacteriological method - isolation of the pathogen from the blood. The isolaton of the S. typhi ia possible already in the first week of the disease - 80%. Isolation of the pathogen from feces (stool) or urine (urinoculture) occurs in the 2nd - 3rd week of illness. It is necessary to take into consideration that the pathogen can be isolated from feces and urine not only in the sick, but also from carriers as well.

Serological diagnosis is based on the quantitative analysis by Widal reaction, which is positive at the end of the first week of the disease. Determination of anti-Ag is practiced in detection of carriage. IgG and IgM antibodies can be detected by ELISA, CFR, IAR or coaglution reaction.

Epidemiological surveillance

Epidemiological surveillance of typhoid fever includes observance of sanitary-hygienic in providing the population with drinking water, food, knowledge about hygiene habits in the population, ensuring conditions for observance of sanitary and anti-epidemic measures, including personal

hygiene. An important element in epidemiological surveillance is detection of typhoid fever carriers. However, epidemiological surveillance is aimed to assess the dynamics of morbidity and risk factors, the conditions for the development or maintenance of the epidemic. Epidemiological situation assessment (epidemiologic diagnosis) is based on retrospective epidemiological analysis and by comparing the results obtained with other time periods.

Control measures

Prophylactic measures

Preventive measures directed to the source of pathogens consist of surveillance and investigation of populations to detect chronic carriers (from catering sectors, water supply, care units for children and specialized units especially in the animal sector). It is important to prevent the transmission of pathogen from carrier persons in these institutions. The control is performed periodically before the employment and during after the employment. All persons are serologically exanimated in order to confirm the presence of antibodies in the blood and Vi-Ag and O-Ag (signs indicating the presence of the pathogen in the body), and a bacteriological investigations of feces. People with negative results are admitted to work. In case of obtaining positive serological results persons are considered to be chronic carriers therefore are not engaged at work. In case of obtaining negative results of feces culture but positive for the presence Vi-Ag in blood, the serological investigations is repeted. Additionally is investigated the gallbladder to identify the pathogen.

The measures of prevention of typhoid fever are based on interruption of the fecal-oral mechanism transmission. In this aspect more results are measures to prevent contamination of of water or food, ensuring hygienic measures in catering enterprises, functioning sewage and sanitation settlements. Particularly important is the formation of epidemiological knowledge and hygiene habits among the population, especially workers in the public system for the preparation and marketing of food products. It is also important disinfection and prophylactic measures.

Vaccination in typhoid fever has a secondary role. As a preventive measure, it is recommended for population with high risk of contamination: people in communal sanitation services, maintenance of sewerage networks, people from bacteriological laboratories working with live strains of S. typhi, the infectious disease hospitals, etc. Vaccination is

practiced mainly in endemic areas or disaster. In the prevention of typhoid fever are used: S. typhi killed vaccine by heat or formalin or alcohol, administrated subcutaneously. The effectiveness of vaccine is 51-77% for a period of 2.5-3 years. The vaccine is reactogenic frequently, producing a series of systemic reactions (fever, headache, malaise) and local (pain, swelling); live attenuated vaccine strains prepared from Ty-21a, without virulence antigens Vi. It is taken orally, and does not induce significant side effects. Vaccine effectiveness is 29-96% over a period of 2 years. Vi polysaccharide vaccine, administrated subcutaneously, has an efficiency of ≈ 65% for a period of 2 years. Side effects are minor.

Antiepidemic measures in focus

Centre for Public Health (CPH) shall be informed about typhoid fever cases both by telephone or information system and by filling in - 058/e notifications form.

Hospitalization of patients or persons suspected typhoid fever is mandatory because of possible complications (perforation peritonitis, gastrointestinal bleeding). Patients are discharged from hospital after resolution of clinical signs is performed three negative results of urine and feces culture. The first investigation is carried out not earlier than 5 days after resolution of fever following - at an interval of 5 days. All convalescence, regardless of profession or occupation, are subject to the clinic supervision over 3 months with the medical examination and temperature measurement once a week during the first month and not less than once in two weeks over the next two months, after discharge. At the end of this term are performed two bacteriological investigations, with an interval of 2 days and a serological investigation. If it is negative results, people are removed from the dispensary, and if it is positive results, these people will be monitored and will be conducted two investigations during the year. In case of positive results, they are considered to be chronic carriers.

People with epidemiological risk (depending on activity) are not allowed to work within a month after discharge. During this period they are subject to a number of bacteriological and serological investigations. In case of negative results, these people continue to be investigated bacteriological over a year, once every 3 months with an investigation to establish the presence of antigene. If the result is negative, there are removed from the surveillance. If the isolation of S. typhi is registered after 3 months, people are passed to chronic carriers group and are removed from work.

All persons confirmed as chronic carriers, shall be permanently trained on appropriate behaviour and rules for conducting current disinfection.

The epidemiological investigation of typhoid fever outbreak is carried out, with identification of the source of pathogens, factors and ways of transmission, early detection of patients, including those with atypical forms. All contacts are subject to epidemiological surveillance during 21 days, with clinical and laboratory investigation. For the prevention of disease among contacts is used bacteriophage, and vaccination of population. Terminal disinfection is performed after the hospitalization of patient.

1.1.1.2. Shigellosis

Short history

The disease dates back antiquity, and it has been known as "dysentery" over the years. Today it is recognized as "dysentery syndrome" (mucosanguineous stools, abdominal cramps and frequent stools) specific for many diarrheal diseases. Since the last century it has been called "bacillary dysentery".

In 1891 A. Grigoriev identified the causative agent of Shigella in Russia. Later (1898) it was studied in details by K. Shiga during the epidemic with 90000 cases and 25% lethality in Japan. The causative agent was called Shigella dysenteriae.

In 1900, S. Flexner isolated another type of shigella which was called Shigella flexneri. The third species of Shigella was isolated and described by W. K. Kruse and Sonne 1907 - 1915, it was called Shigella sonnei. In 1929, in India, Mark Frederick Boyd isolated the fourth species, later it was called Shigella boydii.

The causative pathogen

Shigella belongs to the Enterobacteriaceae family, genus Shigella, which is classified according to the antigenic structure and biochemical properties into 4 groups: group A - Shigella dysenteriae - includes 16 serovariants (1-16), including Sh. Grigoriev-Shigae (S. dysenteriae 1), Sh. Schitzii (S. dysenteriae 2) and Sh. Large - Sachs (S. dysenteriae 3-7); Group B - Shigella flexneri - includes 8 serotypes (1-6); Group C - Shigella boydii - includes 18 serovariants (1-18); Group D - Shigella sonnei - includes one serotype, but it differs according to the biochemical variations.

Only Shigella Grigoriev-Shigae produces neurotoxic exotoxin. Other types of Shigella contain endotoxin.

Shigella is gram-negative pathogen, aerobic which does not form spores. There are varies by antigenic structure, biochemical activity, pathogenicity and virulence.

Sh. Dysenteriae Grigoriev-Shigae, which eliminates exotoxin with hemolytic properties possess the highest virulence. Shigella flexneri possesses high virulence and less pronounced virulence is in Sh. sonnei. At the same time, the biochemical activity is indirect proportional to their virulence. Infected dose for adult is accumulated in milk within 8 - 24 hours. Another feature of Shigella Sonne is resistance to antibacterial preparations.

Shigella has a moderate resistance in environment, it depends on the species. The most resistant type is Sh. Sonnei. Sh. sonnei and Sh. flexneri resist longer (2-14 days) in water, dairy products, in food at room temperature. At the same time, they are sensitive to high temperatures. Heating at a temperature of 60°C kills the pathogen in 10 minutes and boiling kills it immediately. They are sensitive to ultraviolet light and usual disinfectants.

The reservoir and sources of pathogen

Shigellosis is a typicall anthroponosis. The source of pathogen is a sick person with acute or chronic form of infection. Patients with mild or asymptomatic from of infection, especially people working in risk professions belong to a group of high risk.

The pathogen is eliminated from the patient's body with feces from the onset of disease and during 7-10 days and 2-3 weeks during convalescence. Elimination of the pathogen often lasts for several months. Chronic infectious process is more specific for Sh. flexneri and less for Sh. sonnei.

Mechanism, ways and factors of transmission

The mechanism of transmission is fecal-oral. The transmission routes are via by water, food and close contact with sick person. Shigella Grigoriev-Shigae is transmitted mainly via close contact, Shigella flexneri – via water, and Sh. sonnei - via food, especially - milk and other dairy products.

Risk factors

The spread of Shigellosis is determined by the economic status of the

population, poor living conditions, lack of drinking water, use of food of poor quality, poor sanitation, low level of hygiene, emergencies: military actions, floods, centralized drinking water supply breaks, etc.

Manifestations of epidemic process

Receptivity to shigellosis is general, but children are more receptive than adults. In adults, the most common form is asymptomatic. Sh. Sonnei has reduced virulence and needs higher dose for contamination.

Postinfectious immunity lasts 2-14 years. Population immunity can develop as a result of habitual premonition, especially in endemic areas.

Shigellosis is recorded on all continents, in all geographic areas with a significant incidence of digestive diarrheal diseases (according to the WHO terminology) and constitutes a serious problem especially for countries with weak economy.

Shigellosis is manifested in the form of epidemic and it is determined by the activity of pathogen, whose intensity is conditioned by social, cultural level and climatic conditions.

In the 40s and 50s of the XX centery up to 80-90% of deseases were caused by Sh. flexneri, while in the second half of the twentieth century Shigellosis was mainly caused by Sh. sonnei. Lately, Shigellosis is caused by Flexner. This is due to the biological properties of the causative agent, and the evolution of socio-economic conditions of the society at different stages of development.

Shigellosis affects children aged 2-7 in 65-70% of cases *(Figure 2)*.

The infection is more common in the urban population. Most of caeses occur in the warm season of the year. During the summer - autumn months 70-80% of annual morbidity are recorded. In summer the need for drinking water increases, thus creating favorable conditions for multiplication of Shigella in food, especially in milk. It can lead to sporadic morbidity and outbreaks. Outbreaks were reported more frequently in children institutions, schools, children's camps, refugee camps, institutions for people with mental retardation, military units, etc.

It is important to pay attention to flies as mechanical transmitters of pathogens as a source of food contamination during the warm season.

About 78% of general morbidity of Shigellosis is determined by the circulation of Sh. Sonnei in Moldova.

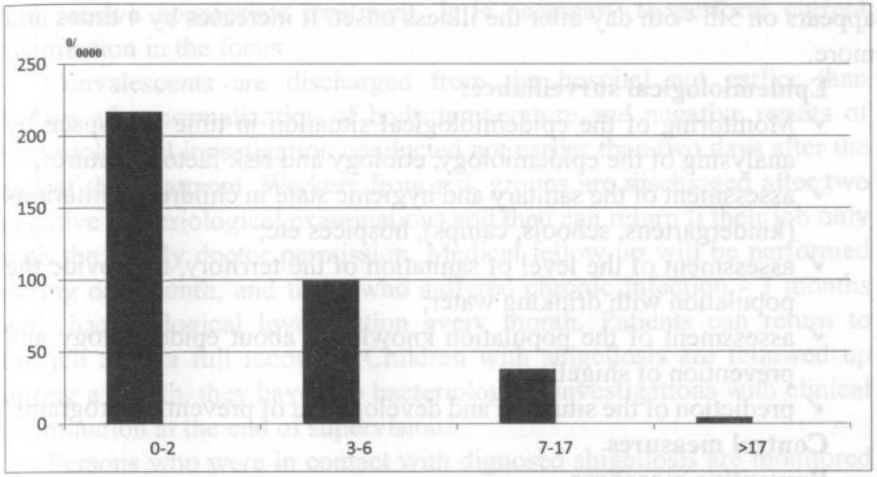

Figure 2. *Incidence of shigellosis according to the age.*

However, there is an increase of illnesses caused by Sh. flexneri.

The manifestations of infectious process

The incubation period is 1-6 days, with the average of 2 - 3 days. The onset is sudden, with fever 38-39°C, in severe forms - up to 40°C, but it can be lower (37.5 to 38°C) or absent in mild forms. Digestive disorders are manifested by nausea, vomiting, abdominal pain, in particular in the large intestine, diarrhea (15-20 times a day) with mucus, sometimes with streaks of blood, tenesmus of defecation. In young children moderate dehydration may occur. In cases of untreated illness, it is lasts 9-10 days. Currently, mild forms predominate (80-90%), including asumptomatic. Lethality is about 0.01% and are recorded, mainly, in infants.

Laboratory diagnosis

Bacteriological diagnosis is important to identify asymptomatic forms of Shigellosis. Feces (stool) are used for bacteriological confirmation at early stage of disease. The concentration of shigella in feces is higher before the administration of antibiotics.

Immunofluorescence method is applied for rapid diagnostic. The result is given in 2-3 hours.

Serological diagnosis is carried with detection of specific antibodies in Indirect Heamaglutinin Reaction. The diagnostic is considered positive when the titter of Sh. sonnei is 1: 100, that of Sh. flexneri - 1: 200 and it

appears on 5th - 6th day after the illness onset. It increases by 4 times and more.

Epidemiological surveillance:

- ✓ Monitoring of the epidemiological situation in time and space by analysing of the epidemiology, etiology and risk factors features;
- ✓ assessment of the sanitary and hygienic state in children institutions (kindergartens, schools, camps), hospices etc;
- ✓ assessment of the level of sanitation of the territory, to provide the population with drinking water;
- ✓ assessment of the population knowledge about epidemiology and prevention of shigellosis;
- ✓ prediction of the situation and development of prevention programs.

Control measures

Preventive measures

General nonspecific measures indicated in digestive diseases are directed to interruption of transmission mechanism. Particular attention should be paid to providing the population with quality drinking water, good functioning of centralized waterr sipply system, aqueducts and sewage systems, compliance with the hygiene rules in food businesses, compliance with hygienic regime in children institutions, increasing the level of sanitary culture of population, knowledge about risk factors and compliance with the rules of personal hygiene. The consumption of water from unknown sources, unboilled milk are prohibited to be used. Prophylactic measures include systematic disinfection in children institutions, military units, hospices, homes, crowded places, wells, water pipes etc.

All persons are investigated before employment in canteens, water supply institutions, children institutions, etc., in order to identify carriers of bacterial infections from digestive group. These people are not allowed to work if the pathogen of intestinal group including Shigella is detected. They will receive a specific treatment.

Antiepidemic measures

It is necessary to inform the Centre for Public Health about detection of Shigellosis patient.

The hospitalization is performed according to clinical and epidemiological indications.

Patient is informed about the methods of care is he is isolated at home

and receive appropriate treatment. It is necessary to perform current disinfection in the focus.

Convalescents are discharged from the hospital not earlier than 3 days after normalization of body temperature and negative results of bacteriological investigation conducted not earlier than two days after the end of the treatment. Workers from risk groups are discharged after two negative bacteriological examinations and theu can return ti their job only with the family doctor permission. Medical fellow-up will be performed during one month, and those who suffered chronic infection - 3 months with bacteriological investigation every month. Patients can return to the job after a full recovery. Children with shigellosis are fellowed up during a month, they have two bacteriological investigations with clinical examination at the end of supervision.

Persons who were in contact with dignosed shigellosis are monitored clinically during 7 days. People with high risk from specific professional activity (canteen) are subjected to one bacteriological investigation at the end of supervision. In case of positive result these people are suspended. Children from kindergarten with recorded shigellosis outbreaks, are allowed to attend the institution, but are subjected to medical surveillance and bacteriological investigation at the end of monitoring.

Current and terminal disinfection are performed in the outbreak. It depends on the presence of the source of infection in the focus.

1.1.1.3. Cholera

Short history

Since the early years of XIXth century, there is no written document attesting the presence of cholera.

The first pandemic of cholera, which occurred from 1817-1823 was associated with the movement of military troops from India (Bombay) to the Persian Gulf. This pandemic of cholera covered the teritory of Iran, Iraq, the Persian Gulf, Turkey, Russia, Egypt, African coast of the Mediterranean, and Asian countries - Nepal, Burma, Thailand, Malaka, Singapore, Indonesia, China and Japan.

The second pandemic (1829-1851) started from Astrakhan (Russia), India, Afghanistan and Iran. Despite strong protection, cholera reached Moscow in 1830. Then the epidemic spread to the western and southern Russia, Bulgaria, Finland, Poland, Austria (movement of military troops), Germany, England, France, Belgium, the Netherlands and Norway in

1832. Later, cholera spread to Syria, Palestine, Egypt, Tunisia, Turkey and Romania from Arabia (after Mecca pilgrimage). In 1832, it spread to Canada, then to New York, South Carolina and Louisiana.

The third pandemic (1852-1859) had onset in Iran and was extending to western and northern Europe (Turkey and Greece are affected as a result of the movement of French military troops in during Crimean War), then to Canada, USA and Mexico.

The Sanitary Conference started in Paris in 1851 and was followed by other 14 conferences held till 1938. In 1903, the International Sanitary Convention stipulated the obligation to notify of all alerts of cholera. In 1907, the Joint International Office of Hygiene was founded, and later, in 1920, was founded the Health Organization for League of Nations, but in 1948 World Health Organization was founded.

The fourth pandemic occurred in the period from 1863 to 1879 years and it was caused by the pilgrimage to Mecca. It was the largest cholera epidemic with 30000 deaths out of 90000 pilgrims. The spreading started from Arabia to Egypt, the southern Mediterranean and France, including Balkans, Central Europe, Middle East, southern Italy, Switzerland, Germany, North Africa, Eastern and Central Europe, the US, Central and South America. The epidemic was very severe with a lot of people affected. Only in Europe hundreds of thousands deaths were recorded. The change occurred in 1866 when cholera began to be considered as a social problem that required the improvement of living and working conditions (safe drinking water, sewage removal, good sanitation, use of disinfectants during epidemic etc.).

The fifth pandemic of cholera (1881-1896) was as severe as the previous one, reaching America, Asia and Africa from Europe.

The sixth pandemic of cholera (1899-1923) started from India and then included Afghanistan, Egypt, western Asia, Middle East, Persia, Russia, Europe, and United States.

Between 1926 - 1960 years epidemic of cholera was present in endemic areas of South East Asia, Afghanistan, Iran, the Persian Gulf, and Egypt.

The seventh pandemic of cholera began in 1961 and it is extended till now. It is the longest pandemic evere recorded. It was started from Indonesia, the Celebes islands (Sulawesi). It is supposed that the area had been endemic (for Vibrio eltor) since 1937, where were reported cases similar to cholera.

Later on, cholera, continued to be manifested as pandemic, epidemic and endemic in many regions of the world. According to the WHO, by 2010 there were 3-5 million cases of cholera, with 100-130 thousand deaths in the world. Endemic outbreaks of cholera were established in Africa and Latin America. The largest outbreak of cholera was recorded in Haiti in 2010. There were recorded more than 200,000 patients with cholera, of which 3,300 died by 31th December, 2010.

The fast spreading of cholera is determined by the movements of population, military troops and fast transports. The administration of rehydration treatment in cholera patients reduced the lethality from 50% to 10.7%.

The causative agent

The pathogen of cholera was unknown in the first 4 pandemics. In the 5th and 6th pandemics, the cholera bacteria that belongs to the Vibrio genus was revealed.

Vibrio cholerae or *V. comma* that was called by R. Koch in 1884, was classified in group 0:1 and non-cholera group 0:1. The differnce is based on antigenic structure, somatic lipopolysaccharide antigen 0, that is specific for each group.

The specificity of somatic 0:1 antigens is common to all strains with epidemic potential, enterotoxigenic, pathogenic, able to determine the most serious diarrhoea, "cholera" and the group non 0:1 – without the capacity to cause the disease.

Strains with specific somatic 0:1 antigens belong to the three known serotypes: Inaba (antigen A and C), Ogava (antigen A and B), Hikojima (antigen A, B and C).

Till 1960, the scientific community considered cholera vibrios group 0: 1 – serotype Ogawa, Inaba or Hicojima – as single determinant pathogens of epidemic cholera ("true" cholera, "Asian"cholera).

Since 1961, El Tor- cholera bacterium was considered etiologic pathogen of "true" cholera. It was the beginning of the 7th pandemic, caused by V. cholerae 0:1 biotype El Tor.

The cholera group non 0:1 includes all strains detected in the environment or from people with similar characteristics or identical serological group 0:1, but cannot agglutinate cholera serum 0:1. Therefore, it was called Vibrio Non-agglutinogen.

Over the past two decades the number of non-agglutinogen serotypes

0: 1, 0: 2, 0: 3, etc. has increased continuously to 0: 138. 1993 was marked by the first appearance of enterotoxigenic Vibrio group non-0:1 (able to determine cases impossible to distinguish clinically from group 0:1) and has diffusion capacity to spread very fast in the Indian subcontinent. The non-cholera bacteria of 0:1 group that was serotype in Japan demonstrated the differnce from other known serotypes (0:2-0:138), being rated to preserve the continuity of the Vibrio 0:139 serotype. It was called Bengal serotype 0:139 to mark the initial occurrence and distribution from the Bay of Bengal.

Cholera vibrio is a mobile bacillus without spores, it is gram-negative. It grows easily on simple medium (peptone water, nutrient agar, gelatin) with alkaline reaction, and it is an aerobic bacillus.

Cholera vibrios are resistant to the external environment: they survive in water 7-15 days, in pools - several months. The resistance to water depends on genetic features (biotype El Tor and Vibrio 139 Bengal are more resistant than the classic one), the concentration of nutrients and oxygen, temperature, alkalinity (pH), salinity, presence of organic substances and the influence of protective sun rays. They are resistant into food, fruit and vegetables within 2-8 days. Cholera vibrios easily resist to low temperature and freezing. They are less resistant to usual chemical and physical agents. Boiling within one minute at 60°C – kills cholera vibrios in 10 minutes. They are sensitive to dryness and pH less than 6.0. Cholera vibrios are sensitive to low concentrations of acid (hydrochloric acid, sulfuric acid) 1:10,000, especially chlorine compounds. The content of 0.2-0.3 mg/l of free chlorine in water determines the destruction of vibrios within minutes.

Cholera vibrios are sensitive to tetracycline, chloramphenicol, and aminoglycosides. The sensitivity may be different depending on the species and strains. Strains resistant to tetracycline and other antibiotics are detected commonly.

The source of pathogen is a patient with symptomatic or asymptomatic forms, carriers of Vibrio cholera, recovery person or healthy carrier with elimination of the pathogen in the priod from several days up to a month.

The results of laboratory investigations of environment (salt or fresh water and aquatic fauna) indicate the existence of the external cycle of cholera bacteria in aquatic systems.

The enterotoxigenic cholera bacteria can pass into a latent form, under unfavourable conditions in the environment (low temperature,

low substances, increased salinity) and there are transformed into small cells that require extremely low metabolic demands. It is supposed that these small cells may return into active state, reproduction under certain favourable conditions, maintaining clinical and epidemiological potential.

Manifestations of infectious process

Cholera remains to be the most severe acute infectious diarrheal disease, characterized by massive loss of body fluids and vomits. Manifestations vary from subclinical form of cholera, which often can hardly be distinguished from carriage or asymptomatic form to severe manifestations, with acute dehydration and death of the patient in the early days.

The incubation period of cholera lasts from 1 to 6 days, often 1-2 days. The disease has an acute onset. The first clinical sign of cholera is pronounced diarrhoea, which occurs unexpectedly. Defecation is usually painless. The stool becomes watery from the onset in most cases. Diarrhoea lasts from a few hours to 7-8 days. A massive loss of body fluids occurs within the first 12-24 hours, after which it progressively decreases.

Vomiting occurs usually after stool, and quickly becomes aqueous. Diarrhoea and vomiting are usually not accompanied by pain in the abdomen. Hypothermia is typical for cholera.

Dehydration starts because of significant loss of liquid and damage of the gastrointestinal tract: hypovolemic shock, hemoconcentration, hypotension, peripheral cyanosis, anuria, skin turgor decreases.

There is a significant difference in the rate of clinical forms (severe, moderate, mild and asymptomatic) generated by the classic biotype compared to El Tor. Severe and moderate forms predominate in classic biotype (26% versus 7% in El-Tor), but in El-Tor cholera mild and asymptomatic forms (93% versus 74% of classic biotype) prevail.

However, clinical and laboratory investigations of the so-called "asymptomatic" forms showed that 80% of them are affected by subclinical cholera.

Manifestations of epidemic process

The mechanism of transmission is faecal-oral. Transmission factors are vomit, feces, water (including sea water, river, lake, residuals, bath, refreshments etc.), food (vegetables, fish and other hydrobionts), hands and contaminated objects, flies.

Humans are contaminated with cholera during care of the sick, from convalescence or carrier of Vibrio cholera, during swimming, using water

for drinking from the sea, rivers, canals, lakes, ponds, vegetables use (a major irrigated with water from the river, ponds or canals contaminated with cholera or leaking sewage), seafood (fish, crustaceans, molluscs, etc.), milk and other contaminated food products.

Water has had the significant role in spreading of cholera since the first pandemic, as it has been used for drinking and for other needs (laundry, hand washing, rinsing the mouth etc.). It is the main risk factor. At the same time, areas with irrigation channels have epidemiological risk (Stefan Voda districts, Slobozia). It is dangerous to store water in tanks, buckets or other containers.

Cholera vibrios enter the human body, usually with water and contaminated food. Total destruction of vibrios is possible in the acid PH of the stomach at low infectious dose of 10^5-10^6 vibrios in 1 ml.

Responsiveness to cholera is general, without distinction of age, sex or race. However, the incidence is higher in young people due to the lack of immune antibodies, and people with unhygienic life style. Severe form of the disease usually occurs in pregnant women which determines abortion or premature birth due to severe shock phenomena.

The infection determines the production of antibodies after 4-5 days from the onset, reaching maximum titers between the 7th and 14th day of the illness and then decreases in the next 4-6 weeks, maximum in 3-7 months.

However, the number of people with antibodies is increasing in areas with intense endemic of cholera, developing the latent immunization among the population. The imported cases of cholera from endemic cholera areas lead to the infection among adults.

El Tor cholera disease can be manifested sporadically and as outbreak, which usually occurs during the summer months. The spreading decreases during cold season.

The epidemiological situation of foci in India and Bangladesh caused by Bengal Vibrio 0:139 indicates the absence of any immune status in the affected population (endemic to vibrios 0:1). A higher morbidity was recorded among adults being mostly manifested sporadically. Transmission from person to person through contaminated food or objects remains theoretically possible and is similar to that of group 0:1.

Prevention and control measures

The prevention is ensured by epidemiological surveillance of diarrhoea organized through a continuous program of supervision and control at the

national level *during pre-epidemic period*. The risk time begins at the end of spring and lasts until early autumn.

In this period a measure of great importance is the collection of morbidity data weekly, mortality, confirmation of suspected cases of cholera, ensuring of prompt intervention to limit the spreading.

During this period general measures of non-specific prevention of disease are applied through:

- surveillance of drinking water consumption by applying the rules of sanitary protection of water sources and conditions control of potable water installations, including local;
- surveillance of municipal wastes and rural irrigation water, bathing water, and surface water (rivers, lakes, canals etc.);
- supervision of collection, removal and neutralization of solid wastes;
- control of markets, crowded places, public toilets, means of transport, tourist areas and temporary communities (camps, agricultural teams etc.);
- surveillance of food safety (preparation and marketing of food).

Nowdays, training of health care specialists in the diagnosis and treatment of cholera, creating of reserve means for the treatment and diagnostic, including bacteriological investigation are important measures.

It is important to inform the population about the mode of transmission and prevention, clinical signs of cholera, and early reference to a doctor. The population must be provided with soap, detergents and disinfectants.

The epidemiological surveillance program provides for bacteriological laboratories supplied with necessary equipment and competent staff in order to detect and confirm fast cholera.

Special attention is paid to multiple cases of diarrhoea outbreaks during pre-epidemic period that may occur in different communities and among the staff of the risk group (water supply, milk, catering employes, etc.). The same attention is paid to cases with dehydration that require hospitalization even if they are solitary cases. In all these situations laboratory exam of stool is compulsory for various enteric pathogens, including cholera bacteria and epidemiological investigation of the outbreak.

An early detection of patients (vibrio carriage) and their hospitalisation without delay are the main antiepidemic measures during *the epidemic period* (from the first confirmed case till the 10th day of the record, isolation or death of the last confirmed patient with cholera). Provisional isolation is justified in case of people suffering from intestinal dysfunction or chronic

gastrointestinal diseases, as well as persons suspected of cholera detected at different stages of care. All these people will be monitored in the outbreak with three bacteriological examination of feces.

Isolation of contacts is justified in case of close relation, from the patient entourage (vibrio carriage) and have equal risk of the contamination. These people will receive tetracycline during three days. Chemoprophylaxis among population from the affected districts can cause disbacteriosis and resistance of vibrios with recovery phenomena of short length. It complicates the early diagnosis of diasese, by changing of clinical symptoms.

The detection of vibrio carrier's is performed by bacteriological examination of persons who were in contact with the patient doing three laboratory tests of feces.

Specific prophylaxis (vaccination) has a secondary role among all measures because it does not prevent the carriage state and spreading of cholera.

1.1.1.4. Viral hepatitis A

Short history

Viral hepatitis A (HAV) is known from the Hippocrates time, when he described symptoms of so-called "jaundice epidemic". In 1855, the disease was called "catarrhal jaundice", considering that it is an inflammatory disease of the bile ducts. In 1912, Cockranye made a scientific description of the disease, using the notion of "infectious hepatitis" for the first time. The disease became common in military campaigns, and was called „soldier's jaundice".

Two major outbreaks were recorded during the Second World War (1942), which included 49,230 cases, of which 28,000 were associated with vaccination against yellow fever applied to US soldiers. Another epidemic affected German and French soldiers' campaign in North Africa. It allowed to distinguish two entities of infections: "epidemic hepatitis" and "serum hepatitis". In 1947, John McCallus introduced the notion of hepatitis A and hepatitis B in the medical literature. Later on, studies performed in England and the US on volunteers allowed to found the "gate", incubation period, contagiousness and prevention with immunoglobulin.

Studies of molecular biology led to major advances in knowledge about the structure of HAV, organization of the genome and pathogenic mechanisms. Epidemiological studies made possible to understand the epidemic process in HAV.

Causative agent

Hepatitis A (HAV), isolated by S. Feinstoume in 1973, belongs to Picornaviridae family, genus Hepatovirus, single representative of this genus. It is an RNA virus, non-enveloped of cube symmetry, with localization in hepatocyte cell cytoplasm. Virus capsid is composed of 32 subunits (capsomeres), the same for the viral proteins (VP1, VP2, VP3 and VP4). The diameter of the virus is 24-30 mm. There is no oncogenic potential.

From the serological point of view, strains of HAV are identical in different parts of the world, which means that the structure is not changeable. Thus, anti-HAV antibodies that are produced by virus hepatitis A, protect against all strains of this kind.

VHA is stable to external environment. The virus survives up to one year in the food, in water - 3-10 months, on different objects at room temperature - a few weeks, in feces - up to 30 days. It is resistant to acid and alkaline medium, to chloroform. It is sensitive to UV light, high temperatures and the action of formalin and chlorine. It is destroyed in 1 min by autoclaving, boilling during 5 minutes, at a temperature of 80°C - 10 minutes, temperature of 60°C - 12 hours; under the action of formalin (1: 4000) it is inactivated after 72 hours, while chlorine destroys it within 30 minutes.

Source of pathogens

Only a sick person with acute HAV can be as a source of infection. The viral particles are absorbed by the gastrointestinal mucosa after ingestion, where it reaches the bloodstream. HAV is located in the hepatocytes of the liver, only that virus recognized by the receptor on the membrane of hepatocytes and invades the cell. The virus lose a capsid inside the cell, releases RNA and starts the replication. New viruses are packaged in vesicles and released from the cell into the bile. The vesicle membrane is dissolved in the bile, releasing particles of HAV, which get into the digestive tract, where they are eliminated by feces in the environment.

The HAV has four periods of infection development: incubation period between 15 and 45 days, sometimes up to 50 days, prodromal - 1 to 3 weeks, jaundice and convalescence. The infectiousness of the patient starts in the last 10-15 days of incubation period and is extended throughout the prodromal period and ends on 6th - 7th day after the jaundice onset. In severe forms it starts at the end of the 2nd week of jaundice. It was

found that at the end of the incubation period, prodromal period and the first day of the jaundice period - 1 g of feces contains up to 10^8 viruses or more. Contagiousness is highest in the prodromal period. The virus is not eliminated in convalescence and in the recovery period. So, the chronic carriage of HAV does not exist. That means that, the convalescent and the person who has suffered in the past of HAV can not serve as a source to the HAV.

The HAV infection can develop different clinical forms: typical (or jaundice form) and atypical (non-manifested without jaundice). Patients without jaundice manifestation are considered to be the main sources of contamination by HAV. Moreover, these forms of the HVA are difficult to detect. They remain undetected without laboratory testing.

Mechanism, ways and factors of transmission

The mechanism of transmission of HAV is mainly faecal-oral. According to the Committee for the Prevention of Viral Hepatitis, the rate of this mechanism is about 95%. The parenteral mechanism is not excluded, which occurs mainly among intravenous drug users or following administration of blood and blood derivatives contaminated with HAV. The incubation period is the same during the parenteral contamination. The dose of HAV must be the same.

The HAV is eliminated from the host-organism by feces. Its high resistance in the environment determines factors of transmission of infection: the water used in food (fountains, aqueducts, reservoirs), water from opened and closed basins, the food, especially vegetables, household items, contaminated hands, flies, sea products (oysters, mussels, clams, shrimp, etc.). Thus, the primary route of transmission is water, followed by food and habitual contact. Outbreaks related to drinking water from the centralized aqueduct, open water tanks, and contaminated food are described. Habitual way of transmission predominates in home conditions and children institutions.

Contributing factors:

Social factors are: unfavourable sanitary-hygienic territory, open water basins, mainly used for recreation, providing with poluted drinking water, non-compliance with sanitary and hygiene rules in catering and food trade, non-compliance with sanitary-hygienic regime in children institutions, insufficient knowledge of hygiene skills, particularly among children.

Natural factors: temperature and humidity environment, the amount of atmospheric precipitation.

Manifestations of epidemic process

The HAV is characterized of ubiquitous spreading. However, the incidence depends on social and natural factors of the territory. The death rate is directly proportional to the density of population and birth rate, the number of preschool and school institutions, and the number of children attending these institutions. Following an analytical ecological correlation study (Sophrony V., Prisacari V., 2000) a strong indirect correlation of HAV incidence with the drinking water quality from aqueducts ($r = 0.93$) and wells ($r = 0.94$) and the level of pollution of surface waters ($r = 0.91$), food ($r = 0.71$), sanitary and hygienic conditions in schools ($r = 0.77$) and kindergartens ($r = 0.94$), and the amount of nitrates in the water from wells ($r = 0.89$) were established.

Late detection of patients with HAV is a key factor that contributes to the spreading of hepatitis A virus in the human population. It results in failure or delay in elimination of the outbreak (isolating of the sick, prophylactic or current disinfection, contact supervision with detection of the source of infection). It is known that 95% of patients with HAV are diagnosed during jaundice, when the infectiousness decreases essentially. It is more important to detect HAV during the prodromal or at the end of incubation period, when contagiousness of these people is higher. Patients without jaundice can be detected only on the basis of laboratory investigations.

HAV endemic countries are considered to be Africa, Central Asia, South America. However, the general trend of the epidemic is decreasing. The epidemic is characterized by cyclic development, with a morbidity periodicity of 5-6 years, which is confirmed by the evolution of the epidemic in Moldova *(Figure 3)*.

Children and adolescents prevail in the structure of HAV morbidity that is 87.0% of general the HAV morbidity *(Figure 4)*. HAV is more frequently registered among children attending kindergarten and schools. The incidence practically falls to zero among people over the age of 60, because of premonition phenomena in the population and immunity in individuals who suffered HAV in the past.

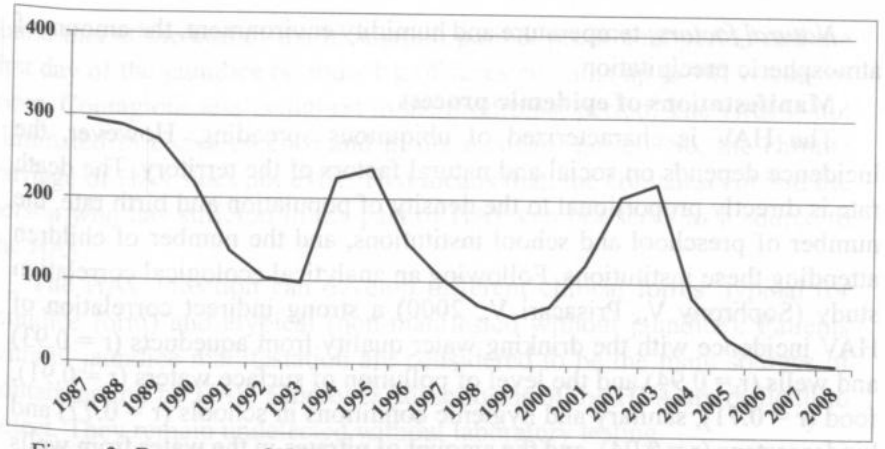

Figure 3. *Dynamics of HAV morbidity in Moldova, in the period 1987 - 2008 (per 100 000 of population).*

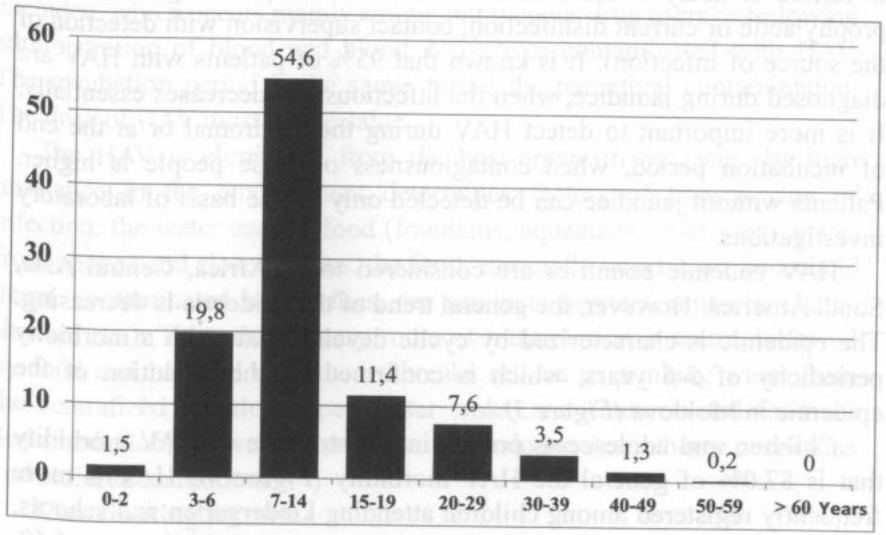

Figure 4. *Distribution of HAV morbidity by age (%).*

The seasonality of the HAV is autumn-winter, with the peak in October-November *(Figure 5)*.

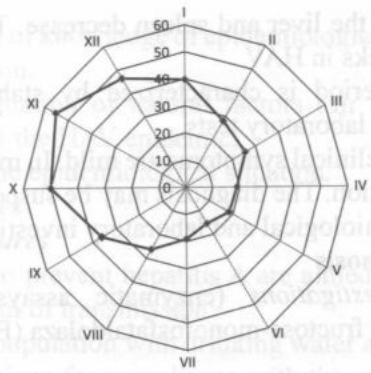

Figure 5. *The seasonality of HAV.*

Social and natural factors determine the increase of seasonal incidence of the HAV: the need for drinking water during the warm season is much higher than in the cold season, water often supplied from unknown sources, abundance of vegetables and fruit, access to the river, activation of the population migration, summer camps, high air temperature and heavy rainfalls. All these factors favour the contamination by the HAV during warm season of the year. Increase of illness in October-November is due to the long incubation period of HAV (45 days) and extension of the epidemic to the winter months determined by habitual contact way of transmission in outbreaks.

The incidence of HAV is higher in the rural population and is more intense in cities located close to the rivers and lakes with low levels of sanitation, and low levels of living conditions and compliance with hygiene.

Manifestations of infectious process

The prodromal period of typical forms of HAV begins suddenly with fever 38-39°C, general weakness, headache, decreased appetite, nausea, vomiting, abdominal pain and other digestive disorders (diarrhoea, constipation). The onset of illness is often similar to that of food poisoning or respiratory infection. Dark urine and pale stools are indicators of infection that occur 2-3 days before jaundice.

The digestive disorders diminish after the jaundice appearance The first is scleral icterus initially, and later it spreads on the skin (1-3 days), persist for 2-5 days, then gradually decreases. The liver has enlarged dimensions, being sensitive to touch. Jaundice is accompanied by pale stools and dark colored urine. Further, jaundice gradually decreases, urine and stool return

to their normal color, the liver and spleen decrease. The average duration of jaundice is 2-3 weeks in HAV.

Convalescence period is characterized by stabilization of normal clinical condition and laboratory tests.

In atypical forms clinical symptoms are mild. In most of cases there are not any signs of infection. The diagnosis may be suspected and found only on the basis of epidemiological and laboratory investigations.

Laboratory diagnosis

Biochemical investigations (enzymatic assays): alanine amino-transferase (ALT) and fructose-monofosfataldolaza (F-1-FA) contribute to the early diagnosis.

The values of enzymes increase by 8-20 times in acute form, even in the last 10-15 days of the incubation period.

Testing of other enzymes in the blood as aspartate aminotransferase (AST), fructozodifosfataldolaza (F - P - FA), alkaline phosphatase, serum proteins and determination of serum cholesterol are useful for the differential diagnosis of other hepatitis. Assesment of isoenzymes can be used for this purpose.

Detection of HAV in feces is possible in the last days of the incubation period, prodromal period and the early days of jaundice period, but it has no practical relevance because test results are delayed.

In the diagnosis of HAV detection of IgM antibodies (anti-HAV IgM) is of a greater importance. IgM antibodies appear in the first days of illness and disappear 3-6 months, sometimes in a year after the onset of disease. It is a specific indicator of acute infectious process. IgG HAV antibodies (anti-HAV IgG) appear later, but they persist for many years, being the markers of immune status. So, detecting IgM anti-HAV in the patient's blood confirms the disease and the occurrence of IgG anti-HAV antibodies indicates the recovery of a patient and the patient becomes immune to HAV. Anti-HAV IgG investigations can be used when the vaccination of people over 40 years of age is necessary.

HAV epidemiological surveillance includes the following objectives:

- systematic epidemiologic diagnosis - as a basis in prevention and combat measures;
- quality assessment and diagnosis of diseases by all forms of hepatitis, as a basic element in the taking anti-epidemic measures in time;
- assessment of sanitary-hygienic state, water sources, food enterprises, children institutions;

- assess the level of knowledge of epidemiological and hygienic habits of the population;
- continued evaluation of natural factors (air temperature, rainfall, etc.), favouring the HAV epidemic;
- prognoses of the epidemiological situation.

Control measures

Preventive measures

Basic measures to prevent hepatitis A are aimed at the elimination of faecal-oral mechanism of transmission:

- providing the population with drinking water and safe food;
- creating conditions for compliance with the requirements and rules on the production, transportation, storage, processing and marketing;
- ensuring continuous compliance with sanitary and hygienic rules in industrial trade and public catering establishments, compliance with sanitary and anti-epidemic regime in children institutions;
- teachiong the population, especially nursery staff, people working in the food industry, children and adolescents the rules of hygiene.

An important measure to prevent the spread HAV is early detection of patients, based on knowledge of first clinical signs and laboratory investigations. The enzymatic analysis is already positive in the incubation period, with 10-15 days before the prodromal period.

Currently, vaccination is used as a prophylactic measure all over the world. There are three types of anti-HAV vaccine: live attenuated vaccine, inactivated vaccine and recombinant vaccine.

In some countries (USA, Spain, Israel, Italy, Slovenia, partially Russian Federation) vaccination is carried out within the planned schedule of vaccinations Other countries, including Moldova, the vaccination against HAV is envisaged according to epidemiological indications.

Vaccination is given to children living in areas with high HAV morbidity (endemic), kindergarten employees, workers responsible for cleaning and repair of sewage systems, people who travel to endemic HAV countries.

Antiepidemic measures

Notification sheet form no. 058/e is transmitted to the Center for Public Health for each patient with detected or suspected HAV.

Patients with mild form can be isolated at home. Hospitalisation is performed according to clinical indications (severe forms and secondary infection) and epidemiological ones – lack of possibility to isolate a

patient at home, compliance with antiepidemic regime. Discharge from the hospital is performed after the clinical recovery.

The epidemiological investigation of the outbreak is carried out with the determination of the source of infection, contamination conditions, and contact people in the outbreak.

Persons who had the contact with a HAV patient are under the medical surveillance (measuring temperature, state of mucous membranes, sclera and skin, the colour of urine and feces, palpation of the right side of the abdomen) once in 10 days and in children institutions. It is performed daily during 35 days from the last contact with the patient. Laboratory examination of persons who were in contact with the patient is performed to determine the HAV enzymes (ALT) and, in case of epidemiological indications - determining of specific markers of the HAV (anti-HAV IgM). Children who were in contact with a HAV patient are not allowed to be transferred to another institution, groups or classes for 35 days from the last day of the contact with the sick.

The current or terminal disinfection is carried out in the outbreak using disinfectants according to methodical instructions provided for this disease (Health Ministry Order no. 41 of 14.07.2004).

Emergency prophylaxis is performed with immunoglobulin or vaccine among those were in contact according to the epidemiologist indication.

1.1.1.5. Hepatitis E Virus

Short history

Hepatitis E virus (HEV) causes an acute infection with epidemiological features simillar to HAV. It was discovered in 1980 as a pathological entity that is transmitted enterally, and belongs to the group of non-A non-B hepatitis. In 1956 the first outbreak with 29300 cases occurred in Delhi, and the second one was recorded in 1978-1979 in the Kashmir Valley, characterized by an incubation period different from the HAV icubation. It affected young adults and high lethality among pregnant women was recorded. 90% of adult population of India were protected from HAV by the vaccination or were infected in the past.

Nine major epidemics of HEV were reported after 1982 in China. It was in the largest region - Xinjiang (1986-1988) with over 100000 cases. In the last two decades, epidemics of HEV were reported in other parts of the world. In 1983 M. Balayan made self-contamination with the

HEV having protective antibody against the HAV. The HEV appeared in 28-45 days in feces, thus demonstrating the HEV entity.

Pathogen agent

The Hepatitis E virus is spherical (27-30 nm) with icosahedral symmetry, without coating. Genomic RNA is surrounded by a capsule with two proteins, one of which is RNA polymerase. HEV is heterogeneous with three genetic types that comes from South-East Asia (Burma, India), North and Central Asia (China, Pakistan, Kyrgyzstan) and North America (Mexico). HEV is less stable than HAV in the environment, but much more resistant in water contaminated with feces and house waste. It is sensitive to heat and clorine substances.

The source of pathogen is a sick person with asymptomatic form. The incubation period varies between 60 and 75 days. The virus is eliminated from the host body with feces during the last week of incubation period and continuie virus elimination 2 weeks after disease onset. HEV disappears in a few weeks after jaundice onset. A chronic carrier of HEV is not described.

The latest studies showed that HEV can infect some species of wild and domestic animals under natural conditions (rats, pigs, chickens and ducks, cattle, cattle, monkeys). Genotyping of HEV isolated from humans and animals is an important proof of the possibility of transmission of the virus from animals to humans.

Factors and transmission routes

The main mode of transmission of HEV is fecal-oral and it is caused more frequently by water contaminated with feces. Practically, all recorded outbreaks were caused by contaminated water in majority of countries. The transmission factors can be contaminated food, objects or more rarely hands. Vertical transmission from mother to child is not excluded. This kind of transmission is confirmed by the presence of viral genome in the umbilical cord and blood of a newborn.

The factors that contribuite to the disease spread are socio-economic (wars, migrations, lack of drinking water, improper maintenance of water system suplly), natural disasters (floods, earthquakes), migration processes and development of tourism.

Manifestations of epidemic process

The infection with HEV can evolve sporadic, endemic and epidemic manifestations. Sporadic cases (less than 1% of cases) occur in economically developed countries. These cases occur mainly ing travelers

that return from endemic areas. The spread has endemic and epidemic features in poorer countries, where from single cases to severe epidemics can be recorded. The outbreaks of HEV that are caused by consumption of contaminated water have explosive character. Usually, outbreaks occur after floods caused by heavy rains or melting snow.

Distinctive features of HEV include: high spread of diseases in persons age 15-40, the prevalence of men in general morbidity, severe evolution of disease in pregnant women, no seasonality. The outbreak occurs mostly in families, with transmission via water. It is recorded commonly in endemic countries with tropical and subtropical climate.

Recentlly, publications about infection spread in endemic and non-endemic territories have appeared. HEV markers are detected in people who are engaged in zootechnics. T. N. Bistrova et al. (2009) found an obvious predominance of anti-HEV IgG in persons at the age of 30-39 in 5.8% of cases and at the age of 50 and over - 7.6% of cases. The frequency of positive results of HEV IgM and anti-anti-HEV IgG among workers who work with pigs constituted 6.3% and 18.9% respectively.

Manifestations of infectious process

The symptoms are similar to those of hepatitis A.

The prodromal period lasts 1-9 days (the average is 3-7 days) with appearnace of dyspepsia (loss of appetite, nausea, vomiting, pain in the right hypochondrium), asthenic syndrome (general malaise, moderate headache, tiredness), flu syndrome with fever, hepatomegaly, splenomegaly, dark urine and acholic stool. The symptoms can be absent in 1/5 of patients during the prodromal period and the disease starts with jaundice and urine of dark colour. Jaundice does not improve the general condition of the patient. Jaundice occurs 2-3 times during 10 days. It lasts from one to six weeks. General weakness persists longer.

The HEV may have a severe form in pregnant women, who can develop fulminant hepatitis. It occurs in 20-25% of cases and lethality may occur in 30-40% of cases, especially in the last trimester of pregnancy.

The diagnosis is based on epidemiological data collected from the foci, the development of viral hepatitis with complications in pregnant women and confirmed by serologic and biochemical investigations. Anti-HEV IgM appears in 1-4 weeks after the onset of clinical symptoms and it disappears in three months. Anti-HEV IgG appears later, but it persists

for years. Detection of RNA - HEV by PCR and hybridization procedure of feces in the early period of the disease is positive in 50-75% of cases.

Epidemiological surveillance does not differ essencially from that of HAV. It is required to collect the information about the situation of centralized water supply system, quality of water in fountains, migration processes, particularly, in endemic territories (India, Nepal, China, Hong Kong Taiwan, Chile, Turkey, Brazil, Tajikistan, Kyrgyzstan, etc.). Early detection of the first cases of HEV has a special role in epidemiological surveillance. Severe hepatitis E has a great impact, especially in pregnant women without HAV, HBV and HCV antibodies.

Preventive measures are basically the same as in other digestive infections: providing the population with qualitative drinking water, protection of water sources from contamination with feces and waste, individual hygiene, well cooked food, epidemiological surveillance of people with high risk. The risk of infection will be reduced by compliance with these measures. The vaccine against hepatitis E is in clinical trial stage.

Anti-epidemic measures in the focus are simillar to those of HAV and refer to epidemiological investigation, detection of sick, isolation of patients and reporting to the CPH, detection of people who had contact with the sick, epidemiological surveillance and disinfection of the focus.

1.1.1.6. Enteroviruses

Enteroviruses are a group of infectious diseases, widespread in the world and with the ability to develop epidemics and pandemics.

The history of Enteroviruses is simillar to that of many other infectious disease groups of polyetiologic character (salmonellosis, shigellosis, leptospirosis etc.). The first discovered pathogen was the most virulent and aggressive.

Enteroviruses belong to the *Enterovirus* genus in the *Picornaviridae family*. There are four subgroups of enteroviruses with 72 representatives according to the original classification:

- ✓ polio virus was discovered in 1908, it was the first representative of enteroviruses;
- ✓ Coxsackie viruse is simillar to polio virus, it was isolated first in 1948 in the town Coxsackie (USA), from which it takes the name. Later, this type of virus has been isolated from patients with aseptic meningitis (1949), epidemic myalgia (1950), summer influenza

(1950), herpangina (1951) and myocarditis (1953). Coxsackie viruses are divided into group A with 23 serotypes and group B with 6 serotypes. These groups of enteroviruses vary by their effects. The group A of Coxsackie viruses cause paralysis in mice, group B viruses have lethal outcome in mice;

✓ In 1953 the ECHO viruse was isolated for the first time from patients with Poliomyelitis. It is cytopathogenic and does not affect laboratory animals. There are 31 known serotypes. The ECHO virus it is the abreviation for the words Cytopathogenic Human Enteric Orphans. This serotype was not related to a specific clinical manifestation, being isolated from patients with myalgia, aseptic meningitis with viral respiratory infections, myocarditis, etc.

✓ Other enteroviruses (68-72 types).

Types 68 and 69 cause respiratory and gastrointestinal diseases. Type 70th is the causative agent of acute hemorrhagic conjunctivitis. Type 71 causes meningitis and meningoencephalitis. Enterovirus of type 72 is recognized as the causative agent of hepatitis A, that was defined later as a separate genus - *Hepatovirus.*

Enteroviruses are resistant to the environment. They remain viabile for years in freezing conditions, at a temperature of 4°C – for ome months, at a room temperature – for a few weeks. They are sensitive to ultraviolet radiation, high temperatures (over 50°C), to clorigene and some oxidants disinfectant.

1.1.1.6.1. Poliomyelitis

Short history

Polio is also known as "spinal paralysis". It was a major public health problem in the past because of paralysis. Although, it was reported from antiquity (Hippocrates described an outbreak of paralytic polio-like disease), the disease was first described in 1789 by Michael Underwood, who called it *debility of the lower extremities.* In the nineteenth century, polio was described by German orthopedist Heine (1840) and Swedish pediatrician Medin (Heine-Medin disease). They described an epidemic of poliomyelitis which took place in Stockholm (1890). In 1908, Landsteiner and Popper Karl described the viral etiology of disease and demonstrated it by reproducing the disease on monkeys. In 1949, the discovery of cell cultures allowed Webler and Frederick Robbins to isolate the polio virus.

Over time, numerous and severe polio epidemics were recorded in

the USA, and Scandinavia. After the Second World War, polio spread with severe clinical manifestations throught the world. In 1951-1955 approximately 28,500 cases of polio in children were recorded in European countries each year.

The universal spread and severe social consequences imposed the intensification of research to obtain the vaccine. In 1950, J. Salk (US) obtained the inactivated polio vaccine by using cell cultures and killing the virus by treatment with formalin. Later, live attenuated virus vaccine was descovered by A. Sabin, as a trivalent antigen, which ensures a complete immunization after orally administration of 4 doses. In 1959, M.P. Chumakov discoverd the same strain of inactivated polio vaccine administered orally, which was used in the polio epidemic in Estonia.

The situation changed for the better after polio vaccines discovery and its wide use in preventing. It was a good example of action against human infectious diseases. It also confirmed the exceptional role of vaccines in preventing and combating such infectious diseases as smallpox, diphtheria, tetanus, pertussis, measles, rubella, hepatitis B, etc.). In 1961-1965 in European countries about 7,700 children contracted polio annually. It was four times lower compared to the previous vaccination (1951-1955). In 1975 the number of polio cases decreased to 1119 in all European countries, and in 1980 – to 209 cases in 29 countries.

However, in 1978-1979 an outbreak of polio was recorded in Denmark, because a community of people refused the vaccination on religious grounds. In 1983 there were 25 cases of polio in Spain and in 1985 9 cases of polio were recorded in Finland. In 1988, 16 patients with polio were recorded in Israel.

The same situation was registered in Moldova. In 1958, the implementation of vaccination against polio led to the decrease of incidence from 456 cases at the beginning of vaccination to 2 cases in 1969. No cases of poliomyelitis were recorded in Moldova from 1970 to 1981. However, in 1980, the rate of vaccination dropped to 48,459 and revaccination to 274,643 compared to 73-75 thousand vaccinated and 330-346 thousand revaccinated in previous years, which contributed to a severe outbreak of polio in 1982 with 93 cases and in 1983-1984 with 38 cases. The intensification of vaccination and revaccination of children against polio reduced the morbidity to single cases. The last case of polio was recorded in 1998. Polio has not been recorded in Moldova since 1999. This point

was a solid argument for the WHO to certify the Republic of Moldova as a country free of polio. In 2002 Moldova was certified as country free of Polio together with other European countries.

In spite of the resolution approved by the WHO about polio eradication in the world and elimination of the infection in over 170 countries (North and South America, Europe, China and Australia), some countries of Asia and Africa remain endemic. It makes posiblle to invade polio by unvaccinated people from these countries. The eruption of wild polio virus recorded in Tajikistan between December, 26th 2009 - April 20th 2010 is an example. There were recorded 120 cases of polio among children, of which 108 were under the age of 5.

Therefore, to avoid complications the vaccination and revaccination of children according to the schedule of vaccinations are cecommended.

Pathogen agent

There are three main serotypes of polioviruses (I, II, III). Frequently Type I polio viruse is recorded. It is resistant to the environment – in water it retains viability up to 100 days, in the feces - up to 6 months, in fruit and vegetables - up to 3 months. It is ease supports both freezing and drying. They are sensitive to high temperatures (boiling), UV radiation, such disinfectants as clorine substances, potassium permanganate and hydrogen peroxide.

Source of pathogens

The only source of polio infection is a man (sick or carrier). Patients with mild or inapparent form of the disease have a greater epidemiological risk. Their number exceeds the number of patients with manifested clinical forms of poliomyelitis. The patient is contagious already in the incubation period, in 36 hours after the contamination through throat excretion and after 72 hours - through faecal excretion. It is highly contagious during the clinical manifestations of infection. The virus is excreted by feces in the period of several weeks to 3-4 months. Its contagiousness is ≈ 90%.

Factors and transmission routes

The main way of transmission of the pathogen from the host body to others is fecal-oral. The spread of the virus occurs through the water, food, contaminated hands and flies. Waste water from the town central water sewerage system is contaminated. Vegetables and milk play an important role. In polio pathogen transmission occurs in the contact with house objects, by direct contact with sick or carrier, or by contaminated

hands as well. Airborne contamination can occurs during the last days of the incubation period and first 5 days of illness when the virus is in the nasopharynx.

Manifestations of epidemic process

From 80% to 90% of cases of polio are recorded among children aged unde 5. The infection leads to the production of type-specific and long-lasting immunity. Postnatal immunity lasts up to 3 months. The disease appears both in urban and rural areas, usually among unvaccinated children. Its seasonality occurs in autumn-winter, and it is manifested at the sporadic level. The risk of import of poliovirus from endemic countries persists because of the intense migration of population, that makes possiblle the occurrence of new cases of polio or even outbreaks.

Manifestations of infectious process

The incubation period varies from 5 to 35 days (average 7-12 days). Incubation is the digestive stage in the development of infection, when the virus multiplies in the epithelial cells of the throat and gut and in their lymph tissues (tonsils, lymph nodes, Peyer's patches). Sometimes, respiratory or digestive signs appeare during this period.

The prodromal period (prodromal or "minor disease") coincides with the state of viremia lasting 1-5 days and is characterized clinically by fever, which may reach 39°C, headache, myalgia, nausea, anorexia, rhinitis, pharyngitis, tracheitis, abdominal pain, diarrhea.

After the prodromal period, the latent period begins lasting 2-4 days. It is characterized by the normalization of temperature. The virus is spreaded by the nervous system to the spinal cord or brain in approximately 1% of infected people. The invasion persists 1-2 weeks. It starts suddenly with high fever and development of paralytic manifestations, which can be divided into two stages:

- *Preparalytic stage* lasts from few hours to two weeks and is characterized by nervous manifestations, headache, myalgia, arthralgia, meningian syndrome, muscle weakness and sometimes paralysis of the abdominal and neck muscles, muscle spasms, apathy;
- *Paralytic stage* starts 2-5 days after the increase of fever when flaccid paralysis of the lower limbs occurs. Paralysis may be different and asymmetrical; they predominate in the deltoid, spinal, tibial, respiratory, facial, and abdominal muscles. Bladder paralysis occurs with retention of urine.

The convalescence period may be accompanied by healing or paralysis in 10-15% of cases. Death occurs more frequently as a result of paralysis of respiratory muscles of the chest. Paralyzed limbs do not develop properly, which leads to disablement.

Epidemiological surveillance

The purpose of epidemiological surveillance in polio is to assess the situation in the country and in all the world and to develop the complex of measures aimed to prevent the import of wild polio virus in the country and new cases of illnesses.

The main objectives of epidemiological surveillance in polio include:

- early detection of new cases of polio that can be imported from other countries, with epidemiological, clinical and virological confirmation (as it is defined in the standard case). It is important to detect and investigate every case of acute flaccid paralysis (PAF);
- monitoring of all non-polio enteroviruses that circulate in the environment and among population.
- to control the vaccination coverage among target ages and the quality of vaccination;
- laboratory control of immunity state against polio (serological monitoring);
- epidemiogical forecasting of the situation.

At the moment the basic element is the surveillance of acute syndrome of flaccid paralysis in polio and emergency information about each case of PAF.

Knowledge of epidemiology, clinical signs and virological investigations to confirm the diagnosis are particularly important.

The intensifiication of epidemiological surveillance of polio enteroviral infections associated with vaccination is an important element of epidemiological surveillance at present, especially in polio-free countries.

Control measures

Preventive measures

Specific prophylaxis includes vaccination with inactivated virus vaccine (IPV) and oral live attenuated polio vaccine (OPV) composed of trivalent antigen. The efficiency is 96-100% in case of administration of 4 doses of the vaccine.

Both kinds of vaccine have advantages and disadvantages. The advantage is the safety of inactivated vaccine. However, this vaccine is administered parenterally and induces humoral immunity but without secretion of local secretory immunoglobulin (intestinal). So, it prevents the replication of the virus in the intestinal mucosa. Vaccinated people, practically, cannot not catch the disease, but they can release the virus in the environment.

Oral live attenuated vaccine, provides the production of humoral immunity, and the local one blocks the entrance gates, makes impossible the replication of wild poliovirus in the intestine. But this type of vaccine can cause poliomielytis associated with the vaccine, namely, Vaccine Associated Paralytic Poliomyelitis (VAPP). It is estimated that the risk of VAPP is about 1 to 2.6 mln of administrated doses, as a result of failure in child protection after the vaccination or administration in the time interval less than 30 days after the vaccination. The risk is higher after the first administration of live attenuated vaccine.

However, polio immunization among children, which is performed according to the national schedule of vaccination remains the main measures. Oral polio live attenuated vaccine (OPV) is used in Moldova according to the following scheme: at the age of 2 months - VPO1 is adminstrated, at the age of four months - VPO2, at the age of six months - VPO3, at the age of 22-24 months - VPO4, at the age of 6-7 years - VPO5, at the age of 15-16 years - VPO6.

The main point of immunoprophylaxis against polio is full coverage with the vaccination that must be not less than 95% of all children.

According to the WHO recommendations, after worldwide polio elimination, the vaccination of children will be performed with inactivated polio vaccine in order to exclude the possibility of invasion of the vaccine virus type among nonimmune population.

Anti-epidemic measures in polio focus

Detected patients with polio or persons with suspected polio, and all PFA cases are compulsory hospitalized to infectious diseases hospital for 40-42 days. On admission, two faecal samples are collected for virological investigations with interval of 24-48 hours and two blood samples are collected for serological investigations - first test on admission and the

second one - in three weeks. The final diagnosis is based on clinical signs, epidemiological anamnesis and laboratory results according to the standard definition of Polio, PAF and VAPP. The treatment is administered according to the standard protocol. The patients are discharged only after a full clinical recovery, if there are no changes in the cerebrospinal fluid. People who were contaminated are admitted back to the job, only after a full clinical recovery, but not earlier than 40 days after the disease.

The institution which detected (or suspected) the person with polio, VAPP or PAF is obliged to inform the Center for PublicHealth (CPH) by telephone or by electronic information system immediately and fill in the notification form no. 058/s within 24 hours of detecting the patient.

Terminal disinfection is carried out in the focus.

All contacts, especially children aged under 5 are investigated clinically by a family doctor, pediatrician and neurologist to detedt clinical signs that are specific for polio or PAF and virological examination of feces is made. The surveillance of contacts lasts 21 days.

The examination of persons who arrive from polio endemic areas is required and it is necessary to check the vaccination and revaccination status of children.

Children institutions where polio case were deteced are closed and are quarantined during 21 days after the isolation of the last polio case.

1.1.1.7. Rotavirus infection

Short history

In 1973 in Australia Rotavirus was first identified as a result of microscope examination of isolates from duodenal biopsies from children with acute non-bacterial gastroenteritis. Later, rotavirus was isolated from patients with acute diarrhea in all geographical areas and was widespread in all over the world.

Pathogen agent

Rotaviruse belongs to the *genus Rotavirus* in the *Reoviridae family*. Under the microscope it looks like a spherical particle with a well-defined contour with radial arrangement of capsomeres, with the stump in the middle. Four groups of antigens of rotavirus were detected. The main antigen belongs to VP2 group. All rotaviruses are divided into seven groups: A, B, C, D, E, F, G. Rotaviruses that affect humans are from A, B and C groups.

However, the majority of the human rotaviruses refer to group A, and commonly produce outbreaks both in developed and low income countries. Within group A there are subgroups and serotypes which differ by the specific surface protein capsid. Rotaviruses heterogeneity explains the reinfection with this infection among humans. The probability of reinfection with rotavirus is 30% of cases in the first year of life, about 70% - in the second year. 30% of infants are reinfected up to 3 times, and 20% of infants – up to 4 times.

Rotaviruses are resistant to the environment, where they can survive for more than 10 days at low temperature and humidity. They may be found in drinking water or ponds for recreation, residual water, resisting for several weeks. Rotaviruses are resistant to organic solvents, ether, chloroform and ultrasound. Multiple freezing does not kill them. The virus can be destroyed under the action of 95% of ethanol, chloramine, formaldehyde, hydrogen peroxide, acid or alkaline substances, ultraviolet rays and high temperatures. Rotaviruses are killed at a temperature of 70°C within 10 minutes, at a temperature of 80°C - 1 minute, at a temperatures of 100°C - momentary.

The source of pathogen

Although some types of rotaviruses that are isolated from humans are similar to those isolated from animals, there is not any reliable information regarding the animal as a source of infection for humans. Therefore, it is considered that the reservoir and source of pathogens are humans – a patient or carrier. Patients with manifested or subclinical forms are the main source of pathogen. These patients eliminate viruses by stool in significant quantities – up to 10^{10} - 10^{12} virus particles in 1 g of feces.

The incubation period varies from 1 to 7 days. The virus is eliminated with feces 2 days before the appearance of clinical signs, with a highest concentration on the 3rd to 6th day of the clinical manifestations, and continues until the 20th day of the disease. Rarely, the viruses are eliminated by people with immunodeficiency in the period up to 30 days from the disease onset.

Carriers of rotaviruses present an epidemiological risk as well. The level of carriages among children aged 3 is about 1.5 - 9.0% of cases. 71% of them are children of the first year of life. Carriage among adult population is about 2.5%. It has a greater epidemiological significance in

children under the age of 12 months, who are infected with the virus from carrier mothers most frequently.

Factors and transmission routes

The mechanism (mode) of transmission is fecal-oral. Transmission factors are water, food, contaminated hands and objects. The primary route of transmission is via water, as it was demonstrated by frequent eruptions spread via water and isolation of rotaviruses in different water sources.

There is a direct correlation between the number of the sick and the amount of rainfalls that lead to pathogens movement from the environment surfaces into the water basins. Milk and milk products present a greater danger.

Contamination from contact objects or hands occurs more frequently in families, hospitals or nurseries. Airborne transmission is not excluded because rotavirus infection starts with catarrhal signs in 50-75% of children. Detection of virus in the saliva of patients by PCR method during the first days of illness can be used as an indirect confirmation of the possibility of airborne transmission.

Manifestations of epidemic process

Rotavirus infection is prevalent worldwide. About half of all diarrheal diseases appear in children in developing countries. Gastroenteritis occurs in 9 - 73%. Rotavirus infection affects people of all ages, but it is recorded more frequently in children under the age of 5, with a higher incidence in children aged 6 months to 2 years. It is the main cause of severe diarrhea and dehydration in infants. According to the WHO, from 1 to 3 mln of children with rotavirus infection die annually in all over the world. Rotavirus infection causes 25% of traveler's diarrhea. Rotavirus infection is a public health issue in Moldova as well. It makes 17% of all cases of Diarrheal Acute Diaseases (DAD) affecting 28% of children under the age of 2.

The incidence of rotavirus infection has a cyclic character. The morbidity of rotavirus infection increased from 0.05 cases in 2005 to 21.78 cases per 100 000 population in Moldova in 2012.

In 2013, the morbidity decreased to 10.11 per 100 thousand of population because of implemention of vaccination against rotavirus infection in 2012 *(Figure 6)*.

Rotavirus infection epidemic is manifested as sporadic cases, epidemic or outbreak.

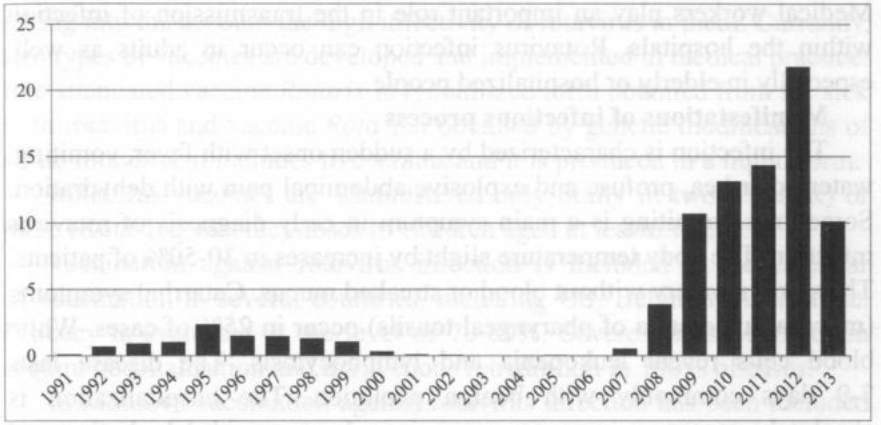

Figure 6. *The morbidity of rotavirus infection in Republic of Moldova, 1991-2013.*

The annual rate of rotavirus infection increases in winter, causing winter gastroenteritis outbreak *(Figure 7)*. During three months of winter (from December to February) 45.5% of annual morbidity are recorded.

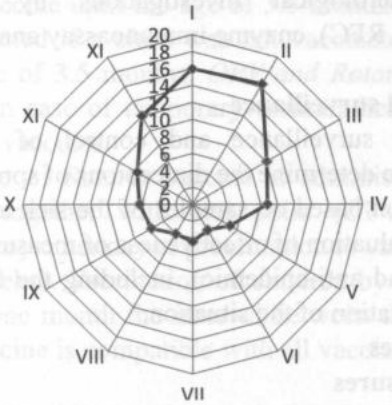

Figure 7. *Seasonality of rotavirus infection.*

Rotavirus infection occurs frequently in children, especially in kindergartens. Eruptions of rotavirus infection are often recorded in hospitals, maternity and children's hospitals. Nosocomial rotavirus infection is recorded, generally, in infants with low birth weight, artificially fed children, who suffer from chronic diseases, with immunodeficiency.

Medical workers play an important role in the transmission of infection within the hospitals. Rotavirus infection can occur in adults as well, especially in elderly or hospitalized people.

Manifestations of infectious process

The infection is characterized by a sudden onset with fever, vomiting, watery diarrhea, profuse and explosive abdominal pain with dehydration. Sometimes, vomiting is a main symptom in early diagnosis of rotavirus infection. The body temperature slight by increases in 30-50% of patients. The stool is watery without blood or streaked mucus. Catarrhal symptoms (mucosal hyperemia of pharyngeal tonsils) occur in 25% of cases. White blood cells reveal leukopenia and lymphocytosis. The disease lasts 7-9 days commonly with benign evolution. The hospitalization is absolutely necessary in severe cases, in order to avoid dehydration and electrolyte imbalance. Death or prolonged convalescence may occurs in severe clinical forms.

Rotavirus infection evolves as a benign diarrhea or even in asymptomatic form in adults.

Diagnosis can be made based on clinical picture, epidemiological, virological and serological investigations by radioimmunoassay examination (RHAI, RFC), enzyme immunoassay analysis and coaglution reaction.

Epidemiological surveillance

Epidemiological surveillance and control of rotavirus infection include monitoring to determine the dimensions of spreading of morbidity, rotaviruses circulation based on samples of the sick and the objects from the environment; evaluation of effectiveness of measures performed in the focus (preventive and anti-epidemic), including the level of vaccination coverage; prognostication of the situation.

Control measures

Preventive measures

Prevention of rotavirus infection is based on the provision of the population with drinking water, maintenance of hygiene in water sources used for public recreation, food, compliance with hygienic and anti-epidemic rules in groups of children and hospitals, individual hygiene of children, promotion of education and training of the population responsibile for health and social care of children.

Vaccination of young children is considered to be an effective prevention

taking into the account the high infectivity of rotavirus in them. Currently, two types of vaccines are developed and implemented in medical practice: live attenuated vaccine *Rotarix* in lyophilized form obtained from the sick with rotavirus and vaccine *Rota Teq* obtained by genetic modifications of cattle rotavirus, it includes five strains and it is produced in a liquid form.

Rotavirus vaccines are administered only orally in two (Rotarix) or three (Rota Teq vaccine) doses to children aged at least6 weeks.

Vaccination against rotavirus infection is included in the calendar of vaccination in several countries, including US, Belgium, Austria etc. Efficacy is evaluated at the level of 70-80%. Severe forms of infection require hospitalization and intravenous rehydration in 100% of cases.

In Moldova vaccination against rotavirus infection has been included in the calendar of vaccinations since July of 2012 by Order No. MS. 662 of 28. 06. 2012 „The implementation of vaccination against rotavirus infection of children in Moldova".

This order provides the first dose of *Rotarix* vaccine together with oral polio vaccines (OPV) and DTP-HepB-Hib pentavalent to children aged 2 months. Children who were not vaccinated at the age of two months will be given the vaccine until the age of 3.5 months (15 weeks). It will no longer be performed if a child was not vaccinated against rotavirus infection till the age of 3.5 months. *OPV and Rotarix* vaccines will be administered only, in case of temporary contraindications to DTP-Hep-pentavalent and Hib vaccines.

The second dose of *Rotarix* vaccine is administered at the age of 4 months usually together with oral polio (OPV) and pentavalent DTP-HepB-Hib, or any other day of presentation to vaccination till the age of 7 months (32 weeks). Vaccination of children by individual schemes requires minimum one month the interval between the doses of *Rotarix* vaccine. Rotarix vaccine is compatible with all vaccines given to children aged 2-7 months.

The vaccine is contraindicated to children:

- with hypersensitivity to any substance in the vaccine or if they had a reaction to the previous dose of the given vaccine;
- with congenital defects of the bowel that could predispose to intussusception;
- with severe immunodeficiency states and receive treatment with immunodepressants.

In children with acute diseases and exacerbation of chronic diseases vaccination should be postponed.

Anti-epidemic measures

Family doctor must inform the CPH about the sick or person suspected of rotavirus infection.

Patients with mild infection that have the possibilities to compliance with the anti-epidemic regime are isolated and treated at home. Patients are hospitalized to infectious diseases hospital based on clinical (severe and moderate forms) and epidemiological indications (lack of conditions for isolation at home).

Patients are discharged from the hospital after clinical recovery. Sick children who attend kindergarten, food company emploees undergo clinical surveillance during a month, with a daily control of the stool. The virological investigation is recommended at the end of the surveillance after the disapearance of clinical signs. The follow-up is not performed.

Current disinfection is carried out in case of the outbreak if the patient is isolated at home. Terminal disinfection is performed if patient is hospitalized. Epidemiological investigation and medical surveillance of persons who had the contact with the sick is performed during 7 days.

Measures of quarantine are organized in children institutions within 5 days from the last sick isolation. Immunoglobulin can be used as an emergency prevention.

1.1.2. Respiratory infections

1.1.2.1. Diphtheria

Short history

The disease was first described in the XVIth century in Spain. The descovery was based on the publications made by Spanish doctors about widespread infection recorded in Spain at that time. The disease was called "garrotillo" because this infection is manifested by asphyxia which leads often leads to a patient's death. Diphtheria was widespread in Italy after the beginning of XVIIth century as well. In that period tracheostomy was used to rescue the patients. That was why it was named "cannula respiratory disease."

In the XVIIIth century it became known in France, Holland, England, Germany, Russia, and since 1752 - in the USA. A very wide spread of disease in the late XVIIIth and early XIXth century diphtheria was recorded

in France, which obliged the French Government to establish a special committee to combat the infection. In June 14, 1807 the decree on the study of the nature of croup was adopted. In 1815 Breton described clinical symptoms of the disease in detail and he used for the first time the term of "diphtherit" (Greek. "Diphtheron" = membrane). Later in 1846, it was replaced in "diphtheria" by Trousseau.

In 1883 the pathogen was identified by Edwin Klebs in fibrinous membrane obtained from the diphtheria patient's throat. Later in 1884 it was isolated in pure culture by Friedrich Löffler. In 1888 Pierre Alexandre Yersin and Roux isolated diphtheria toxoid, and in 1890 Emil von Behring obtained diphtheria serum in Germany and he cured the first patient with diphtheria in 1891. In 1901 he received the Nobel Prize in Medicine for this discovery. In 1923, in France Gaston Ramon obtained toxoid as a means of prevention used against diphtheria. The application of serotherapy contributed greatly to the decrease of lethality from 30-50% to 1-10%. The application of toxoid as a prophylaxis contributed to the decrease of morbidity worldwide.

Causative agent

The pathogen agent is bacillus of Corynebacterium diphtheriae. It is an aerobic gram-positive microogrganism. It belongs to the genus Corynebacterium (from the Greek "Corynebacterium" that means swelling in the extremities of bacteria), which includes also such species of corynebacteria as C. ulcerans, C. pseudotuberculosis. Only C. diphtheriae has toxigenic and pathogenic potential to infect humans. C. diphtheriae can be divided into three biotypes according to the culture and biochemical properties: gravis (R form), intermedius (intermediate form) and mitis (S Form). They differ by virulence. The most virulent is the gravis biotype.

Toxigenicity of C. diphtheriae is caused by specific phage that contaminates the bacteria. The genetic material of bacteriophage invades the chromosome, attaches as a fragment (prophage), and the bacteriophage causes changes in the biological properties of bacteria. Bacillus diphtheria that carriers the prophage (it is marked with the sign *tox*) releases toxic substances (exotoxin) in the environment.

Diphtheria toxin is the main factor of pathogenicity. It is produced only by certain phages with lysogenic action, that are carriers of genes responsible for toxin synthesis (*tox* + type). Non-lysogenic bacteria do not produce diphtheria toxin, and it cannot causes diphtheria, respectively.

Diphtheria is caused by toxigenic strains only. The *tox* + type of bacteria (it produces exotoxin) refers to all three biotypes of C. diphtheriae: gravis, intermedius and mitis. The gravis type causes epidemics and severe clinical manifestations because they possess high toxicity compared to the other two types.

Diphtheria bacillus is resistant to the external environment. It resists on environmental objects for up to two weeks, it survives in water and milk during 6-20 days. It is highly resistant at drying up and low temperatures. Dry diphtheria membrane resists at a room temperature for 5-6 months, in dry soil – for about 3-5 months. Diphtheria bacilli are killed by heating (at a temperature of 60°C within 10 min., and by boiling - within 1 min). Ultraviolet rays destroy bacteria within 5-10 minutes, 10% of hydrogen peroxide - 3 minutes, 50-60° alcohol - in a minute. It is easily destroyed under the action of disinfectants that contain chlorine. Exotoxin is easily destroyed by light and heating.

Source of pathogen

Diphtheria is an anthroponosis. The source of pathogen is the patient with clinical manifestations or carrier of C. diphtheriae. The incubation period lasts for 1-7 days. Diphtheria bacillus locates in the pharynx, larynx, nasopharynx, skin and ear, where it multiplies and secretes a strong toxin. The toxin produces cell necrosis and fibrinous membrane (pseudomembranous or false membrane). It spread throught the body and causes lesions in various organs and it induces antibody production. Diphtheria bacilli multiply only at the gate and they do not invade the blood. The patient with diphtheria is contagious throughout the disease from the first day. The patient with typical clinical form of manifestation is the most contagious because of the massive elimination of infectious pathogens in the external environment. However, patients with typical forms of diphtheria are easier diagnosed and they are isolated and treated. That fact decreases their epidemiological risk in the transmission of the disease. The patients with mild forms of the disease easily contaminate the external environment, thus they have a greater epidemiological role. It is noted that the patients cannot serve as a reservoir of C. diphtheriae in nature when sporadic cases of diphtheria are recorded. In such circumstances, the role of reservoir and maintainance of diphtheria bacilli in nature belongs to carriers who in spite of the fact that they excrete fewer bacteria, are more numerous and are active carriers. They can be divided into convalescent and healthy (immune) carriers.

The sick of diphtheria become *convalescent carriers* in 5-10% of cases. The carriage duration lasts 2-4 weeks. Duration may be up to 3 months in some severe cases.

The carriage of toxigenic bacteria is conditioned by the body's immune status. The repeated contamination of people who had the disease in the past or people who are vaccinated against diphtheria can develop mucosal colonization in the nasopharynx because the immunity against diphtheria is determined only by antitoxin antibodies (antibacterial immunity is very short and it lasts up to a month). The bacteria do not cause the disease because exotoxins are neutralized by antitoxin antibodies.

It was found that the carriage state rate among the population depends on the epidemiological situation (epidemic, outbreak, sporadic morbidity) and the pathologies in the nasopharynx. In children with normal pharyngeal mucosa the carriage rate may be 24% while in children with the pharyngeal mucosa with chronic inflammatory processes the carriage rate may be 54%. The individuals from the first group release the corynebacteria during 2-3 weeks, those with chronic inflammatory processes during 4-6 months.

The number of carriage of toxigenic corynebacteria in the human population correlates with the epidemiological situation in the territory. Favorable conditions are considered to be in case of few cases of carriage state, and unfavorable situations are considered to be in case of high record of carriers of C. diphtheria (4-40%).

Way and factors of transmission

The pathogen colonizes the nasopharynx. The main mode of transmission is airborn. The pathogen transmission is primarily by air, via inhalation of liquid aerosols (droplets, nasopharyngeal secretions spread by air by the carriers or sick when speaking, coughing, sneezing) and solid (by particles of dust contaminated with C. diphtheriae). The contamination can also occur indirectly through external environment objects (toys, dishes, linen etc.) and contaminated hands, but also by food and by alimentary products (milk, cream, salads, etc.) contaminated usually by diphtheria carriers. Cutaneous diphtheria is transmitted through direct contact with patients who have cutaneous diphtheria ulcers.

Manifestations of epidemic process

Diphtheria is a worldwide spread infection. In the past diphtheria was one of the main public health problems because of the large number of cases (150-250 cases per 100 thousand of population). It presented a

serious consequences (high lethality and disability). Diphtheria evolves epidemics during the pre-vaccination period with a periodicity of 10-15 years. The incidence of this infection was significantly reduced after the implementation of vaccination against diphtheria. It was reduced to zero in many countries (where immunization is compulsory). An example can serve the morbidity dynamics of diphtheria in the world *(Figure 8)* and in Moldova as well *(Figure 9)*.

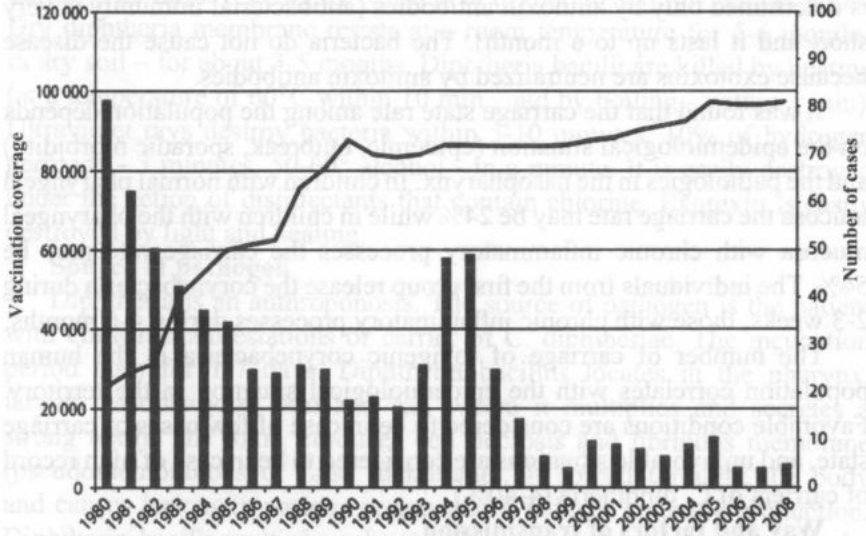

Figure 8. *Number of diphteria cases recorded in the world and vaccination coverage according to WHO (N. I. Briko et al., a. 2013).*

In 1876, there were recorded 6,138 of cases of diphtheria, and 2,417 deaths (39.4%) in Bessarabia. In 1878 there were recorded 11,248 patients and 3,705 cases of deths (32.9%). In 1909 cases of diphtheria in 32517 patients and 135 deaths were recorded, in 1910 - 52033 cases or 212.8 cases per 10,000 inhabitants, in 1911-41896 cases (169.4 per 10,000 inhabitants). In 1914, the morbidity decreased to 8775 diphtheria cases and 935 deths (10.65%). The application of diphtheria serum to the sick has decreased the morbidity since 1895 (P. Iarovoi, 2012).

The vaccination of children against diphtheria introduced in 1951, contributed to an essential decline of deaths due to diphtheria. As early as 1970, 20 years after the implementation of vaccination in Moldova, diphtheria morbidity decreased by 420 times. There were recorded sporadic

cases during the 70s-80s. The number of vaccinated and revaccinated adults reduced in the late 80s - early 90s. Therefore, it led to the gradual loss of immunity in adults and further increase of deaths due to diphtheria. Only within 7 years (1991-1997) there were recorded 1,011 cases of diphtheria and 47 of deaths (4.64%).

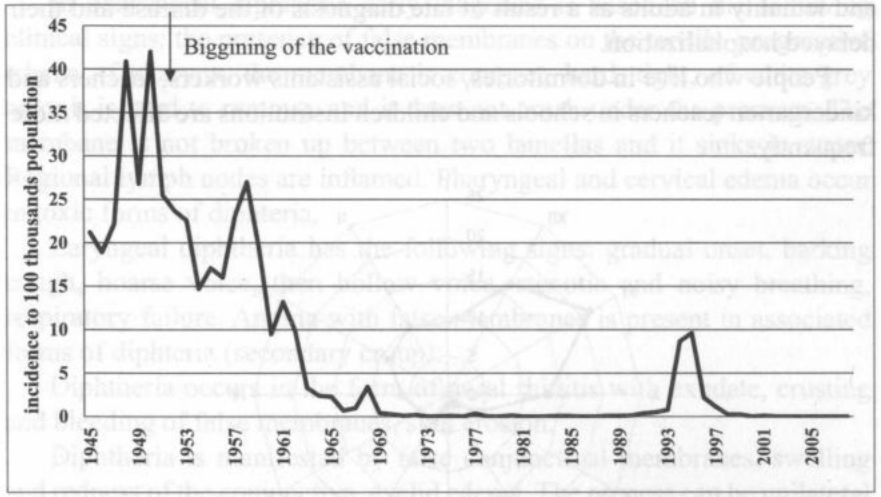

Figure 9. *Diphteria morbidity in the Republic of Moldova, 1945-2005.*

In 1995, mandatory vaccination and revaccination against diphtheria of all ages and till the age of 60 reduced the morbidity. It caused the elimination of diphtheria cases in the country. During 2006-2013 diphtheria cases were not recorded in Moldova.

Response to diphtheria is general. Children aged months have natural passive immunity from the mother. In pre-vaccination period diphtheria was usually recorded in kindergartens and schools. The incidence of diphtheria was significantly reduced in children after the introduction of vaccination of all population, according to the schedule of vaccination. During the vaccination period, diphtheria was recorded practically only among unvaccinated children. However, in the last three decades its incidence increased among adults. For example, during the epidemic of diphtheria in the period of 1991-1997 in Moldova 38% of diphtheria cases were recorded in adults. The incidence of diphtheria increased in adults because they lost the artificial immunity within 10-15 years after the vaccination.

Usually, the incidence of diphtheria is higher in the urban population. But in the period of increase the morbidity (90s of the XX) the rate in urban and rural population was approximately equal, with a higher incidence in children under the age of 14 from the rural areas.

A direct correlation was observed between the increased incidence and lethality in adults as a result of late diagnosis of the disease and their delayed hospitalization.

People who live in dormitories, social assistants workers, teachers and kindergarten teachers in schools and children institutions are affected more frequently.

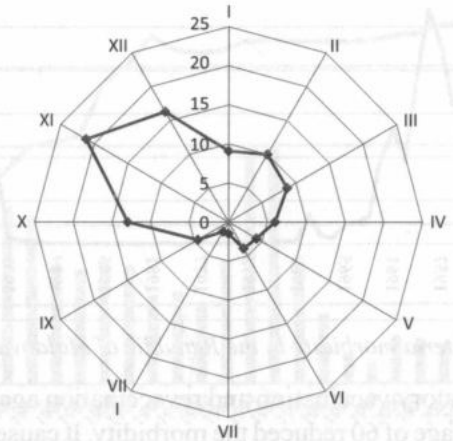

Figure 10. *Seasonality of diphtheria in Republic of Moldova, 1991-2002.*

Diphtheria morbidity has a seasonal character *(Figure 10)*. The incidence of diphtheria cases and carriage are more commonly recorded in the cold season and less cases are recorded in spring and summer months. The annual growth rate increases as a result of the formation of groups of children, and it reaches the highest peak in November and December. The morbidity decreases since February. The autumn-winter seasonality of diphtheria is caused by chronic inflammatory processes detected in the nasopharynx. It leads to high frequency and duration of carriage.

Manifestations of infectious process

Clinical manifestations of diphtheria include the following forms:

- pharyngeal diphtheria - 86-90%;
- laryngeal diphtheria (croup);

- nasal diphtheria;
- other sites (cutaneous, conjunctival, otic, vulvovaginal diphtheria).

Early diagnosis of diphtheria is an absolute necessity to avoid the development of complications. The patient is contagious from the first days of the disease onset.

In pharyngeal diphtheria early diagnosis is based on the following clinical signs: the presence of false membranes on the tonsils, progressive edema of pharynx, the membane is compact, hard, tight, of white grey color,it is hard to remove, and it does not crush under the pressure. The membane is not broken up between two lamellas and it sinks in water. Regional lymph nodes are inflamed. Pharyngeal and cervical edema occur in toxic forms of diphteria.

Laryngeal diphtheria has the following signs: gradual onset, barking cough, hoarse voice, then hollow voice, stenotic and noisy breathing, respiratory failure. Angina with false membranes is present in associated forms of diphteria (secondary croup).

Diphtheria occurs in the form of nasal rhinitis with exudate, crusting and bleeding of false membranes, skin erosion.

Diphtheria is manifested by false conjunctival membranes, swelling and redness of the conjunctiva, eyelid edema. The process can be unilateral and bilateral.

Diphtheria can be transmitted via skin wounds and other injuries excoriation, via edema and fibrinous membrane. Otic diphtheria and vulvovaginal diphtheria are suspected if false membrane is present.

Non-vaccinated children develop toxic forms (65%). Lethality varies from 3 to 5.8% of cases. Vaccinated children suffer the disease easier without complications and usually death is not recorded.

In adults diphtheria can evolve mild and severe forms. The most common clinical form of diphtheria is the pharyngeal one that results in follicular angina, signs of intoxication. It is accompanied by pain during swallowing, and fever is peresnt in adults. The throat mucous membranes are congested, the tonsils are swollen, they are covered with white or yellowish-gray membrane, that is compact and often it is easily detached, sometimes unilaterally. Typical membranes can be absent in 2/3 of patients. The disease progresses easily in a majority of patients, but sometimes the disease may occur in severe form and even with fatal complications during the second and third week of the disease.

Laboratory diagnosis

Laboratory investigations are performed in all clinically suspected diphtheria cases. Bacteriological investigations are required if there are depositions on the tonsils. Isolation of toxigenic diphtheria bacilli confirms the diagnosis. The negative result of bacteriological examination does not exclude the diagnosis of diphteria. It can be induced by a carriage state of atoxigenic diphtheria bacilli in patients with diphtheria.

Agglutination reaction is used to determine the diphtheria toxin in the serum for early diagnosis. Laboratory diagnosis via serological tests include: the reaction of hemagglutination of red blood cells, toxoid indirect specific diagnostic, immuno-enzymatic analysis, polymerase chain reaction (PCR).

Epidemiological surveillance

Epidemiological surveillance provides systematic evaluation of the situation, including the intensity of the epidemic, immune carriers who are maintained by toxigenic C. diphtheriae The screening of vaccination coverage in population is carried to determine the immunological status in different groups of the population. It is necessary to detect all cases of diphtheria in a short time (early detection). The effectiveness of the measures taken in the focus and the vaccination coverage are evaluated.

The situation is assessed as unfavorable when indigenous cases of the disease and carriers of toxigenic C. diphtheriae are recorded in outbreaks of diphtheria. The unfavorable situation is also considered to be in case of low level immunity against diphtheria in the human population.

Control measures

Preventive measures

The prophylaxys is directed to neutralize the source of pathogens by detection and treatment of toxigenic C. diphtheriae carriers. The bacteriological investigation of children is necessary to perform before the admission to children institutions. It is also performed for children from orphanages, boarding schools, employees of these institutions, people with otorhinolaryngology pathologies, particularly angina.

Monitoring of carriage and patients with angina, laryngitis, laryngotracheitis and other diseases is carried out compulsory for early detection of patients. All kinds of pathological deposits on the tonsils or paratonsillar abscesses are investigated bacteriologically for the confirmition of toxigenic diphtheria within 24 hours after the first visit to a doctor.

Interruption of the transmission mechanism is accomplished by carrying out systematic prophylactic disinfection in children institutions and hospitals.

However, the main measure to prevent diphtheria is to keep the vaccination status at a high level. It is considered favorable when the immune level is high (85% >) in the community.

Diphtheria toxoid is administered as a component of vaccines: DTP (diphtheria, tetanus, pertussis), DT (diphtheria and tetanus for children), Td (tetanus and diphtheria for adults). In many countries pentavaccine is used against diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type b (Hib).

According to the schedule of vaccination in Moldova, vaccination against diphtheria is performed by three inoculations with tetanus diphtheria, tetanus, pertussis (DTP) in dose of 0.5 ml at the age of 2, 4 and 6 months, intramuscularly. The first booster vaccination with DTP is applied at the age of 22-24 months and the second booster vaccination - at the age of 6-7 with DT vaccine that contains diphtheria and tetanus. The dosage of administration is 0.5 ml. The mode of administration is similar to that of DTP vaccine. The third booster vaccination at the age of 14-15 and the following revaccination in adults ages 20, 30, 40, 50 and 60, is carried out with Td vaccine, tetanus toxoid and diphtheria containing purified reduced dose. The scheme and dosage of administration are the same as for DT and DTP vaccines.

Anti-epidemic measures

Medical institutions are required to notify immediately the Centre for Public Health and notify the event by filling in the form. 058/e.

The patient is hospitalized mandatory and undergoes serotherapy. Epidemiological investigation of the outbreak, is performed usually by epidemiologists.

All persons who had the contact with diphtheria patients are subjected to bacteriological examination in order to establish the presence of toxigenic C. diphtheriae in the nasopharynx. Clinical surveillance of patients is performed during a period of 7 days, with daily examination of the pharynx and temperature measurement. The clinical examination is done by otorhinolaryngologists (ORLists).

People with follicular angina, paratonsillare abscesses, stenosing

laryngotracheitis and carriers of toxigenic corynebacteriae are subjected to hospitalization since appearance of the first signs.

Patients with diphtheria are discharged from the hospital after clinical recovery and two bacteriological examinations that confirm negative results of C. diphtheriae. The examinations are carried out at an interval of 1-2 days and not earlier than 3 days after the antibiotic therapy.

Patients who sufferd of diphtheria or carriers of bacillus toxigenic diphtheria and still eliminate the pathogen may be allowed to resume the work with the staf fonly if all the persons of the staff are vaccinated against diphtheria. Medical surveillance of the staff is performed untill they stop eliminating pathogens. Only the persons immune to diphtheria will be admitted into the group during this period.

In the outbreak the terminal disinfection is carried out after the hospitalization of the patient (or carrier).

In the outbreak diphtheria vaccination is done to all people who had contact with the sick, children and adults not vaccinated against diphtheria, all persons who are in the period of revaccination and persons with the level of antibodies against diphtheria less than 0.03 IU / ml.

1.1.2.2. Whooping cough

Short history

The first case of pertussis was described in the sixteenth century by C. Baillou (1578) in France. Its more detailed description was made in the XVII century English doctor T. Syndenham, who called it pertussis. In the early twentieth century (1906) Y. Bordet isolated and identified the pathogen of whooping cough.

Causative agent

Coccobacillus Bordetella pertussis is a gram-negative and aerobic microorganism. It refers to the Bordetella genus, which includes nine species of microorganisms. Only B. pertussis and B. parapertussis are pathogenic to humans.

B. bronchiseptica was isolated from animals and birds (dogs, cats, pigs, horses, rabbits, guinea pigs, mice and rodents).

Pertussis species are heterogeneous. The virulent form is isolated, as a rule, from the patient in Phase I, and their virulence decreases on the culture media in Phases II - V.

B.pertussis antigen has a complex structure, consisting of several

agglutinogens which varies depending on the combination of the four serovars circulating in the following groups: I - 1, 2, 3; II - 1, 2, 0; III - 1, 0, 3; IV - 1, 0,0. The mentioned varieties of serotypes are isolated worldwide. Their rate varies on territories or periods of time. Serotypes I and II may be considered pathogenic, and they cause severe forms of the disease. Other serotypes develop usually a mild form.

Currently, strains with reduced virulence prevail as a result of immunization of children against whooping cough. However, unvaccinated children may develop a severe form of the disease.

B. pertussis produces biologically active substances with certain properties in the pathogenesis and protection: filamentous haemagglutinin, exotoxin, tracheal cytotoxin, dermonecrotic toxin, factor promoter of lymphocytosis, adenylate cyclase, protective factor.

B.Pertussis is the only species that produces the pertussis toxin.

Exotoxin plays the leading role in the pathogenesis of infection and immunity development. The pertussis toxin is toxic for the the human body cell, it causes necrosis of epithelial tissues, promotes lymphocytosis, histamine sensitivity with the action on different systems, particularly the Central Nervous System and vascular system. The toxin possesses the immunogenic property.

Filamentous haemagglutinin is a factor of adhesion and invasion of respiratory cell, it determines the coccobacillus virulence at the same time.

Tracheal cytotoxin has an action on tracheal and bronchial epithelium, produces ciliary stasis and prevents the regeneration of damaged cells.

The promoter factor of lymphocytes produces lymphocytosis and activates the toxin (factor antiphagocytic to respiratory mucosal permeability and other toxins).

Adenylate cyclase reduce defenses, causing inflammation of the epithelium of the respiratory tract, activates cell metabolism and produces local edema.

Protective factor is responsible for the formation of stable immunity.

B. pertussis is less resistant in the environment. Its resistance to the external environment is very low and lasts up to 30 min. Light or sunlight kill B. pertussis bacteria within 1-2 min. It is easily destroyed under the action of disinfectants. Boiling kills the pathogen immediatly.

Bordetella parapertussis causes similar diseases. But this coccobacillus

is recorded more frequently compared to B. pertussis and usually it causes mild or even asymptomatic forms.

Source of pathogen

Pertussis is a typical anthroponosis. Only a sick person with typical or atypical forms can be the source of pathogen. Carriers of coccobacillus can rarely be the source of infection.

B. pertussis attaches the cilia of tracheal and bronchial epithelium after contamination, and it destroys them. B. pertussis does not penetrate the blood. Only exotoxin produces excitation of the nerve endings in the epithelium of the airways, causing paroxysmal attacks of spasmodic coughing, removal of mucoid secretion that contains a significant dose of pathogen in the external environment. Pertussis has the incubation period of 3 to 14 days (average 10.8 days), the prodromal period (catarrhal) of 3-14 days, and convulsive period of 2-4 weeks. The convalescence lasts 2- 4 weeks.

The patient is contagious in catrrhal period and two weeks of seizures period. Therefore, the period of infectiousness of the patient with whooping cough lasts four weeks, but at the end of this term the risk of contamination from the sick is minimal.

Factors and transmission routes

The mode of transmission is typically via respiratory mechanism, by inhaling liquid drops excreted by the sick when coughing. Although, the pathogens are eliminated heavily via liquid droplets in the external environment, the contamination is possible only in close contact with the patient at a distance of 2 meters. The transmission through household objects practically does not occur because of instability of pathogen in the external environment. The transmission is more effective in children's communities and in overpopulated areas.

The risk factors are: insufficient immunological protection of children as a result of low vaccination coverage, density of children in institutions, low temperature during the year.

Manifestations of epidemic process

According to the WHO, every year 50 million people are infected by whooping cough and 0.5-1 mln die. The infection is spread worldwide, but in different countries and continents the morbidity varies essentially from solitary cases up to 100 thousand. The morbidity depends on the national immunization programmes and it is directly determined by the level of vaccination coverage of the population.

During the pre-vaccination period, the morbidity of pertussis costituies between 200,000 and 350,000 cases per 100,000 of population in Moldova, It was the first cause of death among children under the age of one. Following the implementation of vaccination (1958 year) of children against pertussis, the morbidity dropped to 107.0 cases per 100,000 of population in 1960. In the last decade, the incidence of whooping cough varies between 0.11 and 213 cases per 100,000 of population *(Figure 11)*.

Figure 11. *Dynamics of morbidity of whooping cough in the Republic of Moldova, 1951-2011.*

The dynamics of morbidity of whooping cough is characterized by cycles of increase and deacrese of morbidity with periodicity of 3-4 years. The phenomenon is caused by the circulation of virulent strains that intensify the increase of susceptible individuals as a result of the high rate of unvaccinated children. The incidence of pertussis corelates directly with the vaccination coverage.

The whooping cough seasonality is characterized by high a incidence during summer months and it continues to spread till the autumn-winter period. It is determined both by the formation of children groups, cold weather, and intensification of respiratory pathologies.

The receptivity to whooping cough is universal and reaches

90-100% of population. However, the epidemic process in whooping cough varies according to the age structure of morbidity. Although, the overall responsiveness to whooping cough is high. Babies costituie the major risk group because of lack of specific immunity. B.pertussis antibodies are not transmitted transplacentally from mother to child, even if the mother has immunity tot pertussis. So, the high level of whooping cough morbidity is recorded among children under one year of age, then it decreases with the age. People who had whooping cough have lifelong immunity. So, cases of whooping cough are very rare among adults. However, during the vaccination period, the increased rate of infected children and adults is recorded. The morbidity of whooping cough among adults is usually manifested via atypical forms.

From the epidemiological point of view, adults who work in children's and maternity institutions present a higher risk. There are no differences by gender structure.

The pertussis vaccination was implimented during decades. It leds to evolutionary changes in the development of clinical manifestations of the infection. The disease evolves more easily, it reduced significantly the lethality.

Manifestations of infectious process

The period of catarrhal cough begins with mild and dry rhinitis. The body temperature remains normal and rangies between 37 to 37.5°C. The cough gradually becomes more frequent, it is predominantly nocturnal and of spastic nature. Sometimes, it is followed by vomiting, pale skin, hyperemia of conjunctiva and eyelid edema.

The seizure period is characterized by repeated attacks of persistent and spasmodic cough, it is the main symptom of whooping cough. The cough lasts 1-5 minutes and ends with difficult expectoration of viscous mucus and vomiting. The number of coughing attacks is variable during 24 hours: 10-15 attacks occur in mild forms and from 15 to 30 and more in severe forms. The patient's face becomes swollen and cyanotic. Conjunctival and eyelid bleeding may occur. The patient's general condition is satisfactory and the body temperature is normal between the attacks.

The cough becomes rare and less intense in the convalescence period.

Whooping cough develops severe and mild forms, usually, in infants. The incubation period is shorter (3-5 days) and the catarrhal period lasts 2-6 days. The catarrhal period is manifested by fever and rhinitis among

infants. It is manifested by apnea, total cyanosis and myoclonus of mimic muscles and coughing become worse. Vomiting, face edema, hemorrhagic syndrome and bacterial complications occur more commonly. Bacterial pneumonia is the most frequent complication and the patient has the high risk to die. The lethality is of about 1%.

Vaccinated children develop atypical or asymptomatic form of whooping cough. The convulsive period is absent. The cough is mild and dry with a duration of 5-7 weeks. Complications practically do not occur.

The disease progresses in adults, but mild forms prevail in most of cases.

Laboratory diagnosis

B.pertussis is isolated from nasopharyngeal secretions. Regan-Lowe and Bordet-Gengou media are recommended for the isolation of pathogen. The result is positive in 90% of cases during the catarrhal period (till the antibiotics administration), and it is positiv in 20-30% of cases in the 3rd – 4th weeks. B. parapertussis is isolated only on Bordet-Gengou medium, but it can subsequently be cultivated on usual media as well. B. bronchiseptica iss isolated only on the media that contain blood.

Agglutination reaction on lamella with anti-B.pertussis sera and anti-B. parapertussis confirms the identity of isolates and identifies serovars of B. pertussis. It is an epidemiological marker. Determination of IgA, IgM, IgG by Enyzmatic Immune Agglutination (EIA). Antibodies of IgA and IgM are indicators of the recent infection with whooping cough. IgG and IgM antibodies appear after the vaccination.

The reaction of indirect heamaglutination (RIHA) and complement fixation test are used for serological investigation. Although, positive titres appear late (in the 3rd week of illness), their dynamic growth confirms the diagnosis of whooping cough.

The diagnostic by Gene amplification technique (PCR) is useful if patients have started the treatment with antibiotics.

Epidemiological surveillance

Epidemiological surveillance of whooping cough has the following main objectives:

- assesment of the immune status among children and their vaccination coverage;
- evaluation of morbidity in groups of population, territories and risk time for the development and maintenance of epidemic;

- effectiveness of early diagnosis and anti-epidemic measures performed in the outbreak;
- monitoring of the B.pertussis strains circulation among the human population.

Control measures

Preventive measures

The whooping cough prevention is ensured by active immunization of children performed with the DTP vaccine against diphtheria, tetanus and pertussis (DTP). Pertussis component of DTP vaccine presents a suspension of inactivated *B.pertusis strains*. The vaccination is carried out by administration of three doses of vaccine of 0.5 ml administrated at the age of 2, 4 and 6 months, intramuscularly, and re-vaccination is performed at the age of 22-24 months. The DTP vaccine that contains pertussis can be administered untill the age of 7. The vaccination coverage must be over 90% and protective immune status should be at least in 88% of the population.

Active detection of the sick among patients with persistent cough is provided by bacteriological investigation. This is an important element in prevention of the infection spread.

Anti-epidemic measures in outbreak

Cases of whooping cough are reported to the Center for Public Health (CPH) by notification of all cases in the electronic system alert and filled in form. 058/e. The patient is subjected to compulsory isolation. Patients with mild form of infection are isolated at home and they receive an adequate treatment. Patients with severe form, especially children from orphanages and boarding schools, children aged up to 2 months and people without conditions for isolation are hospitalized.

Children aged up to 7 years who had contact with patients without signs of coughing are monitored during 14 days from the last contact.

Adults who work in kindergartens, schools, orphanages, boarding schools, hospitals or nursing homes for children, and who had contact with patients with whooping cough or coughing patients are removed from the job and they are admitted after two negative results of bacteriological investigations (one day after another or every other day).

Terminal disinfection is not performed in the focus of whooping cough. Wet cleaning and ventilation are carried out in the room.

Vaccination of persons who had contact with the sick in the focus of

whooping cough is not performed. Unvaccinated children who had contact with the sick with whooping cough may receive anti-toxic pertussis immunoglobulin, regardless of the term that passed from the contact with the patient.

1.1.2.3. Streptococcal infections

Short history

The first description about streptococcus was performed in 1874 by T. Billroth at a patient with tissue erysipelas, who proposed the name of *"streptococcus"*. In 1879 L. Pasteur mentioned the presence of streptococcus in the blood of a patient with puerperal sepsis, and in 1883 Fehlein isolated the microorganism in pure culture from a patient with erysipelas. It was called "*Erysepelatus Streptococcus"*. In 1884 Rosenbach called it *Streptococcus pyogenes*. Later, significant findings were made about the detection and classification of different types of streptococcus. In 1903 Schöttmuller separated all strains of streptococci according to their hemolytic activity on blood agar. In 1919 Bown introduced terms of α, β and γ-hemolysis. In 1933 Lancefield suggested the antigen classification of streptococci.

Feature of streptococci

Streptococci are part of Streptococcaceae family of Streptococcus genus, which includes 240 species, according to the National Center for Biotechnology (NCBI, USA). There are 49 species of streptococcus that have medical significance, which are considered important in human pathology development. There are *S. pyogenes, S. agalactiae and S. pneumoniae.*

Streptococci are divided according to the appearance of hemolysis on blood agar into:

- β-*hemolytic streptococci* - there are form colonies that are surrounded by a clear zone of complete haemolysis;
- α-hemolytic streptococci - form colonies that are surrounded by a greenish area where red blood cells are only partially lysied;
- γ-hemolytic streptococci - therea non-hemolytic streptococci.

Streptococcus are divided according to Lancefield antigene classification that is based on the specific group antigene:

- Group A - W Streptococcus (excluding I and J).
- Non-group streptococci do not have antigen.

The majority of β-hemolytic streptococci that are pathogenic to human

are separated according to antigenic groups A, B, C, F and G. Groups E, L, P, U and V include the streptococcus species that are rare among humans. D group of streptococci (γ-hemolytic) were divided into a separate gene that is called *Enterococcus*, and it includes 12 species, including *E. faecium and E. fecalis*.

Other groups of *Streptococci* are part of the normal microflora of the human or animals. There are non-pathogenic, but under the special conditions (immunodeficiency) can cause non-specific manifestations of the disease, such as nosocomial infections.

Streptococci bacteria are of spherical or ovoid form, they are developed in pairs (diplococci) or in chains. The bacteria are Gram-positive and asporulate. Streptococci are resistant to the external environment, they can easily survive to drying and dry sputum or pus during months. At the same time, they are sensitive to heat and disinfectants.

1.1.2.3.1. Infections with Streptococcus pyogenes

Pathogen agent

S. pyogenes is β-hemolytic streptococcus of A group (only one representative), with significant risk in development of human pathology. It was found that streptococcal pyogenic cause a higher number of diseases both suppurative (angina, acute pharyngitis, scarlet fever, phlegmon peritonsillar, otitis, sinusitis, endocarditis, puerperal fever, septicemia, abscesses and necrosis of pleural lung, skin infection, impetigo, erysipelas, Necrotizing fasciitis, wound infections and thermal injury) and nesupurative (rheumatism, acute glomerulonephritis and rheumatic carditis).

Thae species of pyogenic streptococci are heterogeneous according to the antigenic structure and virulence.

The antigen structure of hemolytic streptococcus is very complex. The main components are proteins M and T. M protein largely determines the virulence and type of antigen (according to the amount of the protein M). Antibodies to the protein M provides long immunity against repeated contamination. The structure of M protein includes 85 determinants. There are 150 of pyogenic streptococcal serotypes, which decrease the protective humoral responses. M protein inhibits the phagocytic reactions, it acts directly on phagocytes.

Specific antigenic properties have *protein-T and lipoproteinaza*, as well. The distribution of lipoproteinaza serotypes correspond exactly

to a particular M-type. However, antibodies to T-protein and protective lipoproteinaza are not produced.

The capsule of cell consists of hyaluronic acid and is one of the virulent factor. It protects bacteria from phagocytes action and adhesion facilitates to epithelia. The capsule of pyogenic streptococci is made of hyaluronic acid that are part of the connective tissue. Bacteria can destroy self-contained capsule during the tissue invasion after the hyaluronidase synthesis.

C5a-peptidase is another pathogenic factor, which suppresses the activity of phagocytes.

However, group A of streptococci produces toxins. *Streptolysin O* shows hemolytic activity under the anaerobic conditions. The antibodies titer has prognostic significance. *Streptolysin S* manifests haemolytic activity in anaerobic environments and produces hemolysis in blood. Both hemolysins destroy not only erythrocytes but other cells, as well. *Streptolysin O* destroys cardiomyocytes and *Streptolysin S* destroys phagocytes absorbed by bacteria.

Sources of pathogen

Sources of pathogen are sick man with typical and atypical forms of clinical manifestations (pharyngitis, tonsillitis, angina, scarlet fever and erysipelas) or carrier of pyogenic streptococci (convalescence or healthy). The most important sources are considered to be patients with angina or scarlet fever. These patients are highly contagious. The pathogen is eliminated via coughing, sneezing, speaking, active or nasopharyngeal secretions that contain major virulence factors: capsule and protein M. Therefore, people manifeste acute infection more frequently.

Patients with localized infection (otitis, sinusitis, flegmoane, glomerulonephritis, rheumatism, streptococcal pyoderma, etc.) do not presents significant epidemiological risk because they eliminate less concentration of the pathogen.

The period of infectiousness of the patient depends on the efficacy of the treatment.

Carrier state is direct depending on the quality of the treatment and may eliminate the pathogen during 3 months after the infection. Healthy carriers have higher epidemiological risk. They spread the pathogen for a long period of time (up to one year or more) from the nasopharynx. They contribute to the permanent circulation of pathogenic streptococci among

humans. The level of carriage state among the human population is high and it can reaches 50%, especially in children institutions. At the same time, cultures of virulent *streptococcus A* are detected among carriers more frequently than in sick patients.

Receptivity and immunity

Natural receptivity varies and its depends on the state of macroorganims and and the virulence of pathogen. Antitoxin and antimicrobial immunity is developed after the infection.

M protein antibodies are produced in the 2nd - 3rd week of the disease and they are maintained for a period of 10-30 years. There are detected in newborns as well. Transplacental maternal immunity is short-term (4-5 months). Streptolysin O-specific antibodies appear at the 2nd week of the onset of disease, and it achieve the maximum level of titer at the 3rd - 4th week. Then, it declines rapidly. Non-supurative complications are confirmed when the titer persist at 200 IU/ml and higer over six weeks..

Factors and transmission routes

The primary route of transmission is airborne, which is achieved by liquid drops or, more rarely, solid one. It is necessary to consider that the people are contaminated due to the close contact, because airborne contamination by liquid drops is not carried out at a distance of 3 m from the source.

Contamination is possible through the alimentary products by contaminated food (milk, stewed fruits, salads, etc.) and after the contact with contaminated objects from the external environment.

Manifestations of the epidemic process

Streptococcal infections caused by S. pyogenes is widespread in the human population.

Angina, scarlet fever and rheumatism are registered in cold and frequently moderate climates. Skin diseases prevail in the southern tropical and subtropical climate (streptodermia, impetigo). Since the 80s of last century there is an activation of streptococcal infection and the emergence of new forms of serious invasive infections caused by pyogenic streptococci, such as streptococcal toxic shock syndrome, sepsis, necrotizing myositis, fasciitis etc. This trend is observed in developing countries and in countries with advanced economies. In the US, for example, 10-15 thousand cases of invasive streptococcal infection are recorded, annually.

According to data from BRIKO N. et al. (2013) in Russia, 1.25 million cases of infections with group A of streptococcal etiology are recorded, annually (86.1 to 10,000 population). The prevalence is estimated to 3.1 million of cases (207.1 to 10,000 population), of which 350 thousand are impairments cardioreumatic cases. The tempo of increased morbidity constitutes 2% annually. According to the literature, group A streptococcal infection is recorded among teenagers in 33% and adults 9% - 58%.The increase of incidence, including seasonal is determined by cold temperature and the formation of groups (kindergartens, schools, military units, etc.). Increase incidence of streptococcal infections (angina, pharyngitis, acute respiratory infections) is already observed over 11 to 15 days after the formation of collectives and the maximum amount is after 30-35 days. The epidemic is manifested by sporadic and eruptive cases, less frequently it can be manifested by epidemics. The outbreaks of group A streptococcal infection may occur simultaneously with different clinical manifestations like as angina, pharyngitis, scarlet fever and streptococcal carriers of β-hemolytic group A.

Manifestations of infectious process

B-hemolytic streptococcal gpoup A infections are varied according to clinical forms and the developing mechanism. The most common manifestations of acute streptococcal infections are pharyngeal infection, tonsil, especially anginas, whose prevalence is 60-80 cases per 10,000 of population, mainly among children of 5-15 years, followed by scarlet fever.

The manifestation of streptococcal infection that cause angina or scarlet fever depends on both the quantity and virulence of the pathogen (scarlet fever and glomerulonephritis are caused by toxigenic streptococcal types) and the receptivity, especially the body's ability to produce antitoxin immunity.

The infection is practically limited to carrage or pharyngeal tonsil manifestations in the body with the potential capacity to produce shortly antitoxin antibodies, otherwise it leads to scarlet fever and illness by 1-5% in angina or scarlet fever with late complications, often with autoimmune component such as rheumatic fever, endocarditis, glomerulonephritis, vasculitis, puerperal fever, etc.

Suppurative complications of streptococcal infection, including carrage of S. pyogenes, are otitis, sinusitis, peritonsillar phlegmon, cervical suppurations pleuralpulmonary, endometritis, sepsis and so on.

Streptococcal infection manifests itself by affecting shells skin (impetigo, erizepel), soft tissue (fasciitis, myositis, focal diseases of internal organs, toxic shock syndrome, wound infection, etc.).

Epidemiological surveillance

Epidemiological surveillance of streptococcal infections includes the following basic principles:

- the supervision of the morbidity and lethality of all nosologic forms caused by by hemolytic streptococcus group A;
- to determine the spreading of streptococcal infection in the multiannual and annual dynamics in different regions, age groups and social groups of population;
- to determine the risk factors that contribute to the spread of streptococcal infection, medical and socio-economic impact from these infections;
- microbiological monitoring of pyogenic streptococci spread and heterogenecity among the human population;
- implementation of advanced microbiological methods, including molecular-genetic study of the population of circulating streptococcus group A, diagnostic of all forms of streptococcal infection, especially invasive ones. It is necessary to use methods of typing such as analysis of complex genome using electrophoresis field pulse sequencing genes responsible for synthesis of virulence factors that would complement much knowledge about S. pyogenes strains, circulating feature, their heterogeneity, development mechanism and prognosis of epidemic situation, but also knowledge about invasive infection. An important element of microbiological monitoring is study of the strains of S. pyogenes resistance to antibiotics;
- to assess the effectiveness of measures carried out in the control of streptococcal infections, including early diagnosis and treatment;
- to improve the supervision and control of streptococcal infections, taking into account the diversity and nosologic etiology and pronounced heterogeneity of the pathogen.

All these measures determine the importance of epidemiological surveillance in streptococcal infections. So, the purpose of epidemiological surveillance in the current streptococcal infections is to reduce the morbidity via all forms caused by streptococcal infection group A.

The basic prevention measures consist of active detection of streptococcal infection and its etiotrop treatment. Preparations of the penicillins group are used for the treatment of all forms of streptococcal infections, it has high sensitivity. Preparations of penicillin group provides the prevention of the disease like scarlet fever, rheumatism, they reduce greatly the death rate from angina and acute respiratory infections.

Sanitary-hygienic measures, prophylactic disinfection in children gropus and hospitals diminishes the possibility of contamination by airborne and parenterally transmission.

Infections with *streptococcus pyogenes*. Scarlet fever

The pathogen

Scarlet fever is one of the main forms of group A of acute streptococcal infection. The first description is found in 1554, by Ingrassias, as "Rossana" is still confused with other eruptive diseases such as measles, rubella, etc. In 1676 English physician Thomas Sydenham differentiated these diseases, calling it *"febris scarlatinae"*.

Gabricevski and Savchenko (1905) describe the etiology of streptococcal scarlet fever, later it was confirmed by V. Ioffe, I. Levina and Dick in 30s-40s.

The pathogen is β-hemolytic streptococcus of group A (Streptococcus pyogenes).

The reservoirs and sources of pathogen are sick with angina, scarlet fever, other forms of streptococcal acute respiratory infections and pyogenic streptococcal, healthy carriers or convalescent. Scarlet fever patients are contagious in the first 7-10 days of onset. Patients with tonsillitis, chronic nasopharyngitis, streptococcal diseases other suppurative contagiousness is are contagious longer period (up to 3 weeks). Convalescent carrage in scarlet fever can take up to 3-4 weeks of recovery.

Ways and factors of transmission

As a gateway to the pathogen most frequently serves oropharyngeal mucosa, where the pathogen - β-hemolytic streptococcus - multiplies, and is eliminated the exotoxin. It is causes an inflammatory process (tonsillitis or discharge). Exotoxin diffuses through the blood, but streptococci are eliminated in the external environment, so the realization of epidemic process predominant is realized by respiratory transmission of the pathogen, through aerosols containing pathogens, by coughing, sneezing, speech.

Usually, contamination occurs in conditions of close contact with sick or carrier. Contamination is possible through milk and dairy products, salads, confectionery, but also through casual contact, through contaminated objects or hands, the wounds operators etc.

The incubation period ranges from 1 to 12 days, on average 2-7 days.

Manifestations of the epidemic process

Scarlet fever is prevalent worldwide, but most frequently in moderate and cold climates. The general trend is decreasing morbidity of scarlet fever. Ascents are characteristic in intervals of 2-4 years *(Figure 12)*.

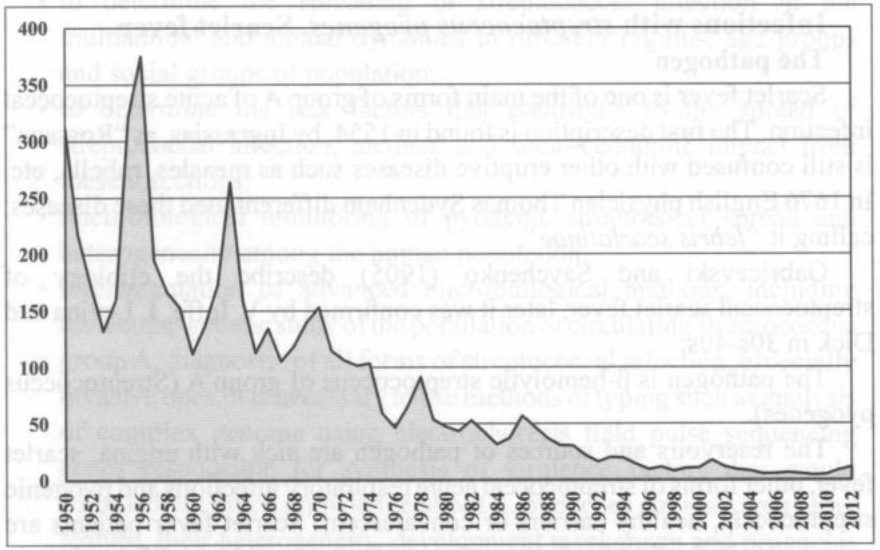

Figure 12. *Dinamics of morbidity by scarlet fever in Republic of Moldova to 100 000 population, 1950-2012.*

Although multi-cyclical dynamics are maintained, in recent years there has been a stabilization in the spread of scarlet fever.

In addition to cyclical epidemic dynamics in scarlet fever, the infection, retains the classic epidemiological features of airborne infection, being uncontrolled by the vaccination. Children get sick more often, especially children aged of 3-4 and 7 years. First - as a result of the formation of groups in kindergartens, the latter - in connection with the formation of teams of students, especially in the early grades *(Figure 13)*. Morbidity decreases with age.

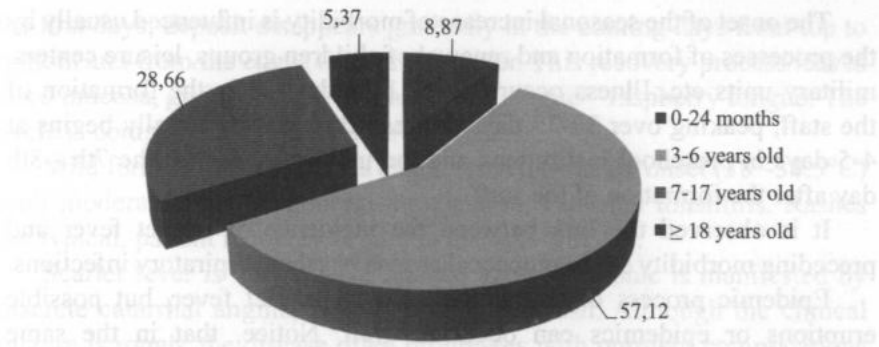

Figure 13. *The incidence by scarlet fever in Republic of Moldova according to age group (%).*

Children aged 3-6 months do not develop scarlet fever being protected by antitoxins obtained transplacentar from mother. Ther are exceptional cases of scarlet fever in children up to one year in adults.

The incidence among children in institutions is about 4 times higher compared to that observed among institutionalized children.

The annual growth rate of the morbidity observed the increasing during the the cold season and a decrease during warm period. The lowest incidence was recorded in August. Vertiginous ascent usually occurs in September that continues far into November, with a subsequent decrease *(Figure 14)*.

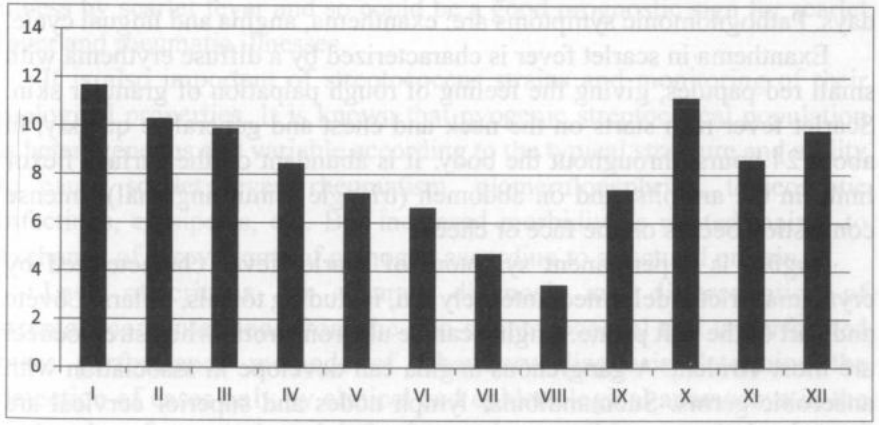

Figure 14. *Annual dynamics of morbidity by scarlet fever in mun. Chisinau, Years 2009-2010.*

The onset of the seasonal increase of morbidity is influenced usually by the processes of formation and renewal of children groups, leisure centers, military units etc. Illness occurs over 11-15 days after the formation of the staff, peaking over 30-35 days. Increased morbidity usually begins at 4-5 days in preschool institutions and the incidence reaches the 7th - 8th day after the formation of the staff.

It is observed the link between the incidence of scarlet fever and preceding morbidity of streptococcal angina or other respiratory infections.

Epidemic process occurs sporadically in scarlet fever, but possible eruptions or epidemics can occur as well. Notice, that in the same outbreak of scarlet fever can be detected angina illness, other streptococcal respiratory infections and even pyogenic streptococcal carriers.

Stable antitoxic immunity is obtained after scarlet fever infection. Repeated cases of scarlet fever are very rare and are from 2 to 3% of people who had the disease.

The manifestations of infectious process

Scarlet fever has sudden onset, sometimes brutally, with a high temperature (39°-40°C), sore throat (erythematous angina), vomiting and headache. Digestive manifestations like: nausea, vomiting, abdominal pain are more common in children. Over several hours (sometimes in the 2nd - 3rd day) appear rash, diffuse edema and hypertrophy of the pharyngeal lymphoid formations. Hypertrophy and sub-maxillary nodes are sensitive. Evolution is benign, with a resolution of phenomena in 3-5 days. Pathognomonic symptoms are: exanthema, angina and lingual cycle.

Exanthema in scarlet fever is characterized by a diffuse erythema with small red papules, giving the feeling of rough palpation of granular skin. Scarlet fever rash starts on the neck and chest and generalize quickly, in about 24 hours, throughout the body. It is abundant on the surface flexor limb, in the armpits, and on abdomen (triangle femuroinghinal). Intense congestion occurs on the face or cheek.

Angina is a permanent symptom of scarlet fever characterized by erythema strictly delimited, intensely red, including tonsils, pillars, Lövéte and part of the soft palate. Angina can be ulceronecrotic when streptococci are most virulent. A gangrenous angina can develope in association with anaerobic germs. Submandibular lymph nodes and superior cervical are swelling and pain.

Lingual cycle shows the following signs: tongue is intense sabur in

the first days; deposit disappears gradually in the coming days from top to bottom and from the edges toward the center. This recovery process leaves a red mucosa, giving a particular aspect of tongue – raspberry tongue. The cycle is complete in 5-6 days after the onset.

Mild form of scarlet fever is characterized by acute onset (38°-38.5°C) with moderate signs of general intoxication, catarrhal tonsillitis. Rashes are typical, patient recovery occurs in the 4th - 5th day.

Scarlet fever is very rare in infants, toxic syndrome is manifested by discrete catarrhal angina, poorly pronounced rash. Although the clinical picture is rough, the disease often progresses with purulent complications (otitis, purulent lymphadenitis etc.).

Most frequently scarlet fever occurs in adults without rashes. Remains acute onset, with signs of general intoxication and mild fever. Rashes are discrete, often unnoticed and remain for several hours. Tonsillitis, peripheral lymphadenitis, lingual manifestations, descuamation may be present, but of short period.

Epidemiological surveillance

It is important to assess the level of scarlet fever incidence by both multi-annual and annual dynamic, cyclicity of epidemic process, taking into account the incidence in children collectives. Simultaneously are supervised the dynamics of morbidity by other respiratory streptococcal infections, increasing incidences that usually precedes or accompanies illness by scarlet fever and so could be a good prognostic sign for scarlet fever and rheumatic illnesses.

It is also important of streptococcus strains and monitoring of their biological properties. It is known that pyogenic streptococcal population is heterogeneous and variable according to the typical structure and ability to cause scarlet fever, rheumatism, glomerulonephritis, toxicoseptic infections, erysipelas, etc. But increased morbidity is related mainly to exchange of serovariants of pathogen according to structural protein M.

Lack of criteria for accurate diagnosis and differentiation of streptococcal infections from the non-streptococococcal and unperformed mass performance methods of laboratory diagnosis determine the detection of cases only by clinical and epidemilogical anamnesys in the outbreaks of streptococcal infection and prescribing prophylactic and curative measures.

Preventive measures (See chapter "Infections with Streptococcus pyogenes")

Anti-epidemic measures

The patient with scarlet fever is hospitalised in infectious disease department or at home.

Reporting of sick or suspected cases of scarlet fever is mandatory. Health worker must transmit the information by electronic notification epidemiological alert system to CPH, and complete and submit sheet (form 058/e) within 24 hours from the detecting patient.

Patients with mild are isolated at home, if conditions permit the observance of anti-epidemic measures. Inpatient hospitalization will be performed according to clinical indications (severe and medium) and epidemiological (unable to provide isolation at home, presence in the family of people who attend or work in collective children aged up to 10 years, workers from surgical hospital, maternity, medical institutions for children (clinic, hospital), in closed institutions, businesses catering and dairy products, in case if it is imposible to isolate them from the sick).

Patient discharge is performed after clinical recovery, but not earlier than the 10th day after the onset.

Convalescents will pass the fellow-up within a month, ensuring the clinical and laboratory examination (blood and urine exam, clinical exam). The person who has suffered of scarlet fever is considered cured after 21 days of illness onset with two consecutive bacteriological examinations of a throat, hemolytic streptococcus is not detected, urine examination was normal, and general clinical examination revealed no pathological changes. The person who suffered of scarlet fever and is detected the pathology during the fellow-up will be supervised by rheumatologists, nephrologists or otolaryngologist, depending on the localization process.

People who have suffered from scarlet fever among children attending preschool and first two grade students - are admitted in these institutions over 12 days after clinical recovery.

Patients with angina or scarlet fever detected during 7 days after recording the last case of scarlet fever are not allowed in the above mentioned institutions for 22 days of the illness.

Current disinfection is carried in the outbreak before the patient hospitalization or during the treatment of patient at home. Disinfection is performed in affected groups or classes during 7 days after the last patient isolation.

The room where was (or is) patient is well ventilated, wet cleaning is performed, processing of utensils, toys, items for personal hygiene of the patient, cleaning the floors with soap and solution of caustic soda of 2%, and solution of chloramine of 0.5%.

Anti-epidemic measures in the outbreak, largely based on epidemiological survey and achievement, primarily, on detection of pathogen sources.

Following the epidemiological investigation are identified all persons who had contact with patients in the family, nursery school during 7 days until the appearance of the first clinical signs (establishing the source of pathogen), people who had contact with patients during illness or 7 days after their isolation.

Medical supervision of contactant people is carried out by family doctor and includes estimating of overall condition, thermometers of the body, examining throat and skin. People who were in contact should inform their doctors about illnesses borne by streptococcal infection (scarlet fever, angina, nasopharyngitis and others), date of appearance, the presence of simillar diseases at work place, school or kindergarten.

Detected cases of scarlet fever in children institutions require measures of restriction. There are not allowed accepting of new children in the group, where was isolated sick with scarlet fever. It is prohibited the transfer of children in this group or others, it is not allowed communicating with children from other groups. People detected with acute respiratory disease (angina, nasopharyngitis, etc.) are examined in order to identify outbreaks and isolate them from collective with announcement of physician. Their admission to the collective will take place after recovery and the certificate on treatment with antibiotics.

Emergency prophylaxis in outbreak

All persons who had contact with patients, and people with chronic inflammatory processes in the nasopharynx are subject to sanitation. It is recommended timocidina within 5 days (rinse or irrigation throat 4 times a day after meals).

In order, to neutralize the rash of scarlet fever in children collectives, it is recommended that all people who contacted to the source of pathogen receive intramuscular: bicillin-5 at a dose of 750 thousand U - preschool children, 1.5 million. U - schoolchildren and adults or bicillin-1 at a dose of 600 thousand U - U preschoolers and 1.2 million - schoolchildren and adults.

In case of eruptions in children collectives all registered contact children will undergo bacteriological investigations to confirm the carrage of pyogenic streptococci. Contaminated people will be treated with antibiotic.

1.1.2.3.2. Infections with group B streptococcus

Short history

Group B streptococcus (GBS) was first known as a causative agent in infections among animals, being identified in the etiology of mastitis in cows. Therefore, the species was named *Streptococcus agalactiae*. Group B strep infections in humans were reported for the first time in 1935 by Fry who described 3 cases of puerperal sepsis caused by this organism. Hare Lancefield identified subsequently group B streptococcus in vaginal secretions of pregnant women.

In the second half of the twentieth century, infections caused by GBS became increasingly common as one of the main causative agent of both sepsis and meningitis in newborns and high death rate among parturient women. In recent years, pathologies caused by GBS has gained an increasing medical and social significance.

The pathogen

Streptococcus agalactiae is a β-hemolytic streptococcus which according to Lancefield classification belongs to group B (the only representative), hence its name of *hemolytic streptococcus B*.

Serologically, group B streptococci are divided into 10 serotypes Ia, Ib, II-IX. Commonly one and the same serotype strains differ according to surface protein C. Recently, there have been described two types of protein C: α and β-protein. Types B streptococcus of human origin differ from cattle ones, and mostly colonize within the female genitalia, perianal region and the lining of the cervix; in males – in prostate secretions; in the newborns – the umbilical stump, groin, nose cavities and pinna.

Serotypes Ia and III GBS regard to CNS tissue tropism and airways, thus frequently causing meningitis in neonates. Pharyngeal colonization with group B streptococcus was reduced (5%).

Pathogen sources

The sick and carriers represent the main sources of GBS infections. Carriers are considered as a main reservoir for maintaining group B streptococcus in nature. Most commonly, it refers to women with

asymptomatic vaginal carriage at the puberty onset and which is estimated to 6-25%.

Mode of transmission

The transmission occurs both vertically and during the childbirth in neonates, being conditioned by the immunodeficiency of the pregnant woman (in mothers the immunoglobulin G level is lower in case of premature birth), as well as in premature rupture of membranes with aspiration of contaminated amniotic fluid, prolonged labor and premature birth. Newborns are very susceptible to GBS and therefore develop severe streptococcal infection (early septicemia or pneumonia).

Manifestations of epidemic process

Diseases caused by group B streptococcus are found worldwide and at all ages, predominating in newborns, which is about 1-3 cases per 1000 live births.

Bacteremia (with no specific source of primary infection) is observed in 30% of sick children; pneumonia occurs in 32-35%; meningitis appears in the rest of cases, 50% of which occurs at the first 24 hours of the child's life. The mortality rate is about 37%. Late manifestations of meningitis and bacteremia are most commonly observed in children with a mortality rate of 10-20%, whereas in 50% of survivors remaining phenomena have been reported. Febrile post-partum disorders are caused by GBS in 15-20% of cases.

Puerperal sepsis resulting from a carriage condition shows an incidence of about 2 cases per 1,000 births.

Clinical manifestations

In GBS the most common clinical manifestations are the neonatal septicemia and meningitis in infants. Early forms occur within the first hours, or up to 7 days after birth and are characterized by pulmonary disorders with respiratory disturbances or severe septicaemia, which is fulminant in 50-70% of cases. Late forms occur after weeks or a few months after birth, resembling meningitis and lead to lethal outcome or serious complications (deafness, blindness, mental retardation, epilepsy, otitis or septic arthritis).

GBS affects women who have recently given birth or after a childbirth, resulting in sepsis, puerperal fever, endometritis, urinary disorders, septic-purulent postoperative complications after a caesarean birth.

Additionally, GBS disease leads to skin and soft tissue infections,

pneumonia, endocarditis and meningitis in adults, as well as being largely responsible for premature births.

Prophylaxis

Prophylaxis refers to general hygiene and behaviour of pregnant women. Special prophylaxis includes investigation of pregnant women to confirm the carriage state of group B streptococcus and its treatment with antibiotics.

The implementation of intrapartum antibiotic prophylaxis among women has considerably diminished the incidence of this disease in neonates.

Specific prophylaxis is now being developed.

1.1.2.3.3. Infections with Streptococcus pneumoniae

Short history

For the first time, Streptococcus pneumoniae was identified in 1881. Being more common in pneumonia, it is called "pneumococcus".

In the early twentieth century, there were identified four serotypes of pneumococcus, being named as 1, 2, 3 and 4. M.D.Felton prepared the first vaccine derived from the capsular polysaccharide structures of the microorganism, which was used in 1938 during an epidemic of infection in patients hospitalized with pulmonary manifestations.

The pathogen

Streptococcus pneumoniae is an encapsulated Gram-positive pathogen, which occurs in culture as diplo- (hence the name of diplococci) or in short chains. Colonies of pneumococcus are surrounded by a greenish haemolysis on blood agar. Polysaccharide capsule present on the cell surface is considered a virulence factor; it inhibits phagocytosis via a mechanism that induces non-recognition of the structures of bacterial cell wall by the the antibodies or complement. Pneumococcus eliminates toxins with cytolytic effect, thus neutralizing the host defense mechanisms.

Surface protein A along with other antigenic structures are essential for increasing the virulence of the organism via an antiphagocytic effect.

Capsular structures enable the classification of pneumococci in serotypes. Currently, there are 91 known pneumococcal serotypes and no more than 40 serogroups.

All of them are pathogenic to humans, whereas types 1, 3, 4, 7, 8, 9 and 10 are more commonly reported. Types 6, 14, 19 and 23 are responsible

for most cases of pneumonia and otitis media in children. This explains the need for pneumococcal vaccines to contain antigens common to the 23 circulating serotypes, which are responsible for 85-90% of invasive S. pneumoniae infections, including the diseases that refers to community-acquired pneumonias.

Pathogen sources are found in individuals with various typical or atypical nosologic forms of the disease (see "Clinical manifestations") and healthy carriers of S. pneumoniae. Pneumococci commonly colonize the mucosa of the nasopharynx, estimated to 5-10% in healthy adults, 20-40% -in children and sometimes to 50-70%, particularly in children groups. The carriage rate is increased during winter and lasts from 2 -4 weeks up to 2-3 years.

The mode of transmission

S. pneumoniae transmission from one person to another is carried out both via a direct contact (e.g during a conversation) or indirectly through contaminated air drops resulting from coughing or sneezing. Close contacts and overcrowded rooms may favour the contamination.

Manifestations of epidemic process

Pneumococcal infections are typical anthroponotic diseases which ubiquitously spread and represent a serious public health problem worldwide. Receptivity to S. pneumoniae is increased in certain categories of persons. The highest risk of catching the disease is in children aged 2 years and in elderly people; people with chronic illnesses (chronic respiratory failure, heart failure, chronic renal disease, liver cirrhosis etc.), individuals with a pronounced immunodeficiency disorder(HIV/AIDS, asplenia, etc.). Therefore, the risk of catching diseases in pneumococcal infections is directly related to the mechanisms of transmission of the causative agent and the body's defense mechanism.

A significant feature of pneumococcal infection is the high morbidity among young children caused by pneumonia, as well as in adults. Both in Europe and in the USA, 76% of pneumonia cases in adults and 94% pneumonia cases in children are caused by streptococcus pneumonia.

Aside from community-acquired pneumonia, S. pneumoniae infections are commonly found in medical institutions as nosocomial infections, especially in intensive and maternity care units, but also in medical and social care institutions. The morbidity ranges between 10% and 60% depending on the age, pathology and associated conditions.

S. pneumoniae also causes 30-50% of otitis and 40-60% of media sinusitis.

The epidemic process of pneumococcal infections shows sporadic and eruptive manifestations. The eruptions are mainly recorded in children groups, medical institutions and those of social assistance.

According to WHO, about 1.6 million people die annually, whereas 0.7-1.0 million are children.

Respiratory infections are ranked 3rd among leading causes of death in children under 5 years old in Moldova, accounting for 13% of deaths, most of cases (93%) being caused by acute pneumonia.

Clinical manifestations caused by *S. pneumoniae* are quite various and characteristic for both non-invasive (media otitis, acute sinusitis, bronchitis, pneumonia) and invasive pathologies, when the causative agent enters into the blood, cerebrospinal fluid or sterile anatomic sites (bacteremia, meningitis, endocarditis, peritonitis, septic arthritis). Pneumonia and meningitis are the most important clinical manifestations caused by pneumococcus. *S. pneumoniae* infection should be suspected in all cases of illnesses in children who present symptoms of meningitis or pneumonia.

Preventive measures

General measures are similar to prevention of respiratory infections and nosocomial infections, especially in intensive and neonatal care units. Before the carriers are being treated with antibiotics, mainly with penicillin, preventive investigations for multidrug resistance will be carried out. Immunoglobulins are given to high-risk individuals, particularly to patients with marked immunodeficiency disorder.

The most effective specific prophylaxis is considered the administration of pneumococcal vaccine that is an antigenic type, which contains pneumococcal capsular polysaccharides. Currently, the prophylaxis of pneumococcal infection is being performed via 3 types of vaccines: 7-valent pneumococcal polysaccharide vaccine (PCV-7), which contains 7 serotypes of pneumococci, and pneumococcal polyvalent polysaccharide vaccine (VPP-23) and 13-valent pneumococcal vaccine Prevenar™ (VPC 13).

Based on Order of the Minister of Health no. 1022 dated 25 September 2013, immunization of children against pneumococcal infection is included in the national vaccination schedule starting from 1 October 2013, via

pneumococcal conjugate vaccine Prevenar™ (VPC 13). The complete vaccination course consists of 3 doses of vaccine by administering sterile suspension for intramuscular injection.

The first and second doses of vaccine (0.5 ml) are administered to children at ages of 2 and 4 months, concomitantly with both oral poliovirus vaccine (OPV) and rotavirus Rotarix (administered orally) and pentavalent B-Aib-PEA injection. Prevenar™ and pentavalent injectable vaccines will be administered via syringes in different anatomical locations (the right and left anterolateral side of the thigh).

The third dose of vaccine Provenar™ is administered to children aged 12 months, together with the vaccine against measles, mumps and rubella (MMR) via syringes in various anatomical sites, as well.

Children, who have not been vaccinated for different reasons at these ages, will receive vaccines indicated in any day, at on appropriate time. Vaccination of children according to individual schemes is performed with an interval of the minimum one month between doses of vaccine Prevenar™ and other vaccines. Prevenar™ is a compatible vaccine and can be administered with all the vaccines included in the calendar of vaccinations.

Prevenar™ vaccine is contraindicated in children:

- who present hypersensitivity to any substance of the vaccine or have had a reaction to previous dose of the vaccine.
- who present severe immunodeficiency condition and are receiving treatment with immunosuppressed remedies.

Vaccination should be postponed in children with acute illnesses and during exacerbations of chronic diseases.

Vaccination against pneumococcal infection is indicated to elderly persons as well.

1.1.2.4. Meningococcal infection

Short history

Meningococcal infection, also known as epidemic cerebrospinal meningitis has been recognized since antiquity (the 5th century BC.) T. Uillis described first clinical symptoms of infection in 1661. During the epidemic in Switzerland (1805), Gaspard Vieusseux described it as an independent nosologic form. A. Weichselbaum (1887) discovered the pathogen and isolated meningococcus from cerebrospinal fluid and called it *Diplococcus intracellularis meningitidis*.

In 1889, Osler U. isolated the causative agent from blood, whereas Kiefer (1896), Albrecht and Khoh (1901) - from the nasopharynx in healthy individuals. That served as evidence that meningococcus causes not only meningitis, but also other clinical forms as those from the nasopharynx to sepsis, as well as in the carriage state. Sulfanilamide therapy, which was first used in 1937, played an important role in the treatment of patients with meningitis, and led to a sudden decrease in the death rate of patients with generalized forms of meningococcal infection.

The pathogen agent

Neisseria meningitidis belongs to the family of *Neisseriacee*, *Neisseria genus*. It is gram-positive and immobile type. Morphologically, under a microscope, it looks like diplococci, shaped as a pair of coffee beans. It has a rather complicated antigenic structure determined by a pronounced heterogeneity. Depending on the structure of the antigen, there were identified 13 serogroups: A, B, C, D, H, I, K, L, X, Y, Z, W135. Not all meningococci are equal according to their pathogenicity. Meningococcal serogroups A, B and C A pose a greater threat on and are responsible for about 90% of generalized forms of meningococcal infection. The strains of meningococcal serogroup A are most frequently causes of rashes or even epidemics, while strains of serogroups B and C, and serogroup Y and W 135 might cause sporadic cases of illness, although in recent years there have been reported eruptions and epidemics caused by these serotypes, as well.

Endemic manifestations are characteristic of meningococcus serogroup B, while invasive clinical forms – of serogroup Y.

N. meningitidis includes a polysaccharide capsule, which allows the differentiation of serotypes and serogroups.

The main pathogenic factor for *N. meningitidis* is represented by endotoxin (lipopolysaccharide). Serogroup B, C and Y are identified in serovariants according to the comprising albumins of the outer membrane.

Currently, there are known 20 serovariants of meningococci. The serovariants 2, 4, 15 and 16 are considered as markers, which characterize the virulence of the pathogen. It is highly significant to determine the pathogen, which belongs to a serogroup or isolated serotype in patients or carriers. Additionally, meningococci contain antigenic components similar to many other types of non-pathogenic Neisseria, localized in the nasopharynx, including *N. lactamica*, which frequently colonizes the nasopharynx of young children.

Meningococci are very susceptible to exogenous factors. Within the external environment, they die after 5 minutes at temperatures below 22°C and higher than 55°C. Dryness and sunlight are also harmful for N. meningitidis. Nevertheless, it resists for a longer period of time (2-3 days) in nasopharyngeal secretions and in high-humidity environment. It is inactive for 2-3 min, under the influence of 0.01% chloramine solution, 1% solution of phenol, 0.1% aqueous hydrogen peroxide.

N. meningitidis is very demanding to cultivation. Isolation of pathological products requires special environments containing blood or serum. The optimum temperature is at 36.5°-37°C, therefore the cultivation of pathological products have to be carried out immediately after the collection and transportation at 37°C.

Largely, due to prevention and treatment with sulfanilamides, currently, there have been recorded up to 50% of N. meningitidis strains, which are sulfamido-resistant, and whose prevalence varies by territory.

Pathogen sources

Meningococcal infection is a typical anthroponosis. Only sick or carrier individuals may be as a source of pathogen.

There are three categories of sources of meningococcus:

1) Patients with generalized forms (meningitis, meningoencephalitis, meningococcemia);

2) Patients with acute meningococcal nasopharyngitis;

3) Healthy carriers or convalescent ones.

Patients suffering from meningococcal disease are already contagious in the last 1-2 days of the incubation period, which varies for meningococcal infection from 2 to 10 days, commonly it lasts for 4 - 6 days. Patients with generalized forms are more contagious during the prodromal period, which is manifested by catarrh phenomena (signs) and lasts for 2-3 days. The patient is highly contagious in the first week and then gradually the elimination of pathogens is decreased; whereas in non-treatment cases lasts up to 3-4 weeks after the onset of clinical manifestations, during which the infectious agent still persists in the nasopharynx. The duration of antibiotic treatment reduces the elimination of pathogen. The same period of infectiousness is characteristic of patients with nasopharyngitis. Convalescent carriers represent about 2% of people who suffered from a meningococcal infection.

Patients with clinical manifestations of meningococcal infection

present a considerable epidemiological risk, since they eliminate large quantities of pathogens into the external environment. It was proved that a sick patient might contaminate by six times more people than a healthy carrier might at a time.

However, the main source and reservoir of the pathogens in nature are considered meningococcal carriers. This phenomenon is due to a large number of healthy carriers among the human population. One patient with meningococcal infection correlates to 2000 - 45-50000 carriers.

The results of experimental epidemiological studies show that patients with generalized forms of meningococcal infection are responsible for 1-3% of the total number of infected patients, individuals with acute meningococcal nasopharyngitis - 10-30% of cases, while meningococcal carriers are responsible for 70-80% of illnesses. The number of carriers essentially increases during epidemics. Population may become carriers of meningitis during an increased level of morbidity rate up to 20%, and during meningococcal infection outbreaks - up to 30-40%. During a silent period of the pathogen, the frequency of carriage states of meningococcal infection in the human population does not exceed 1-4%. The carriage duration is only 2-3 weeks, whilst in individuals with inflammatory processes of the nasopharynx - up to 5-6 months. The carriage state is 3 recorded times more frequently among the latter group than among healthy people.

Only about 5% of carriers eliminate pathogens (meningococcus) for more than six months, some of them - up to several years. It has been observed the significant role of carriers in the spreading of meningococcal infection because of the long-lasting and solid carriage that characterizes the strains (A, B, C). This phenomenon can be explained through the persistent outbreaks of meningococcal infection in children's collectives. The widespread carriage among human population maintains the continuity in the epidemic process of meningococcal infection. It has been found also that the epidemiological states of generalized meningococcal infection are caused by strains of serogroup A, whereas the non-pathogenic strains cause only carriage states.

Factors and transmission routes

Considering the modes of entering and the predominant location of the pathogens, which are present in all forms of meningococcal infections and

carriers - in the mucosa of the nasopharynx, as well as the reduced viability of the pathogens in the external environment, the infectious transmission takes place from the source to susceptible persons, as a rule, via liquid aerosols of the sick or carriers, during speaking, coughing or sneezing. Therefore, the respiratory mechanism transmission and the airborne way of contamination, as well as close contact between individuals are lead to epidemic of meningococcal infections.

Contributing factors:

- presence of chronic carriers within a collective;
- formation of epidemiology strains of the causative agent;
- children's age;
- overcrowded conditions, particularly in children's collectives;
- not following the temperature, ventilation and humidity conditions;
- low specific and non-specific resistance of the body;
- social and economic factors.

Manifestations of the epidemic process

Meningococcus infections are ubiquitous pathologies. It is an important cause of morbidity and mortality worldwide. According to WHO, annually, there are about 300,000 illnesses and 30,000 deaths caused by meningococcal worldwide. Simultaneously, the intensity of morbidity varies in different areas. The annual morbidity rate caused by generalized meningococcal infection ranges from 0.01 to 12.0 cases per 100 thousand population in most of moderate climate countries. Nevertheless taking into the account the severity of generalized forms, high disability after incurring the infection, high morbidity (cerebrospinal meningitis - 10-20%; encephalitis - 90%), even these indices present a serious medical and social problem. The incidence of meningococcal infection is much higher in countries with tropical climates. For example, annual morbidity from meningococcal infection shows over 300-700 cases per 100 thousand population in tropical African continent, the so-called *"African meningitis belt"*. This peculiarity of the meningococcal infection spreading differentiates it from other respiratory infections.

The epidemic process of meningococcal infection is characterised by sporadic level, outbreaks and epidemics. Epidemics have a multiannual evolution, which leads to major upward cycles for meningococcal infection *(Figure 15)*.

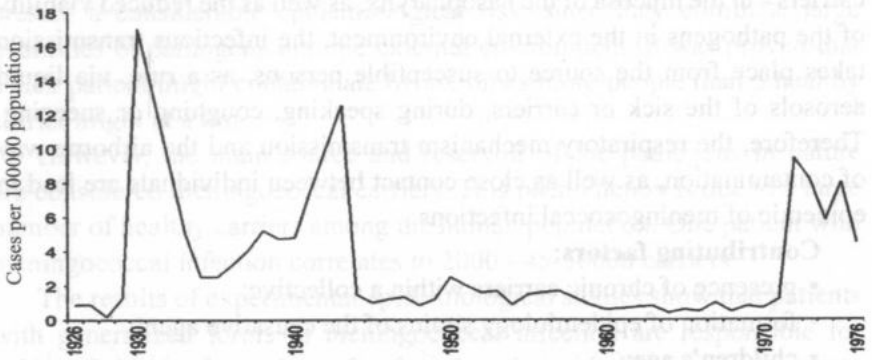

Figure 15. *Multiannual dynamics of morbidity rate caused by generalized forms of meningococcal infection in Uzbekistan, during the period of 1926-1976. (L.P. Zueva, R.H.Iafaev, 2005).*

During the 1945-1969 years, in Moldova, the death rates from meningococcal infection ranged between 0.5-1.2, with an average of 0.98 cases per 100,000 population.

In 1970, a sudden increase in morbidity rate was recorded. The high level of morbidity was present until 1988, with an annual average of 9.25 cases per 100,000 population; then a long period of a decreased morbidity rate occurred until 2005, with an annual average of 3.75 cases per 100,000 people; as well as a stabilization period (interepidemic period) was present with an annual average incidence of 1.2 cases per 100,000 population *(Figure 16)*.

People who got already meningococcal infection acquire a long-lasting immunity. The immune status of adult population increases with age because of frequent causative agent contamination from healthy carriers. Children up to 6 months are not receptive to meningococcal infection since they acquire maternal immunity. Therefore, the most patients prone to develop a meningococcal infection are children up to 14 years, especially those of young age, starting from 7 months after birth. Therefore, a higher level of morbidity rate is recorded in children aged up to 14 years. This group constitutes 70-80% of overall morbidity, of which 50% are children aged up to 5 years. The incidence decreases with age.

Carrier's distribution by age has a reversed tendency compared to morbidity structure.

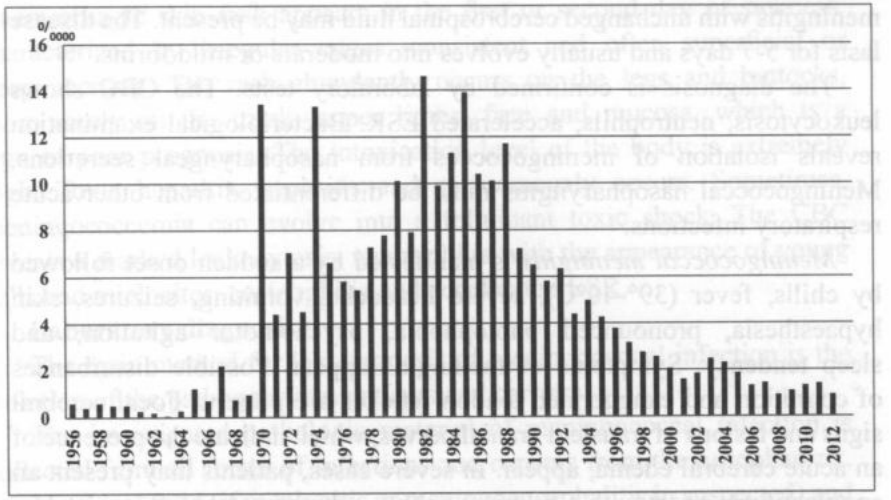

Figure 16. *Multiannual dynamics of morbidity rate caused by generalized meningococcal infection in the Republic of Moldova, in the period of 1956-2013.*

People with immunodeficiencies are at higher risk to develop generalized infections with *N. meningitidis*.

Seasonality of meningococcal infection is characteristic for airborne infections. The maximum level of morbidity is during January – May, while the onset of epidemics takes place in autumn. The seasonality is more pronounced during the epidemics, which is characteristic for airborne infections.

It is important to note, that only 1 in 2,000 children are infected with generalized meningococcal infection (meningitis, meningococcemia). This phenomenon is due to the virulence of the pathogen and the level of immunodeficiency, and according to some authors due to a genetic predisposition as well. It has been found that individuals with B blood type (III group) develop serious forms of meningococcal infections, and patients with O blood type (I group) – milder forms.

The manifestations of infectious process

Meniningococcal nasopharyngitis is the most common form of meningococcal infections, but also the most benign one. It is manifested by sudden onset, chills, fever, headache, myalgia, vomiting, pain on swallowing, runny mucopurulent rhinitis and dry cough. Symptoms of

meningitis with unchanged cerebrospinal fluid may be present. The disease lasts for 5-7 days and usually evolves into moderate or mild forms.

The diagnosis is confirmed by laboratory tests. The CBC shows leukocytosis, neutrophils, accelerated ESR. Bacteriological examination reveals isolation of meningococcus from nasopharyngeal secretions. Meningococcal nasopharyngitis must be differentiated from other acute respiratory infections.

Meningococcal meningitis is manifested by a sudden onset followed by chills, fever (39°-40°C), severe headache, vomiting, seizures, skin hypaesthesia, pronounced photophobia, psychomotor agitation, and sleep tendency. Symptoms of meningitis appear. Possible disturbances of cognition and exaggerated tendon reflexes are present. Focal cerebral signs and lesions of transient cranial nerves, which indicate the presence of an acute cerebral edema, appear. In severe cases, patients may present an apoplectiform onset with a fulminant coma, which is sometimes mistaken for strokes in adults. The cerebrospinal fluid is hypertensive, cloudy or purulent, containing meningococci. The CBC shows leukocytosis, neutrophils, eosinophilia, accelerated ESR.

The onset of meningococcal meningitis is often atypical, characterized by refusal of breastfeeding in infants, vomiting, fever and diarrhea or respiratory phenomena, but often it develops into serious, long-lasting and exacerbating complications like viral and bacterial superinfections. It is often associated with meningococcemia, encephalitis, and ependimatis. Fulminant forms are frequently recorded.

Ependymal meningococcal meningitis can evolve in children older than one year, usually with poor outcome.

Meningoencephalitis meningitis occurs more frequently in young children. From the first days of onset, there are symptoms of neuraxis: psychic phenomena (mental confusion, agitation or apathy, delirium, unconsciousness, coma) and motor ones (convulsions, myoclonus, and paralysis); cranial nerve impairment: of the eye (diplopia, anisocoria, strabismus, eyelid ptosis), auditory (hypo- or hyperacusis). The reflexes of tendon are exaggerated or abolished. The symptoms of meningitis are present but not pronounced. The prognosis is often unfavorable: death or sequelae (paresis, paralysis, mental disorders, epilepsy, etc.).

Meningococcemia (meningococcal septicemia) is manifested by a sudden onset followed by fever, chills, headache, myalgia and arthralgia.

Haemorrhagic skin rash appears in the first or second day of sickness, characterized by irregular edges, consistent and often superficial or deep necrosis. The rash abundantly occurs on the legs and buttocks, more rarely on the trunk, upper limbs, face and mucosa, which is a sign of poor prognosis. The intoxication level of the body is extremely high. Toxic hepatitis, nephritis and splenomegaly occurs. Sometimes, meningococcemia can evolve into a fulminant toxic shock. The CBC reveals a marked leukocytosis, neutrophilia with the appearance of young cells and mielocites, eosinophilia and accelerated ESR.

Laboratory diagnosis

The main method for lab diagnosis of meningococcal infection is the isolation of the pathogen from patients and carriers.

The investigated pathologic material of meningococcal infection is collected from all forms of infections and carriers from the nasopharynx, cerebrospinal fluid (if meningitis or meningoencephalitis in suspected) and blood (if meningococcemia are suspected).The material is taken from the nasopharynx with a bent tampon, in oder to collect the mucus from the back wall. Taking into the account the low resistance of *Neisseria meningitidis* within the external environment, the material sampling should be carried out at the patient bedside and transported to the laboratory under specific humidity and temperature (37°C) conditions.

In generalized forms, blood or cerebrospinal fluid are cultured in a medium which contains human proteins (blood or serum). The cultivation of material from the nasopharynx occurs in the same medium. The result is obtained within 24 hours.

Serological investigations are used to determine serovariants of meningitis isolated by RLA and the level of antibodies - by IHAR.

Epidemiological surveillance of meningococcal infection includes the following objectives:

• Supervision of both morbidity and lethality levels, as well as the level of multiannual dynamics of the carriage state. It is important to supervise the dynamics of the morbidity rate in affected children, most frequently in the first year of their life. A visible increase of incidence among children is an index of poor outcome.

It is useful to study the level of carriage state, especially in risk groups (children's collective) by double sampling of the material in September and during November-December. High level of carriage state among the

population may serve as a prognostic indicator of epidemics in the near future:

• Supervision of circulation of meningococcus in the population. The increased circulation and determination of the predominant serotype of meningococcus are important indicators in prediction of epidemiological situation in both near future and for the next year.

• Analysis of the epidemic spread intensity in different territories, living conditions and risk groups.

• Assessment of the undertaken measures and the proposed intervention measures (preventive and anti-epidemic).

• Systematic analysis of the resistance of circulating meningococci to sulfanilamide and other antibiotics used in the treatment of meningococcal infection.

Control measures

Preventive measures

It is crucially important to follow a sanitary-hygienic regimen in children's collectives as a prophylactic measure due to overcrowding conditions and long communication factors that favour the epidemic spread of the meningococcal infection. Overcrowding conditions will be avoided, whereas the rooms properly ventilated.

During an increased seasonality, it is important to enhance the follow-up measures. During this period, it is reasonable to limit the mass activities like cultural ones, sports etc. An important prophylactic measure in children's communities is the detection and treatment of meningococcal carriers.

Some countries practice vaccination against meningococcal serogroups A and C with polysaccharide vaccine that provides protection for up to 3 years and an efficacy of 85-95%. The vaccine may be administered at the beginning of the epidemic onset, as well as during this period.

Vaccination is recommended for children of 1 - 8 years old and first year grade students of universities. In epidemic situations vaccination is also recommended for adolescents and adults who go to medical institutions for immunization. Repeated vaccinations may be carried out no more than every three years.

Meningococcal B vaccine is a poorly immunogenic type. Currently, a polyvalent meningococcal conjugate vaccine has been developed, which includes capsular polysaccharides of serogroups A, C, Y and W135.

Using one or another preventive vaccine might be justified only after determining the type of circulating meningococcus.

Anti-epidemic measures

Medical institutions regardless of their type will report the patient with meningococcal infection by recording the case into the electronic system of epidemiological alert and by completing the form (no. 058/e).

Patients with generalized forms of meningococcal infection and nasopharyngitis are subjected to mandatory hospitalization in case of the epidemics, especially children aged under 3.

Home isolation is allowed, in case if there are no preschool children and adults working at children's instititions within the family. Hospital discharge of the patient is allowed only after a clinical recovery and negative test outcomes collected from the nasopharynx mucosa. The bacteriological investigation is conducted not earlier than 3 days after the therapy completion. People who have suffered meningococcal infection will be admitted to preschool institutions, schools, boarding schools, etc., after a negative bacteriological investigation of nasopharyngeal exudate conducted no earlier than 5 days after the discharge or recovery of the home isolated patient diseased with nasopharyngitis. A family physician, neurologist or pediatrician fellow up the persons suffered generalized form of meningococcal infection during 2-3 years with one examination every 3 months during the first year and 2 times per year during the fellowing years.

People who were in contact with patients would undergo a daily medical examination within 10 days. This includes measuring of the body temperature twice a day, examination of the nasopharynx and teguments by an otorhinolaryngologist.

People who contact children in institutions are subjected to bacteriological examination twice at intervals of 3-7 days, and those at home - once.

Carriers of meningococcus undergo the same treatment as patients with meningococcal nasopharyngitis, followed by a bacteriological investigation in 3 days after the treatment is completed. Recurrent meningococcus infections require a repeated course of treatment with the administration of other types of antibiotic. Disinfection in the focus is not required due to the instability of the pathogen within the external medium. It is advisable to perform a wet cleaning of the room, to air it frequently, to reduce the

crowding in children's groups, especially in bedrooms. In order to avoid the occurrence of nosocomial cases, hygienic and anti-epidemic measures must be undertaken by hospitals, where patients with meningococcal infection are treated in.

1.1.2.5. Influenza (Flu)

Short history

Influenza was reported in the Hippocrates' writings in ancient times. Epidemiological and clinical descriptions of influenza were conducted by Hirsch in 1173, Seneca in 1387, and Pasqeur in 1403. Over the times, influenza has been assigned such names as "catarrhal fever", "acute nasopharyngitis", and "epidemic influenza". In the eighteenth century, the severity of some epidemic events led to the name of "petite over" and then it was called flu, from "grippe" (fr. "Gripper" - grab, comprising). The Flu was often described alongside smallpox, plague and cholera according to epidemics and pandemics that they determined.

Throughout the history, there were described over 30 pandemics, most of them started off the Asian continent, but a more accurate description refers to the last seven pandemics caused by influenza type A: 1889-1891 (Russian Flu), 1918-1919 (Spanish flu - the most severe pandemic, estimating 50 million of deaths), 1957 to 1958 (Asian flu), 1968-1969 (Hong Kong flu), 1997-2003 (avian influenza), and 2009-2010 (swine flu). This disease has proven to be one of the communicable diseases with the highest potential of re-emerging, risk of disruption of social activities, inducing major human and economic losses.

In 1933, Michael W. Smith, Christopher Andrews and Patrick Laidlau identified Influenza A, in 1936 Francis et al. - Influenza B, and in 1950 Taylor et al. - Influenza C. Also in 1950, after the discovery of cell cultures, the first vaccine against influenza was prepared.

The pathogen agent

Flu viruses are the causative agents belonging to the family of *Orthomyxoviridae, genus influenzavirus*. The name of *orthomyxoviridae* expresses the affinity for cell surface mucoproteins (myxa - mucus), and influenza expresses the high potential for spreading. Structurally, influenza viruses are spherical in shape with a diameter of 80-120 nm. The genome of the virus consists of 8 segments of RNA, and the membrane includes two antigenic surface proteins: hemagglutinin (HA) and neuraminidase (NA) *(Figure 17)*.

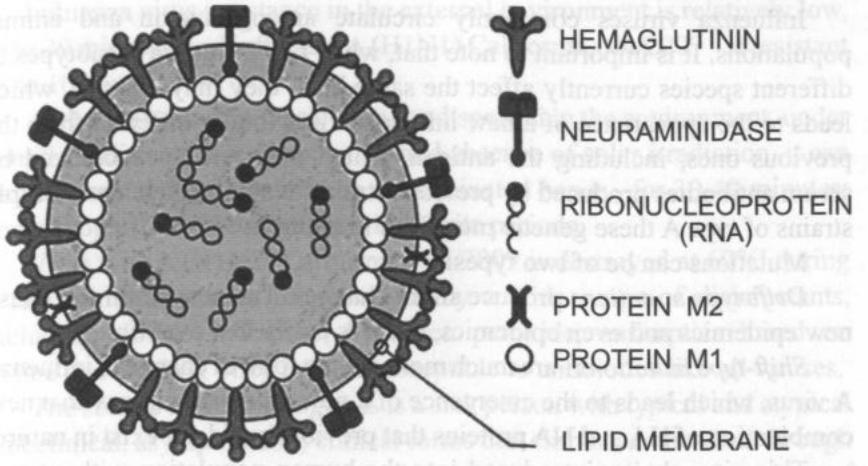

Figure 17. *Structure of the influenza virus.*

According to its variety and based on the differences of the internal specific antigens - nucleoprotein (NP) and the M protein, influenza viruses are divided into several types, whereas based on the characters of membrane proteins HA and NA- in subtypes.

Currently, there are three known types of influenza viruses - A, B and C.

Type A influenza virus has a high virulence and increased epidemiological potential but also a pronounced antigenic variability. The latter, is explained by the high potential of the joint surface proteins, hemagglutinin and neuraminidase, characteristic of this type of virus. There are 16 different HA subtypes and 9 NA subtypes, with multiple possible connections between them (up to 268).

Type A influenza virus is a pathogen for both humans and different animal species, which also constitute a reservoir for different subtypes of virus in nature.

The pathogenic subtypes A (H1N1), A (H2N2), A (H3N2), variant A (H1N1) new type - California / 04-2009 and occasionally A (H5N1) and A (H7N2) are contagious for humans. Subtypes A (H1N1) and subtypes A (H3N2) can cause swine influenza, subtypes A (H7N7) and subtypes A (H3N8) – in horses and subtypes A (H5N1) – in birds.

Influenza viruses commonly circulate among human and animal populations. It is important to note that, when two influenza A subtypes of different species currently affect the same host, they may reassort, which leads to the appearance of a new influenza virus totally different from the previous ones, including the antigen, which may not be recognized by serum antibodies produced by previous strains. Namely, there are multiple strains of type A these genetic mutations in nature.

Mutations can be of two types:

Drift-type mutations produce slight changes. The new strain can cause new epidemics and even epidemics, but it is easily lost over time.

Shift-type mutations –are much more drastic form of change in influenza A virus, which leads to the emergence of a new influenza virus with a new combination of HA and NA proteins that previously did not exist in nature.

This virus, being introduced into the human population without any specific protection, can lead to the development of a major pandemic. Such changes in the antigenic structure of the influenza virus cannot be predicted. They occur at irregular intervals (10-40 years) and are responsible for the development of pandemics, usually with high morbidity and lethality, and high economic losses.

So far, there are seven human pandemics caused by viruses type A *(Table 1)*.

Table 1

Pandemics of flu

Pandemic	Years	Number of deths	Type of the virus
Rusian influenza	1889 - 1891	1 million	A /H_3N_2
Spanish influenza	1918 - 1919	50 millions	A/ H_1N_1
Asian influenza	1956 - 1958	2 millions	A /H_2N_2
Hong Kong influenza	1968 - 1969	0,75-1,0 millions	A /H_3N_2
Avian influenza	1997 - 2003	257	A H_5N_1
Swine influenza (mexic)	2009 - 2010	18500 (laboratory confirmed)	A/ H_1N_1/ 09

Influenza B and C viruses are pathogenic to humans only. Unlike type A, viruses B and C are not classified into subtypes. Virus B causes epidemics, not pandemics. C virus causes mild illnesses and does not lead to epidemics.

Influenza virus resistance in the external environment is relatively low, up to 30 minutes, and influenza A (H1N1) California / 04/2009 - is resistant up to 10 hours.

At the same time, in septic droplets dispersed in the environment, under humid conditions, low temperature and absence of solar irradiation, it can last for several days; whereas on contaminated hands –for 30-40 minutes; under lyophilization conditions - indefinite periods.

Virus type A (H1N1) California / 04/2009 is destroyed at 60°C during a few minutes; it may be destroyed by a wide range of disinfectants, including clorine subtances, hydrogen peroxide, antiseptics based on iodine and alcohol. They are not susceptible to antibiotics like all viruses.

The source of the pathogen is is a sick person with typical and atypical (subclinical, asymptomatic) clinical forms that eliminates the virus through the exhaled air from the end of the incubation period and the first signs of the disease. The incubation period ranges from 1 to 7 days, more frequently for 1-2 days. The patient is contagious in the catarrhal period and within the febrile period. Contagiousness is maximal in the first 5-6 days of illness. Virus elimination ceases in 5-10 days from the onset of the disease.

In influenza A (H1N1) California / 04/2009, the virus was eliminated for up to 10-14 days. Virus elimination is more intense during coughing or sneezing. Healthy or postinfectious carriers have a minor epidemiological importance, inspite of they are reported.

Mode, factors and transmission routes

The primary mode of pathogen transmission from the sick to the receptive person is a respiratory one. Contamination occurs aerogenically upon inhalation of liquid aerosols eliminated by the patient (the main localization of the virus - mucous membrane of the nose) after coughing or sneezing. Contamination occurs in close contact with the patient at a distance of 1 m. In case of cough or sneezing, the distance of possible contamination increases up to 2-3 m. Virus A (H1N1) California / 04/2009 can be transmitted by recently contaminated objects and hands touching the nose or eye mucosa.

Contamination though water or food does not occur. Infection with avian influenza virus usually occurs as a result of human contact with diseased birds or during the cleaning up the hen-roost by inhalation of solid aerosols formed by the drying of feces.

The epidemic is a characteristic manifestation of influenza, especially that of type A, which actually occurs every year, whereas every 3-5 years it might be extensive and severe.

In case of a new type of antigenically modified A strains ("shift"-type mutation),the epidemic process in influenza develops into the pandemic one. The last pandemic began in 2009, when WHO announced the record of confirmed influenza cases caused by an antigenic new variant A (H1N1) in California/04/2009, which is a complex reassortment between human viruses and animals (pigs, poultry), initially called "swine" influenza. A particularity of this virus is the speed of spread. The virus emerged in Mexico in April, and it spread very quickly throughout the world and in October 2009, the pandemic involved already 195 countries. The virus affected all age groups of population, mostly young children and aged people because of the lack of immunity to the new virus. The data of *Figure 20* is a confirmation of this phenomenon.

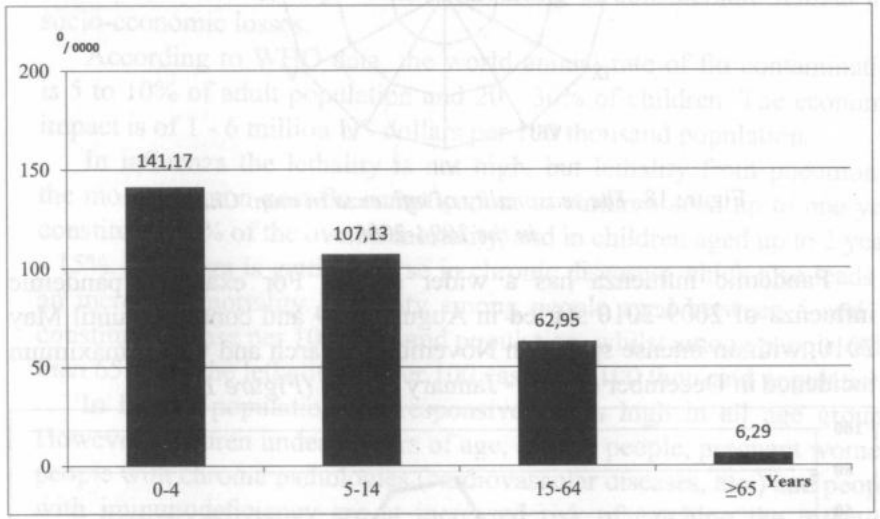

Figure 20. *Morbidity of new type of influenza A(H1N1) according to the age groups in the Republic of Moldova (year 2009).*

Another feature of the new type A (H1N1) influenza virus is the ability to affect the lower airways (uncharacteristic of other types of human influenza viruses) and to cause severe pneumonia especially in children and young adults. Only during the first 5 days after the onset of the pandemic in

Mexico, 2155 cases were complicated by pneumonia, of which 100 cases were fatal.

A higher severity of infection was registered among people aged over 20 and 40 years, but fatal cases were shown to be more common in older people with chronic diseases, particularly cardiovascular and lung diseases. According to WHO data, 80% of deaths due to influenza A (H1N1) California/04/2009 belong to people from this group of population.

The manifestations of infectious process

Influenza infection is accompanied by the following clinical manifestations of acute respiratory syndrome: fever 38°C, headaches, chills, upper respiratory tract symptoms (dry cough, runny nose, sore throat, difficulty breathing), generalized myalgias, arthralgias, fatigue, general dyspeptic symptoms (diarrhea and vomiting). At least two of the symptoms listed above are sufficient to suspect influenza. Clinical symptoms can vary from mild to severe forms according to the severity of the disease. The duration of the disease is in most cases 4-7 days. The Flu caused by A (H1N1) virus California / 04/2009 may result in pneumonia. In avian flu, the infectious process is vice versa and begins with pneumonia.

The following complications are characteristic of the flu: acute chronic diseases, upper respiratory tract disorders (sinusitis, otitis media, laryngotracheal collapse), lower respiratory tract infections (pneumonia, bronchiolitis, asthma), cardiac disorders (myocarditis, pericarditis) neurological pathologies (encephalopathy and acute post infectious encephalitis, febrile seizures, status epilepticus), toxic shock.

Laboratory diagnosis

Confirmation of the diagnosis with influenza virus is performed via the following laboratory tests:

- RT - PCR positive;
- Positive viral culture;
- Positive serological test (increased more than 4 times than the specific antibody titters in two pairs of serum by neutralisation method).

Epidemiological surveillance

Currently, WHO performs a global epidemiological surveillance of influenza viruses through a network of 110 virological laboratories operating in 79 countries (including the Republic of Moldova) and 3 Reference Centers (UK - 1 and US - 2). The main task is to carry out permanent monitoring of the circulation of influenza viruses and influenza

Contributing factors

Rapid spread of influenza in the human population is determined both by the epidemiological features of infection (short incubation period, aerogenic transmission pathway, high responsiveness of the population, especially to new strains of the virus, violation of the protection regime), and other social and natural factors: intense migration of the population, development of transport means, which ensure the rapid movement of the population in different countries and continents. An essential factor favoring the spread of influenza is the cold season of the year, which contributes to crowding in the rooms, the development of respiratory inflammatory processes with coughing and sneezing syndrome.

Manifestations of epidemic process

Influenza is a global infection. Having a high index of infectiousness, it knows no geographic boundaries.

In the flu the epidemic process is manifested through sporadic cases, rashes, epidemics and pandemics, resulting in considerable human and socio-economic losses.

According to WHO data, the world annual rate of flu contamination is 5 to 10% of adult population and 20 - 30% of children. The economic impact is of 1 - 6 million US dollars per 100 thousand population.

In influenza the lethality is not high, but lethality from pneumonia, the most common post-flu complication, in children aged up to one year constitutes 30% of the overall mortality, and in children aged up to 2 years - 15%. Influenza is getting worse in chronic diseases, which also leads to an increased mortality. Lethality among people aged between 5 and 19 constitutes 1 case per 100 thousand population, whilst among people older than 65 years the lethality is over 100 cases per 100 thousand population.

In human population the responsiveness is high in all age groups. However, children under 5 years of age, elderly people, pregnant women, people with chronic pathologies (cardiovascular diseases, etc.) and people with immunodeficiency are at increased risk of catching the infection and developing post-flu complications. The population's receptivity is extremely high to new influenza virus serotypes.

People who have suffered influenza infection and those vaccinated against influenza get a type-specific immunity.

Epidemics developed due to the absence or weakening of immune status in population against different types of virus.

Seasonal influenza causes the increase of the morbidity rate every year in all countries, epidemics - every 2-3 years, pandemics - 10-30 years, respectively.

Although the regularities of the epidemic spread are common, the level of population morbidity in different countries varies and is inversely proportional to the undertaken measures.

Influenza seasonality is noted in cold seasons of the year – from December to April, being more frequently intense in January-March and with a pick in February *(Figure 18)*, although the peak of seasonality may vary: in December (1995), January (2007), and April (2006).

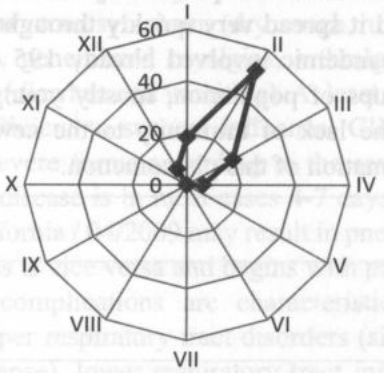

Figure 18. *The seasonality of influenza in mun. Chisinau, in the 1994-2008.*

Pandemic influenza has a wider spread. For example, pandemic influenza of 2009-2010 started in August 2009 and continued until May 2010, with an intense spread in November - March and with a maximum incidence in December (2009) - January (2010) *(Figure 19)*.

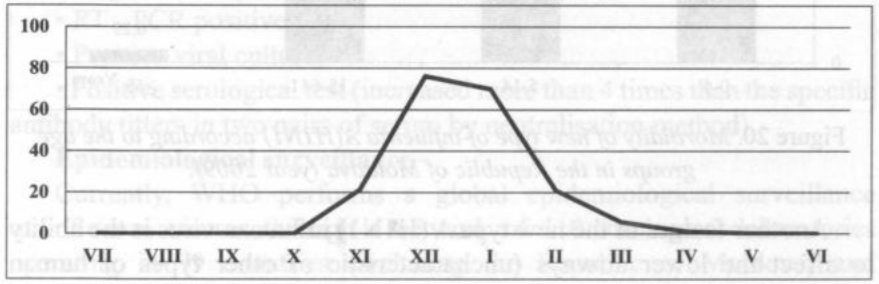

Figure 19. *The seasonality of pandemic influenza A (H_1N_1) new type in mun. Chisinau, in 2009-2010.*

The epidemic is a characteristic manifestation of influenza, especially that of type A, which actually occurs every year, whereas every 3-5 years it might be extensive and severe.

In case of a new type of antigenically modified A strains ("shift"-type mutation),the epidemic process in influenza develops into the pandemic one. The last pandemic began in 2009, when WHO announced the record of confirmed influenza cases caused by an antigenic new variant A (H1N1) in California/04/2009, which is a complex reassortment between human viruses and animals (pigs, poultry), initially called "swine" influenza. A particularity of this virus is the speed of spread. The virus emerged in Mexico in April, and it spread very quickly throughout the world and in October 2009, the pandemic involved already 195 countries. The virus affected all age groups of population, mostly young children and aged people because of the lack of immunity to the new virus. The data of *Figure 20* is a confirmation of this phenomenon.

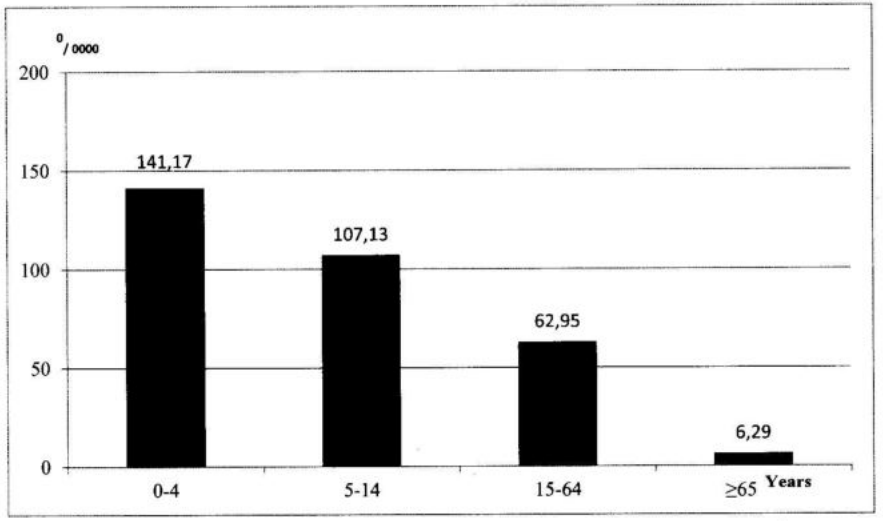

Figure 20. *Morbidity of new type of influenza A(H1N1) according to the age groups in the Republic of Moldova (year 2009).*

Another feature of the new type A (H1N1) influenza virus is the ability to affect the lower airways (uncharacteristic of other types of human influenza viruses) and to cause severe pneumonia especially in children and young adults. Only during the first 5 days after the onset of the pandemic in

Mexico, 2155 cases were complicated by pneumonia, of which 100 cases were fatal.

A higher severity of infection was registered among people aged over 20 and 40 years, but fatal cases were shown to be more common in older people with chronic diseases, particularly cardiovascular and lung diseases. According to WHO data, 80% of deaths due to influenza A (H1N1) California/04/2009 belong to people from this group of population.

The manifestations of infectious process

Influenza infection is accompanied by the following clinical manifestations of acute respiratory syndrome: fever 38°C, headaches, chills, upper respiratory tract symptoms (dry cough, runny nose, sore throat, difficulty breathing), generalized myalgias, arthralgias, fatigue, general dyspeptic symptoms (diarrhea and vomiting). At least two of the symptoms listed above are sufficient to suspect influenza. Clinical symptoms can vary from mild to severe forms according to the severity of the disease. The duration of the disease is in most cases 4-7 days. The Flu caused by A (H1N1) virus California / 04/2009 may result in pneumonia. In avian flu, the infectious process is vice versa and begins with pneumonia.

The following complications are characteristic of the flu: acute chronic diseases, upper respiratory tract disorders (sinusitis, otitis media, laryngotracheal collapse), lower respiratory tract infections (pneumonia, bronchiolitis, asthma), cardiac disorders (myocarditis, pericarditis) neurological pathologies (encephalopathy and acute post infectious encephalitis, febrile seizures, status epilepticus), toxic shock.

Laboratory diagnosis

Confirmation of the diagnosis with influenza virus is performed via the following laboratory tests:

- RT - PCR positive;
- Positive viral culture;
- Positive serological test (increased more than 4 times than the specific antibody titters in two pairs of serum by neutralisation method).

Epidemiological surveillance

Currently, WHO performs a global epidemiological surveillance of influenza viruses through a network of 110 virological laboratories operating in 79 countries (including the Republic of Moldova) and 3 Reference Centers (UK - 1 and US - 2). The main task is to carry out permanent monitoring of the circulation of influenza viruses and influenza

morbidity, to study the evolution of respiratory viruses and to develop recommendations for prevention and treatment. All these centers also select influenza virus strains to produce influenza vaccines, with vaccine components being modified each year.

The epidemiological surveillance system for influenza at a national level includes:

- continuous surveillance of influenza incidence, acute respiratory viral infections and severe acute respiratory infections (acute pneumonia and bronchiolitis in children and infants). In the pre-epidemic period, the epidemiological surveillance of acute respiratory infections is ensured through an ongoing and continuous surveillance and control system at the national level. A measure of great importance is the collection and transmission of morbidity data, including sentinel influenza surveillance system (within a maximum attention period - weekly), in order to detect morbidity increases and confirm the suspected cases of influenza. Sentinel points will collect and report the following data:

- ✓ the number of requests for ambulance services for Acute Respiratory Infections (ARI), influenza, pneumonia and bronchopneumonia in children and infants;
- ✓ use of specific drugs in the treatment of influenza and its complications;
- ✓ number of absences in kindergartens, schools, lyceums and industrial units;
- ✓ number of consultations and home visits by age group;
- ✓ number of (ARI) cases, influenza and pneumonia cases in total home visits and consultations;
- ✓ number of short-term certificates (<10 days);
- ✓ the number of hospitalization by influenza, (ARI), pneumonia and bronchopneumonia, in relation to total hospital admissions;
- ✓ number of bronchiolitis in children under 2 years of age admitted in the previous week, compared to the total number of hospitalization
- ✓ rapid detection of circulating influenza virus serotypes (serotypes).

Criteria for virological investigation of suspected cases during the pre-epidemic period include:

- ✓ cases of outbreaks of acute respiratory infections similar to influenza;
- ✓ acute infections of the lower respiratory tract;

- ✓ severe unexplained respiratory infections;
- ✓ death associated with acute respiratory infections similar to flu or lower respiratory tract diseases;
- ✓ an increased number of deaths related to respiratory diseases;
- ✓ an increased number of severe respiratory diseases in previously healthy adolescents or adults and in pregnant women;
- ✓ a high level of absences from school or workplace;
- ✓ operative assessment of the epidemiological situation and providing measures and financial support for its prevention and control;
- ✓ prognosis for the evolution of the epidemiological situation.

Control measures

Preventive measures

Specific prophylaxis vaccination against avian influenza provided for the population during the pre-epidemic period (rationally in October-November) is considered an important measure in reducing the spread and severity of the epidemic.

The same circulating serotype of virus and vaccine administrated to the population can prevent 80-90% of diseases in children and adults, whereas in case of diseased vaccinated population, influenza evolves easily and uncomplicatedly, which eventually leads to a lower level of lethality.

Vaccination against influenza is carried out according to the epidemiological guidelines and is recommended primarily in population at high risk of contamination and developing influenza infection:

- ✓ workers from health care institutions;
- ✓ children and adults with chronic conditions;
- ✓ pregnant women in the 2nd and 3rd trimesters or at any other stage with inactivated vaccine;
- ✓ staff of social assistance institutions, orphanages, boarding schools, nursing home care, retirement homes for the elderly and disabled;
- ✓ preschool and school-age children;
- ✓ students at universities, colleges and vocational schools;
- ✓ staff of preschool institutions, schools, colleges and vocational schools;
- ✓ staff of the customs service and border crossing points;
- ✓ international airports and crew workers;
- ✓ staff of the Ministry of Internal Affairs;
- ✓ National Army;

✓ railway workers who serve the passengers;
✓ Emergency Service workers;
✓ Any other category of the population indicated by epidemiologists.

Immunization is generally carried out free of charge, in clinics in accordance with the requirements of the vaccine administration instructions, and with respect to medical contraindications for vaccination.

For these purposes, live, attenuated, inactivated, subunit and split vaccines, which typically contain all the three strains are recommended by the WHO for each epidemic season

Nonspecific flu prevention measures are of both individual and social order.

Individual measures include compliance with personal hygiene rules, the use of masks or other means of protection, following a healthy lifestyle, including adequate proper protein enriched diet, vitamins and minerals that will increase the body's ability to fight against influenza, avoiding undesirable effects of cold, moisture, agglomeration and unprotected contact with patients affected by respiratory infections.

One of the most effective methods of preventing influenza illness and other acute respiratory infections is systematic airing and cleansing of rooms while maintaining a proper temperature regime.

Administrative measures to prevent human influenza include:

1. Preventing the influenza import by:
✓ detection of diseased and suspected flu individuals at border crossing;
✓ limiting the trips to countries affected by influenza;
✓ informing the persons who go abroad about the epidemiological situation of the target country and about the preventive measures;
✓ a 7-days medical surveillance of people arriving in the country from countries affected by flu

2. Ensuring that employers acquire individual protection means for employees and create necessary conditions for compliance with personal hygiene.

3. Providing health care professionals with individual protection (masks, respirators, glasses, rubber gloves, alcohol napkins) and medical staff training in aetiology, epidemiology, clinical picture, diagnosis, treatment, prophylaxis and appropriate control measures for influenza infection caused by the virus circulating this year.

4. Informing the population. Local government authorities, medical

institutions, the media provide systematic information to the population upon the situation worldwide and about the preventive measures.

5. Develop prevention programs and instructions, including immunization, according to the characteristics of the seasonal epidemic and national specificity.

Anti-epidemic measures

Medical institutions, regardless of the type, inform the Supervisory Service of the Center for PublicHealth by recording the case into the electronic alert system and by completing the form no. 058/e.

Hospitalization of patients with influenza in the infectious department is carried out according to clinical and epidemiological indications.

Clinical indications include:
✓ severe forms of influenza;
✓ presence of complications;
✓ patients with mederate form of disease, but with unfavourable premorbid status (chronic lung disease, cardiovascular disease or endocrine system).

Epidemiological indications include:
✓ Patients from institutionalized collectives (orphanages, boarding schools, military units, dormitories etc.) or from families where there is no possibility for isolation or home medical care.

Antiviral drug therapy with Oseltamivir (Tamiflu) or Zanamivir (Relenza) is administrated primarily to hospitalized people or people at high risk of complications.

Emergency prophylaxis (post-exposure) with the same antiviral drugs is indicated to people in the risk group who had close contact with the patients and were subject to the exposure over the last 7 days.

The limited use of antiviral preparations in post-exposure treatment and prophylaxis is related to the possibility of the increase of the influenza virus resistance to these preparations.

People who had contact with the patient are to undergo medical surveillance for 7 days after the last exposure. Particular attention will be given to people at high risk of developing the disease (children, the elderly, people with immunodeficiency, pre-existing diseases, chronic diseases, diabetes, etc.). Influenza suspected persons are to be isolated.

Family members limit the contact with the sick person in the focus and

those who care for the patient wear protective equipment. The mask should cover the mouth and nose.

Alpha-interferon at a dose of 2-5 drops in the nose should be used 4 times a day for prophylactic purposes, throughout the risk of contamination, or 0.25% oxolin ointment in the nasal mucosa

Systematic airing of the room, surfaces and hands decontamination are to be performed.

During the emergence of the flu, all mass events are to be avoided

In case of flu emergence, medical and social institutions and staff are to be quarantined.

The suspension of all activities in education institutions for a period of up to 14 days is provided when more confirmed flu cases are recorded. The decision to suspend the work of the institution is the responsibility of the Territorial Extraordinary Anti-Epidemic Commission.

1.1.2.6. Parainfluenza

Short history

Parainfluenza virus was first identified by N. Kuroda (1952) in the city of Sendai, Japan. Initially, it was called "influenza virus D Sendai". Later, Cenok R. (1954, 1957) isolated other Sendai virus-like viruses and influenza viruses. In 1959 these viruses were called "parainfluenza".

The Pathogen

According to the contemporary classification parainfluenza virus infection refers to the genus *Paramyxovirus* belonging to the *Paramyxoviride family*. The external membrane contains two glycoproteins of parainfluenza virus, one of which possesses hemagglutinin and neuraminidase activities, while the second is a fusion protein.

There are known four types of parainfluenza viruses (1, 2, 3, 4). The first three virus types are similar to each other and cause diseases in humans. Parainfluenza virus type 3 shows a higher pathogenicity. Parainfluenza virus type 4 is not pathogenic for humans.

The antigenic assortment of parainfluenza virus is steady enough, compared to influenza virus type A. The antigen structure does not vary.

All influenza virus serotypes possess antigenic properties common to some viruses, like those of mumps and Newcastle disease, which together form the genus Paramyxovirus.

All parainfluenza virus serotypes possess some common antigenic

properties with the viruses of epidemic parotiditis and Newcastle disease, which together form the genus Paramyxovirus.

Parainfluenza viruses have a relatively low resistance to the environment and chemical desinfectants. It retains its viability for up to 4 hours at room temperature. Temperature up to 50°C kills the pathogen in 30 minutes.

The source of pathogen in parainfluenza is the sick person with typical or atypical clinical forms. As the virus affects usually the upper airways mucosa, the dissemination in the external environment occurs through exhaled air during speech, coughing and sneezing. The infectious period lasts for 7-10 days. Diseased patients are at higher epidemiological risk within the first 2-3 days of clinical symptoms.

The incubation period is from 2 to 7 days, more often 3-4 days.

Mode and transmission routes

The mode of transmission is through respiratory droplets. As the virus possesses the low resistance to the external environment, the transmission usually occurs directly from the patient to the healthy person by inhalation of liquid aerosol within crowded conditions or close relationships.

Parainfluenza factors contributing to the spread of infection are similar to those of influenza: agglomerations, cold season of the year, childhood, low resistance to infection.

Manifestations of the infectious process

Polymorphic clinical manifestations of parainfluenza may be confused with those of influenza or other acute respiratory infections. In most cases, the disease develops gradually, with mild manifestations of intoxication, headaches, sleep disturbances, chills, and moderate fever.

The incubation period lasts from 3 to 4 days with variations from 2 to 7 days. As a rule, the disease develops gradually and lasts for 3-5 days. Catarrhal phenomena are not pronounced: cough, mild rhinorrhoea, less frequently a subfebrile temperature may appear. The patient complains of chilliness, headache, and slight fatigue. Hoarseness and chest pain are caused by laryngitis (the most common symptom of parainfluenza) and laryngotracheitis. Nasal breathing is impeded. Serous nasal discharge gradually thickens and becomes mucous or mucopurulent due to a secondary infection. Children, and especially infants, develop severe laryngitis, which is often followed by clinical symptoms of laryngeal stenosis and croup. The entire respiratory tract can be involved in parainfluenza. Bronchitis

develops more severely and is followed by lesions of small bronchi (bronchiolitis) and lung parenchyma (pneumonia).

Laboratory diagnosis

Unlike influenza, toxaemia is not manifested in parainfluenzal infection. Cardiovascular and nervous lesions are absent; the larynx and the lower airways are involved. The diagnosis is checked up in the laboratory via virologic tests (isolation of the parainfluenza virus from nasopharyngeal washings), serologic (blood serum tests) and immunofluorescence.

Manifestations of the epidemic process

Parainfluenza is a ubiquitous infection, which is widely spread all around the world, especially among children population. The actual morbidity caused by parainfluenza viruses within different geographical areas is poorly known. It is considered that, parainfluenza shares about 20% in adults and 30% in children out of the overall morbidity rate caused by acute respiratory infections. The epidemic process is manifested by both rashes and sporadic illnesses. Eruptions occur predominantly in children, being local in character. The illness may occur at any time of the year, but more frequently in the cold seasons like autumn-winter. The cases of illnesses caused by parainfluenza virus type 3 occur more frequently in spring.

The natural susceptibility of the population to parainfluenza viruses is high, being higher in young children of nursery schools and primary schools. Children under 6 months of age have antibodies of maternal origin, which constitute 50-60%. Once being infected, it leads to the formation of specific but short-term immunity. However, recurrent infections evolve more easily. Parainfluenza virus type 1 causes most frequently laryngotracheobronchitis and croup syndrome in children. Serotype 2 also causes laryngotracheobronchitis, which, however, evolves more easily. Serotype 3 causes nearly 50% of bronchiolitis and pneumonia in infants, although maternal antibodies can be determined during this period.

The infection can get a wider spread among institutionalized children, students in colleges, vocational and military institutions.

Parainfluenza **epidemiological surveillance** is conducted under the epidemiological surveillance system of influenza and other acute viral respiratory infections. The main objectives can be highlighted as systematic analysis of the epidemiologic situation of parainfluenza, monitoring the circulation of the causative agents and prognosis of situation.

Preventive measures

The general preventive measures recommended for Acute Respiratory Diseases and influenza are also useful in prevention of parainfluenza. Nonspecific immunostimulatory and antiviral drugs are used in prophylaxis. Specific prophylaxis is not developed.

Antiepidemic measures for parainfluenza are identical to those mentioned in influenza.

1.1.2.7. Adenovirus Infections

Short history

For the first time adenoviruses were isolated by Row U. et al., 1953, in the tonsils and adenoids fragments obtained from the operated cases, and in 1954, R. Huebner et al. isolated adenovirus strains from patients with acute respiratory infections and atypical pneumonia with signs of conjunctivitis. Also in 1954 Enders isolated the same pathogen from the enteric product. An extended study of these viruses established the existence of a large number of serological types and a variety of clinical manifestations caused by human and animal adenoviruses. The oncogenic action of adenoviruses was determined in laboratory animals (Trentin J. et al., R. Huebner et al., 1962).

The pathogen

The causative agent belongs to the *Adenoviridae* family. There are 32 types of adenovirus isolated from man, but only types 3, 4, 7, 14, and 21 cause severe diseases. Type 8 causes keratoconjunctivitis in susceptible persons. Adenoviruses contain DNA. They are more stable to the environment than the influenza virus.

Adenoviruses are resistant to the external environment at low temperatures in particular. Temperature of 30°C retains its viability for 2 months, whereas in normal thermal conditions – for 4 months. Adenoviruses can maintain the viability for 2 years in water at a temperature of 2°C. Inactivation of adenoviruses can be carried out at a temperature above 56°C under the influence of ultraviolet rays and preparations containing chlorine, hydrogen peroxide.

The source of infection is a diseased person, who releases adenoviruses through nasal and nasopharyngeal mucus, sputum and conjunctival discharge during the first 5-6 days of the disease. Virus carriers are another source of infection. At later terms of the disease adenoviruses are eliminated

with feces. The infection is mainly transmitted through air droplets. Since the virus is steady to environment, the infection is spread via contact, food or water (bathing in swimming pools, ponds, lakes, etc.).

Ways and factors of transmission

Adenoviruses multiplication in the human body usually occurs in the cells of the upper respiratory tract. The process gradually involves the lower airways and virus elimination through the expired air. The main mode of transmission is the respiratory one, which is carried out by inhaling contaminated air from a sick person, especially during coughing or sneezing.

However, replication of the virus can occur in the intestinal tract and conjunctival tissues, thus a possible fecal-oral transmission mechanism might interfere in catching the virus from contaminated hands or objects.

Manifestations of epidemic process

Infants aged from 6 months to 3 years are usually infected. Type-specific immunity is produced in convalescents. Adenovirus infections occur as sporadic cases and epidemic outbreaks in children's institutions. The morbidity rate is higher in

autumn and winter. Since the incubation period lasts from 3 to 12 days, the outbreaks of adenoviral infection last longer than those of influenza. Adenoviruses mostly affect the respiratory mucosa, and less frequently the conjunctiva. They can multiply in the intestinal mucosa as well. The lymphoid tissue of the regional lymph nodes is damaged, the vegetative nervous and endocrine systems are also affected by subsequent vascular disorders (pallor, tachycardia).

Manifestation of infectious process

The incubation period lasts from 3 to 12 days, most frequently from 5 to 6 days. Acute adenoviral infection is characterized by the following clinical symptoms: rhinopharyngitis, rhinopharyngotonsillitis, rhinopharyngobronchitis, pharyngoconjunctival fever, membranous or follicular conjunctivitis, and pneumonia. Rhinopharyngotonsillitis and rhinopharyngoconjunctivitis are most common ones. The incubation period lasts 5-6 days. The disease onset is usually gradual (2-3 days). The overall symptoms are marked: malaise, chills, fever, headache. Local symptoms develop at early stages: stuffy nose, hyperaemia of the fauces and the posterior wall of the pharynx, difficult swallowing, cough (dry or with expectorated sputum) and chest pain. Some patients complain of

abdominal pain, intestinal disorders, and sometimes hepatic enlargement may occur. Fever lasts from 2 to 7 days. Malaise and other general symptoms like catarrh symptoms can persist for more than 1-2 days. Acute rhinopharyngoconjunctivitis is characterized by a moderate impairment of the general health condition, inflammation of the respiratory mucosa, fauces and eyes (rhinitis, tonsillitis, pharyngitis, nasopharyngitis, laryngitis, tracheitis, bronchitis, conjunctivitis). The internal organs can be involved separately or in various combinations. The respiratory mucosa and the mucosa of the eyes can be involved simultaneously, but sometimes only pharyngitis or only conjunctivitis may develop. Conjunctivitis lasts from several days to 2 weeks and longer. The eyelids, mucosa of the eyeballs and conjunctiva can be affected with oedema and gentle granularity (catarrhal or follicular conjunctivitis). Membranous conjunctivitis is also possible. The eye secretion is meagre and serous in character. The cornea and the iris are usually not affected. Rare symptoms as nosebleeding, nausea, vomiting and diarrhoea may appear. Additionally,to the mentioned above symptoms, small round foci of corneal opacity develop in several days (to 2 weeks) after the onset of keratoconjunctivitis. The foci sometimes fuse together. The disease lasts 2-4 weeks and usually ends in a complete recovery. Pneumonia is the most severe form of adenoviral infection. It usually affects infants under 1 year of age. Pneumonia can occur along with other forms of adenoviral infection. Focal pneumonia is usually present (bronchopneumonia). The body temperature can remain high for 1-2 weeks and longer. Dyspnoea, cyanosis, and symptoms of toxaemia may develop.

Laboratory diagnosis. Adenoviral infection is characterized by pronounced exudation; toxaemia is absent; conjunctivitis, especially a membranous one, is typical of infection. An accurate diagnosis can be established only in laboratory conditions: virologically (isolation of the adenovirus in tissue culture), serologically, and by immunofluorescent method.

Epidemiological surveillance

The epidemiological surveillance is associated with influenza epidemiological surveillance system. It consist of the analysis the morbidity rate of various forms of adenovirusis, monitoring the circulating adenovirus serotypes, and assessment of epidemic prevention measures.

Prevention and control. Patients with severe course of the disease

should be hospitalized. Other patients should be isolated in home conditions untill they recover completely. Chlorination of water in swimming pools is used to prevent outbreaks of the infection. For other preventive measures see „Influenza".

1.1.2.8. Mumps

Short history

The disease is described for the first time in the 5th century BC. by Hippocrates, who claimed its contagious character, and later in the 1st BC- 1st AD century, Celsius recorded its main clinical and epidemiological manifestations. In the eighteenth century, Hamilton revealed the presence of the orchite in the evolution of the disease, in 1853 Bouchet affirmed the bilateral character of the parotid gland lesions (hence the name mumps), and Trousseau highlighted its epidemic character (epidemic mumps). In 1934-1935 Johnson and Goodpasture replicated the disease in monkeys. In 1945, Habel managed to grow the virus in a chick embryo, which subsequently allowed to obtain live attenuated mumps vaccine.

The pathogen

The causative agent of mumps belongs to the family of *Paramyxoviridae.* Paramyxoviruses are characterized by high contagiosity and low stability in the environment. The virus resistance in the external environment is reduced. It loses its infectious property at room temperature within 30 minutes, whilst at lower temperatures in 3-4 hours. However, it remains long term resistant at temperature minus - 20-70°C and can be easily preserved, although the freeze-thawing variations easily destroy it. It is rapidly destroyed under the action of ether, oxidizing substances, organic iodine derivatives, and ultraviolet rays.

The source of infection

Humans are the source of infection. The patient is contagious from the last days of the incubation period and remains sick for 7-9 days. Patients with an obliterated form of the disease are at higher risk. The infection is transmitted mainly via air-borne route. Susceptibility to mumps is high, but lower than in measles, influenza, or chickenpox. Children aged from 5 to 15 are especially susceptible. Outbreaks of the disease among adults are also possible. Stable immunity is produced in those who had the disease. Seasonal variations in the infection rate are characteristic of mumps: the morbidity is higher during the cold season. Epidemics of mumps occur at 4-5 year intervals.

Mode and routes of transmission

Although mumps virus is eliminated from the host only through the droplets of saliva and not from secretions of the upper airways, the main mode of virus transmission from the source to receptive people is still the respiratory inhalation of contaminated air with liquid aerosols, consisting of saliva droplets, which contain viruses transmitted during patient's speech, coughing or sneezing. Since liquid aerosols formed from the droplets of saliva, which have a low dispersion, do not spread at great distances and settle down quickly, a closer and prolonged contact with the patient is required to have the mumps transmitted. Infectiousness index is about 40%. The transmission of the virus to surrounding people may occur via objects contaminated with the patient's saliva, dishes and also through kissing. There is a risk of transplacental transmission of the virus to the fetus in pregnant women diseased with mumps.

Manifestations of the epidemic process

Mumps spreads unevenly everywhere across countries and population groups, revealing a multiannual dynamics, which largely determines the level of vaccination coverage, at present.

The implementation of mass immunization shows an essential decrease in morbidity of its multiannual dynamics *(Figure 23).*

The annual incidence of mumps during the pre-vaccination period increased to an average of 226.2 cases per 100,000 population, with an average about 7,650 cases per year, showing an increasing trend. The implementation of vaccination within the national vaccination program in 1983 led a decrease in morbidity by 10-11 times.

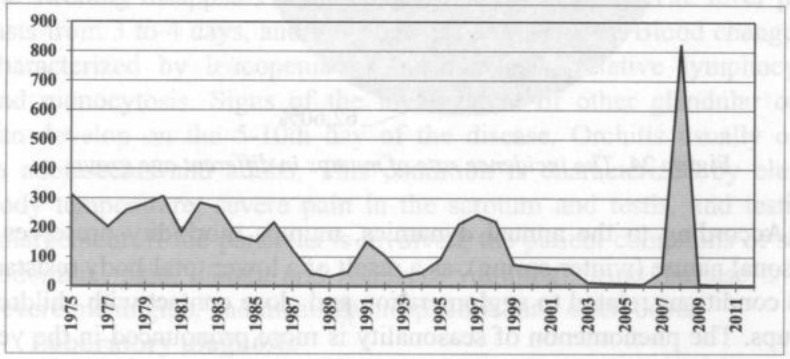

Figure 23. *The dynamics of mumps morbidity in the Republic of Moldova, in 1975-2012.*

Although mass vaccination maintain the cyclic epidemic process, frequency of incidence increased from 2-3 to 8-10 years, being an average 4-5 yearse. Under current circumstances, the major incidence might occur due to the inadequate immunization coverage of the risk-bearing groups.

An example of this may be the epidemic increase of mumps morbidity in the Republic of Moldova in 2007, which is one of the most significant epidemic increases. Within only eight months (October 2007 - May 2008), 30,192 cases were recorded, while the intensive index constituted $745,3^{0}/_{0000}$, affecting mainly children over 14 years and the youth from communities (students, military etc.) *(Figure 24)*. That was mainly due to the deficiencies in re-vaccination of the risk groups.

Following the vaccination and revaccination campaigns for the risk groups, the epidemic has decreased sharply, whereas morbidity is still decreasing, thus it can be assumed that the inter-epidemic period will last up to10-11 years.

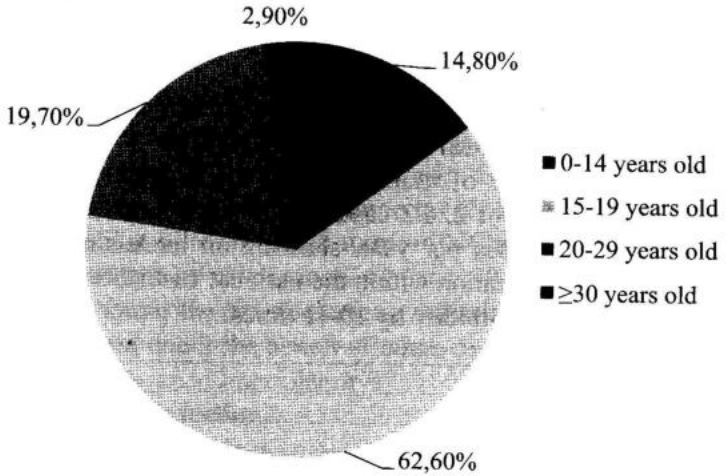

Figure 24. *The incidence rate of mumps in different age group.*

According to the annual dynamics, mumps morbidity preserves its seasonal nature (winter-spring), as a result of a lower total-body resistance and conditions related to agglomeration and close contact with children's groups. The phenomenon of seasonality is more pronounced in the years with epidemic ascents. About 70% of all illnesses are recorded in January-May *(Figure 25)*.

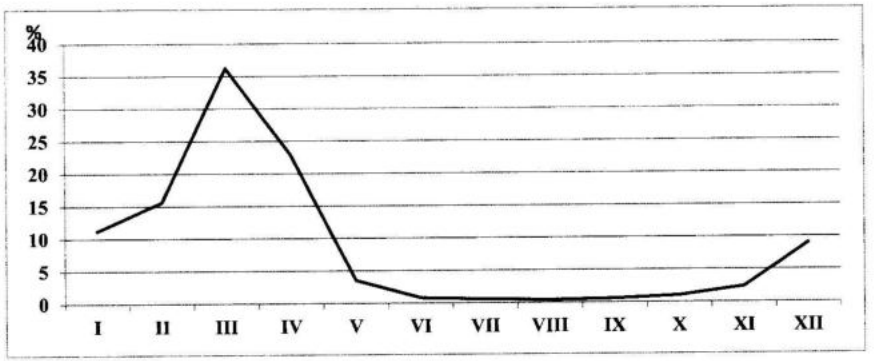

Figure 25. *The annual dynamics of mumps morbidity during 2003-2012 in the Republic of Moldova.*

The manifestation of epidemic process

The incubation period lasts from 15 to 19 days, with variations from 11 to 23 days. The disease begins with a prodromal period that lasts 12-36 hours. The body temperature then rapidly increases to 38-40°C and signs of involvement of the salivary glands become apparent: the gland is swollen and bulges out in front of the the ear, then it extends posteriorly and inferiorly; it is soft to touch; the pain is intensified during chewing, swallowing and talking. Salivation discontinues on the involved side. The parotid gland of the other side becomes involved in 1-2 days of the disease. The submandibular and, less frequently, sublingual glands can also be involved. Salivary adenitis relieves in 3-5 days of the disease and the swelling disappears completely by the 8-10th day. The fever period lasts from 3 to 4 days, and in severe cases - 6-7 days. Blood changes are characterized by leucopenia or normocytosis, relative lymphocytosis and monocytosis. Signs of the involvement of other glandular organs can develop on the 5-10th day of the disease. Orchitis usually occurs in adolescents and adults. This condition is characterized by elevated body temperature, severe pain in the scrotum and testis, and testicular enlargement. If the pancreas is involved, the patient complains of severe abdominal pain, nausea and recurrent vomiting; urine diastase is high. Severe meningitis, and meningoencephalitis may often occur.

Laboratory diagnosis

Typical cases are easy to diagnose. Atypical disease should be differentiated according to epidemiologic anamnestic data. Serologic

tests (complement fixation test, direct haemagglutination reaction) can be performed.

Epidemiological surveillance

WHO refers mumps to eradicable infections via active immunization of the population. It is crucially important to inform urgently about each suspected case of mumps. The main elements of epidemiological surveillance in mumps consist of monitoring the morbidity dynamics in different aged groups; vaccination coverage of all risk groups (according to WHO criteria, a favorable epidemiological status in mumps can be maintained at 95% vaccination coverage of children aged up to one year and revaccination of children aged 6-7 years); determining the immunestatus population by specifying it at different aged groups (a risk of epidemics may occur if the vaccination coverage of the target ages is under 92% and the immune status is below 85%); determining the effectiveness of vaccination.

Control measures

Prophylacic measures

General prevention includes avoiding people crowding, following an anti-epidemic regime in children collectives (detection and isolation of suspected cases, prophylactic disinfection, health education, etc.), protection of women during pregnancy. Prophylactic measures should be intensified during the cold season.

However, the main method of protection in mumps is considered vaccination. The vaccine against mumps is a live attenuated vaccine,which is very effective and harmless. It can be administered as monovaccine or in combination with other vaccines. Since 2002, the monovalent vaccination against mumps has been replaced with the combined vaccine against measles, mumps and rubella (MMR). In Moldova, the vaccine is given to children in the following order: aged 12 months are given primary vaccination (ROR_1), at the age of 6-7 – they are given revaccination (ROR_2), and since 2011 a repeated revaccination (ROR_3) has be introduced for adolescents aged 15. The vaccine is injected subcutaneously in the upper arm, the dose being of 0.5 ml. Children who have got one of the three infections (measles, rubella or mumps) before the vaccination age or have been vaccinated against one or two of those three infections are to be subjected to vaccination with combined MMR vaccine in 6 months after their recovery or the vaccination with monovaccine. Children with

missing data about measles, mumps and rubella vaccination are subjected to combined MMR vaccine.

Anti-epidemic measures

In case of detecting suspected cases of mumps,medical institution should inform the Center for Public Health by recording the case in the electronic alert system and completing form no. 058/e. The patient is subjected to isolation in home conditions or hospital. Hospitalization of patients is performed according to clinical and epidemiological indications. The isolation of patient is carried out within a period of 9-10 days from the illness onset or until the clinical symptoms disappear.

Surveillance of the contact persons is carried out for 21 days from the last contact with the patient.

Immediate prophylaxis with human immunoglobulin (or specific mumps immunoglobulin) at a dose of 3.0 ml is recommended for children under 12 months of age.

The following categories of persons are vaccinated or revaccinated for the purpose of prophylaxis, and within 7 days from the detection of sick:

- persons who had no mumps in the past or who had not been vaccinated;
- persons who ha not mumps, but were vaccinated with a single dose of vaccine, and after the expiration of six months since the vaccination;
- persons with unknown disease or vaccination history.

Persons who had the contact with the mumps patient but who did not develop the infection or unvaccinated persons are not admitted to children's institutions for 21 days after the last contact with the patient. The current disinfection is carried out in the focus.

Wet cleaning and ventilation of the room are performed and personal hygiene is respected. A person who cares for the sick is necessary to wear a mask.

1.1.2.9. Measles

Short history

Measles was known due to its high contagiousness and morbidity in ancient civilizations, being mistaken for smallpox and other exanthematous diseases. The first description of measles as a nozological entity is attributed to a Persian physician Rhazes in the 10th century, who in his turn cited

the authors of the 7th century AD. Even in the Middle Ages, measles was mistaken for smallpox. A clear distinction was made by John Hall, who first described measles in America, reporting about the Boston measles epidemic in 1657. The pathognomonic enanthema of measles was first described by Koplik in 1896, whilst Panum's extraordinary investigations and report about the epidemic of measles in the Faroe Islands identified the incubation period and the lifelong postinfectious immunity. The measles virus was isolated by Y. T. Enders and Peebles in 1954.

The pathogenic agent

The pathogenic agent of measles is *Morbillivirus morbillorum* of the paramyxoviridae family, the Morbillivirus genus. It contains RNA. This genus also includes canine and chickenpox viruses. The measles virus is a helical nucleocapsid with a lipoprotein coating. The particle diameter ranges from 100 to 250 nm (150 nm on average).

Measles virus is very unstable outside. It is rapidly inactivated by heat, ultraviolet rays, lipid solvents and extreme acidity and alkalinity (pH under 5 and over 10). Its instability at room temperature is of major importance during the vaccination period.

The source of the pathogen is an infected person. Measles is contagious from the early prodromal period (3-4 days until an exanthema appears) and during the first 4-5 days of measles rash (in a total of 9-10 days). The day when signs of first exanthema (usually on the face) appears in the affected person, is considered the fourth day of incubation.

The mechanism, routes and factors of transmission

Human-to-human measles pathogen transmission occurs exclusively via wet aerosols. The measles virus is removed from the mucous membranes of the patient's upper airways in the form of a high dispersed aerosol that is produced during coughing or sneezing and spreads into the surrounding environment of the patient, and still remains viable in a suspended state for up to 30 minutes. Children are infected directly from the patient. The virus is not transmitted by a third person. Contamination of environmental objects are also not epidemiologically relevant because of the minor resistance of measles virus in the external environment.

Manifestations of epidemic process

Measles is spread ubiquitously on all the continents and in all climatic zones of the globe. The receptivity is general and practically all people are susceptible to it, but mainly children get sick. In countries where

mass vaccination is not carried out, 85% of cases of measles occur in children under the age of 7 years. In the Republic of Moldova, during the prevaccinal period, the intensity of the measles epidemic process exceedes the index of 1000 per 100,000 population. After the implementation of measles vaccination, the morbidity gradually decreases. No indigenous measles case has been recorded in recent years *(Figure 26)*.

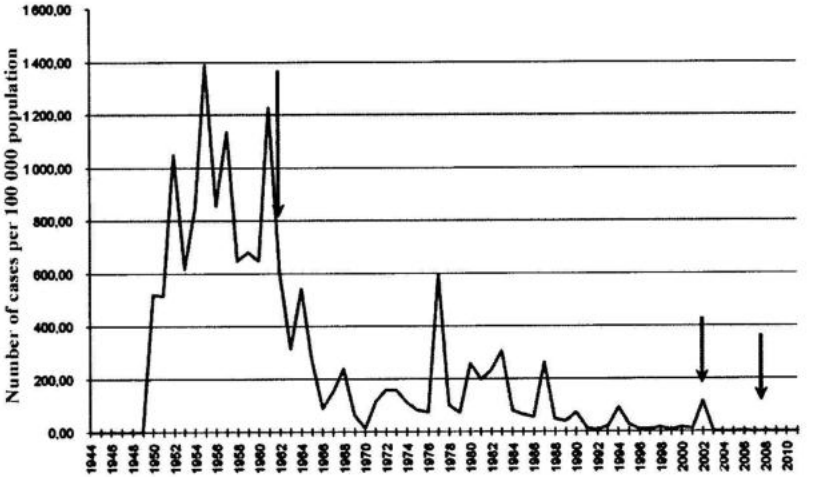

Figure 26. *The measles incidence in the Republic of Moldova (1944-2011).*

The immunity is sustainable during lifetime, therefore adults rarely get sick after they had incurred measles.

In non-immunized populations, probably, more than 90% of people aged 20 years fall ill with measles. Worldwide implementation of specific prevention has significantly changed some of the fundamental features in the current evolution of the epidemic process in various countries and is, in fact, directly related to the level of vaccination coverage in these regions.

Another characteristic of the measles epidemic process is its periodicity, i.e. the periodic increase and decrease of the morbidity intensity every 2-3 years, being dependent upon the number of susceptible individuals in the target population *(Figure 26)*. This increase and decrease in the number of cases is also observed during the year (seasonality) when the transmission mechanism is activated (in winter, spring).

Currently, the measles epidemic intensity and other characteristics of this process are determined by the level of vaccination coverage in many countries, i.e. data about the immune status of the population.

The manifestations of infectious process

The incubation period lasts from 8 to 17 days. The onset is gradual: the body temperature increases to 38.5°C, the patient complains of weakness, moderate headache, myalgia and arthralgia. The symptoms of upper airways catarrh are mild, hyperaemia of the fauces is insignificant, conjunctivitis is moderate, the lymph nodes (suboccipital, postauricular, and the nodes of the posterior neck) are enlarged. Small maculopapular lesions appear on the face and neck during the first or second day of the disease. In a few hours the whole body is involved and a dense rash covers the volar surfaces of the limbs, the back, and the buttocks. The rash persists for 2-3 days and then fades out without scaling or leaving any pigmentation. Haematologic changes: leucopenia (3-4 x 109/1), relative lymphocytosis, increased quantity of plasma cells. Complications are rare: arthritis, encephalitis, myelitis, nephritis.

Subacute sclerosing panencephalitis (SSPE) can occur in children aged over 4-10 years after they suffered measles. SSPE is a degenerative neurological disease of children and adolescents, caused by persistent measles virus in the central nervous system tissue.

Laboratory diagnosis

The diagnosis is based on clinical, epidemiologic and laboratory investigations (blood tests). The people to be examined are the sick, suspects, convalescents, and contact persons in the focus.

Prevention and control. The patient should be isolated for 5 days after rash develops. Children who contacted the patient may remain in groups. Pregnant women, who had no rubella past medical history, should be protected from contacts with the diseased ones. If a pregnant woman has measles in her past history, or had contacts with a diseased person, she is administered immunoglobulin (10-30 ml) to avoid the infection of the foetus. Live attenuated vaccine is used in prophylaxis. The schedule of vaccination is:

- at the age of 12 months (MMRR-1);
- at the age of 6-7 - the first revaccination (MMR-2);
- at the age of 14-15 - the second revaccination (MMR-3).

MMR vaccine is administered subcutaneously in the upper arm at a dose

of 0.5 ml in both vaccination and revaccination. Possible side effects, and undesirable adverse reactions to MMR vaccine are rare. Seroconversion against measles in vaccinated individuals reaches 95%.

Anti-epidemic measures

Registration and information to the Center for Public Health about the suspected or confirmed cases of measles are performed simultaneously on the same day. At the same time, anti-epidemic measures aiming to localize and liquidate the outbreak are performed. The measles patient (confirmed or suspected case) is hospitalized compulsorily in the infectious diseases unit, preferably in a specially designed box. The isolation should last about 4 days from the appearance of exanthema, in case of complications - 10 days.

The measles patient's room is going to be well ventilated and wet cleaned. As the virus does not persist for more than 30 minutes, terminal disinfection is not indicated in the external environment.

The susceptibility of measles contacts is determined. Contact persons are under medical supervision from the 8th day until the 17th day from the moment of contact with the patient. The temperature is measured daily, as well as the skin and buccal mucous membranes examined. Non-immune children are vaccinated during the first 3 days of contact. In case of contraindications, specific immunoglobulin (3 ml for children aged from 3 months to 1 year and 1.5 ml for children aged 1-7 years) is administered.

1.1.2.10. Rubella

Short history

Rubella was recognized only in the middle of the eighteenth century by German physicians, who called it „redness". Manton described the disease as a separate clinical entity in 1815. Later, rubella was considered a mild disease, which rarely produces complications. This view persisted until 1941, when Australian ophthalmologist Norman Gregg revealed the association between intrauterine infection and the presence of cataracts and cardiac defect in newborns. The rationale appeared only after 1962, when the virus was isolated and identified in cell culture.

The pathogenic agent

Rubella virus belongs to the Togaviridae family and is the only representative of the Rubivirus genus. So far, only one distinct immunological type has been described and there are no common serological relationships between rubella virus and other viruses. The virus

is sensitive to heat and chemical action. At 100°C it loses its infectivity in 2 minutes. It is also inactivated in environments with pH levels below 6.8 and above 8.1, under the action of ultraviolet rays, lipid solvents, formalin, and proteolytic enzymes. In the presence of 2% albumin virus viability is maintained for a week and more. Infectivity is rapidly lost during the storage at temperatures between -10 and -20°C.

The only source of infection is a measles patient, who becomes contagious during the last one or two days of the incubation period, during the catarrhal period, and during 7 days following the development of rash. The period of contagiosity can lasts up to 10 days from the day when rash develops, in the presence of complications. The infection is transmitted via air-borne route. The virus can be carried by air droplets to adjacent rooms and flats within a house. All persons who had no rubella or were not vaccinated against rubella are susceptible to this disease.

The mechanism (mode), routes and factors of transmission

Rubella virus is spread by tiny air droplets from the patient's nasopharyngeal secretions, which form wet aerosols with a high level of dispersion. It is also eliminated with the patient's urine and feces. Transplacental transmission from mother to fetus can occur if the mother bears rubella virus during the first three months of pregnancy, thus causing very serious consequences in newborns. The newborn is a source of infection for a long period of time. Infection may also occur when contacting fresh contaminated items with the nose and pharynx, and possibly by blood, urine or feces.

Manifestations of epidemic process

Rubella is widespread on all continents. In most world countries, planned vaccination of the population is carried out for a time period and, as a result, rubella morbidity has decreased significantly, while rare and isolated cases have been reported. In countries where planned vaccinations are not carried out, and in our case during the pre-clinical period, mainy children aged from 1 to 7 get sick *(Figure 27)*.

Caeses of rubella are recorded also in adolescents and adults, including pregnant women. Epidemics and eruptions are reported in nurseries, kindergartens, schools, colleges and barracks. Seasonality occurs in winter-spring.

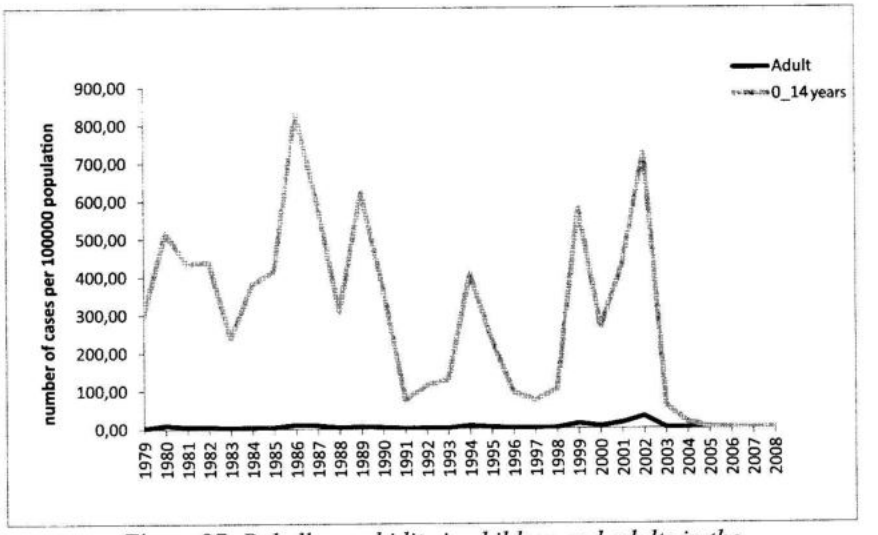

Figure 27. *Rubella morbidity in children and adults in the Republic of Moldova 1980-2008.*

Manifestations of infectious process

The incubation period is 7-24 days, on an average of 16-18 days. The measles onset is insidious. Children may or may not have minor signs of illness, whereas in adults the prodromal period (1-5 days) is accompanied by weakness, headache and moderate nasopharyngitis, mild fever and conjunctivitis. Lymphadenopathy of auricular, suboccipital or cervical posterior lymph nodes is common but not pathognomonic. Rarely lymphadenopathy is generalized. Rash or maculopapular rashes, sometimes accompanied by subfebrile temperature appear at the onset of the disease or a few hours afterwards, but rarely at the end of the first day or the next day. In the 20 - 50% of cases, there are no obvious eruptions. In the first trimester of pregnancy rubella results in spontaneous miscariages, stillbirths or various congenital anomalies (cataract, cardiac defects, deafness, hepatitis, microcephaly). Congenital rubella syndrome is detected in more than 20-25% of children, born from mothers who had rubella during the first trimester of pregnancy, with a gradual reduction of risk rate in the following months of pregnancy.

Laboratory diagnosis

Laboratory investigation methods are identical to those of measles.

Epidemiological surveillance

General and preventive measures to fight off the epidemic of rubella do not show a desired effect. Isolation of children in preschool institutions and schools does not cease the epidemic. Terminal disinfection in outbreaks does not make sense, because of the minimum resistance of the pathogen in the external environment. At present, the strategic epidemiological surveillance and control of rubella, are determined by the the rigorous organization and conducting of planned immunization against rubella according to the National Immunization Program with MMR vaccine (see Measles, prophylaxis) *(Figure 28).*

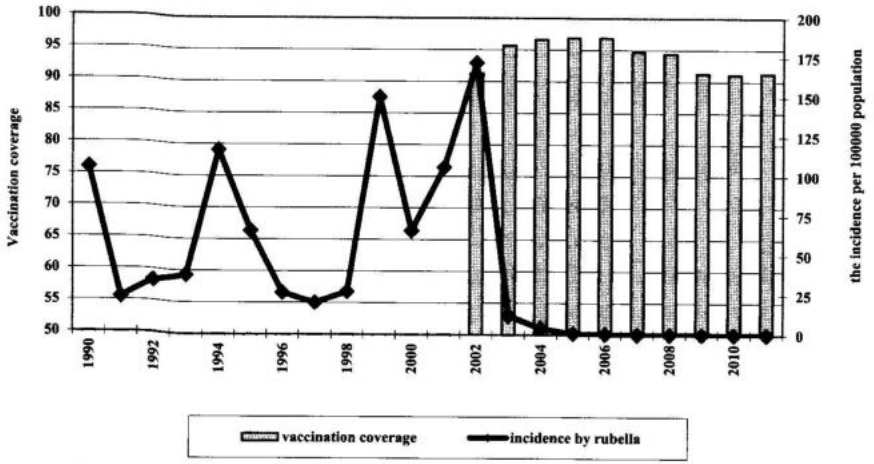

Figure 28. *Dynamics of rubella morbidity and vaccination coverage in the Republic of Moldova (1990-2011).*

Anti-epidemic measures

The Center for Public Health must be informed about suspected cases of rubella. The patient is hospitalized into the infectious diseases unit. Convalescence children are admitted to the kindergarten after their recovery, but not earlier than 7 days after the onset of rash. The patients are isolated in a separate room or behind the screen at home. The unvaccinated people are identified. Special attention is given to pregnant women in the first trimester of pregnancy. They are to be subjected to serological tests and examined by an obstetrician, to make a decision on the issue.

1.1.3. Blood-borne infections

1.1.3.1. Hepatitis B

Short history

The concept of „human serum jaundice hepatitis „ first appeared in 1938 after investigations during a large epidemic of jaundice-like hepatitis, with a total of 28585 cases in soldiers of the US Army during the Second World War in the Pacific, as a result of immunization campaign against yellow fever. Clinical and epidemiological observations (the incubation period, the route of transmission of viruses into the body) led to delimitation of two entities, „serum" hepatitis and „epidemic" hepatitis (hepatitis A). The existence of two viruses of jaundice-like hepatitis was demonstrated by Saul Krugman (1956) and confirmed by Boggs (1970).

In 1963, Baruch Samuel Blumberg discovered a new antigen in the blood of an Australian aborigine,and called it the Australian antigen (Australia) (HBsAg), later determined as a marker of „serum"hepatitis. He was awarded the Nobel Prize for his discovery in 1976.

The term of *Hepatitis B* was introduced by MacCallum in 1947 and adopted by the WHO in 1973, thus replacing the notion of „serum hepatitis".

The pathogenic agent

Hepatitis B virus (HBV) belongs to the Hepadnaviridae family, like Orthohepadnavirus. HBV is a a 42-45nm spherical corpuscle, consisting of an outer coating (envelope) and the central part („core"), forming the virion nucleocapsid.

The viral envelope is of glycoprotein-like nature and contains HBs surface proteins (HBsAg surface antigen, HBsAg):

a) low protein S (S ABS) contains only the surface S;

b) middle protein (M HBs) contains S and pre-S2 domains. Domain S2 promotes the attachment of the virion to the cell and has a high immunogenic epitope;

c) large protein L (L HBsAg) contains the S, pre-S1 and pre-S2 domains. M protein is responsible for recognition receptor, that contributes to efficient attachment to superficial receptors of the host cell.

There are four main subtypes of HBsAg: adw, adr, ayw, ayr. The common antigenic determinant when infecting a subtype, therefore forms cross-immunity in the contamination against other subtypes.

Determination of subtype and genotype is used as an epidemiological

marker in establishing the regional belonging of the hepatitis B virus,and assessing the efficacy of antiviral therapy, as well as obtaining vaccine preparations.

The virus is distinguished by high resistance to physical and chemical factors: low and high temperatures, freezing and thawing, multiple drying, long-acting acid environment. HBV retains their viability in the serum at room temperature for 6 months, at 20°C - for 15-20 years, in lyophilized plasma - up to 25, at a temperature of 60°C, it lasts 10 hours. It is inactivated by autoclaving (120°C/45 min.), in dry heat sterilization (+ 180°C/60 min.), at boiling - during 30 min. The hepatitis B virus is destroyed within 1-2 hours under the action 1-2% of chloramine, in 0.5-1.0% sodium hypochlorite - during 30 min and in 70% alcohol at + 11°C temperature during one minute.

The source of pathogen in viral hepatitis B is the sick person,with various forms (manifested, asymptomatic) of acute and chronic hepatitis B, as well as Ag HBs carriers. The contagious source is determined by the activity of the pathological process in the liver and by the concentration of the antigen in the blood. The virus appears in the blood, hepatocytes, sperm, vaginal secretion, cerebrospinal fluid, synovial fluid, mother's milk, saliva and urine for 1.5-2 months before the clinical disease occurs, and persists for more than 2-8 weeks.

The incubation period is 60-180 days.

On average, the period of infectiousness of acute HBV is of 69-95 days (15-60 days - the incubation period; 10-14 days - prodromal period; and 14-21 days - the clinical manifestation/jaundice). The HBsAg is detected in approximately 50% of convalescent patients, usually in low concentrations. Virus persistence for more than 3 months from the onset of the disease denotes chronic infection or an antigen-carrier status. Asymptomatic HBsAg carriers and asymptomatic patients with chronic hepatitis B virus are the main sources of infection and are a particular hazard if HBeAg is simultaneously detected in the blood. The viral replication in the blood of carriers is increased and HBsAg carriage lasts many years which is quite common in the human population, ranging from 3% to 20% of the population.

Factors and transmission routes

The main mechanism of transmission is a parenteral one, through poorly sterilized medical instruments (dental, surgical, gynecological, urological,

endoscopic, bronchoscopic, acupuncture etc.), transfusion of blood and its derivatives (plasma, erythrocyte mass, platelet leukocyte, fibrinogen, thrombin, antihemolitic factors etc.). The risk of post-transfusion hepatitis is proportional to the number of transfused units or the number of donors used for the preparation of a derivative product unit. It is worth mentioning that the frequency of post-transfusion hepatitis has decreased considerably after the mandatory bacteriological control of donated blood for the detection of HbsAg.

HBV is also transmitted via intravenous drug injection (repeated or collective use of needles and syringes); by sharing shaving instruments, toothbrushes, manicure items, pedicures, etc.; via sexual intercourse.

Sexual transmission accounts for about 30% of HBV contamination. The probability of sexual contamination is directly related to the sexual behavior of the person, the number of sexual partners, but also to the activity of the infectious process and the concentration of the virus in the blood.

In viral hepatitis B the vertical transmission (transplacental) of the virus from mother to child is common. Transplacental transmission from pregnant women infected with HBsAg can reach 5-10% of cases in the first trimesters of pregnancy. Transmission of infection may occur in up to 85-90% of children in the prenatal period, in whom viremia is confirmed after 2 months of delivery and after 2-3 months of their life. These children may remain asymptomatic with subsequent risk of developing acute or chronic hepatitis.

Although hepatitis B virus is present in body fluids (semen, vaginal secretions, cerebrospinal fluid, breast milk, saliva, bile, urine), its concentration in secretions is 100-1000 times lower than in blood. Therefore, the contamination is very rare, while the absolute majority of HVB contamination occurs via blood.

According to the estimates, the amount of infected material sufficient to produce HBV infection may be 0.00005 ml, and the mean volume of blood inoculated during a needle prick is about 0.0001 ml and can hold up to 100 doses of HBV infection. A high risk of HBV contamination is possible by performing a tattoo and cosmetic procedures accompanied by skin damage.

Manifestations of epidemic process

Currently, hepatitis B is a global public health problem. Being a typical anthroponosis, the infection is spread everywhere. Approximately 2 billion

people worldwide show markers of hepatitis B virus, of which 350 million are chronic carriers of HBV and 100 million surffer from liver cirrhosis and primary liver cancer.

The intensity of human hepatitis B infection varies in various regions of the world.

According to the carriage index of HBsAg in healthy population, the territories are classified into 3 areas of endemicity:

• area of low endemicity - up to 2% (Australia, North America, Northern Europe, Central and Western);

• endemic area - 2-7% (South and East, Central and South America, Middle East);

• area with high endemicity - more than 7% (Southeast Asia, North Africa and Equatorial Africa). In South China, Taiwan and Tropical Africa HBsAg carraiage is 20-50%.

In the Republic of Moldova the intensity of hepatitis B infection is 13% on average and varies in different areas: North - 3-4%, Center - 7-8%, South - 15-20%. Viral hepatitis B was first recorded as a nosological form in our country in 1966. By 1987, the morbidity dynamics was characterized by a growing tendency and a periodic cyclicity of 6-7 years.

The selective vaccination of newborns from HBV-carrying mothers in 1989, the implementation of total immunization of newborns in 1995, groups at high risk of infection contamination, and application of disposable medical supplies in parenteral procedures decreased the incidence of HVB morbidity in the Republic of Moldova from 76.60 / 0000 in 1987 to 1.60/0000 in 2012 *(Figure 29)*, which proves the efficacy of the prophylactic measures in fighting off infectious diseases.

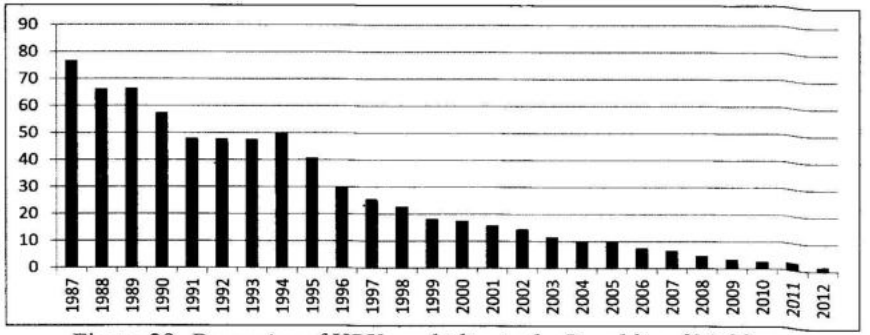

Figure 29. *Dynamics of HBV morbidity in the Republic of Moldova, in 1970-2012.*

A dramatic decrease of HBV morbidity occurred in children. Compared to the pre-vaccination period, the morbidity was reduced more than 1,000 times in children aged 0-14 years, being recorded at a sporadical level over the past decade *(Figure 30)*.

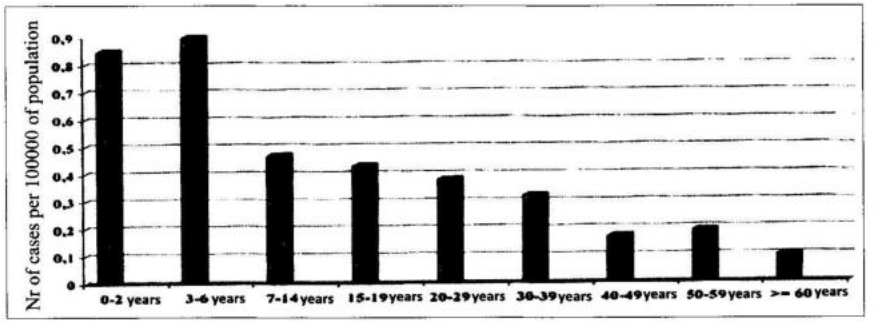

Figure 30. *The incidence of hepatitis B virus in different age groups in the Republic of Moldova, 1990-1992 (P. Iarovoi, 2012).*

Since this phenomenon has significantly decreased the HBV incidence in children, it currently has led to a ratio change of the age groups in general HVB morbidity rate *(Figure 31)*.

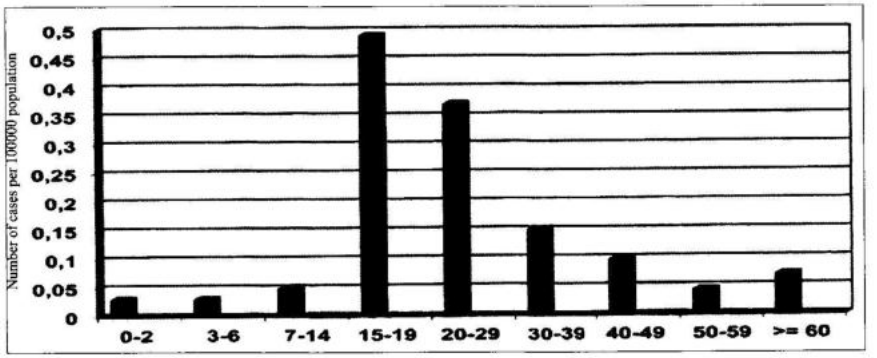

Figure 31. *The HBV incidence rate according to the age group in postvaccinal period, 2002-2004 (P. Iarovoi, 2012).*

During the pre-vaccination period the morbidity rate in children was 30.6%, whereas in adolescents and adults - 69.4%. During the vaccination period the total number of infected children was reduced to 5%, while that of adolescents and adults increased up to 95%. The young persons, aged

15-29, forms the most affected group due to the spread of injecting drug addiction, on the one hand, and risky sexual behavior on the other hand.

The incidence of acute HBV is about 2 times higher in the urban population compared to the rural population.

However, HBV morbidity indices in Moldova much exceeds the recorded indices of most European countries.

At the same time, due to a decrease in the number of acute HVB cases, there is an increase in morbidity of chronic viral hepatitis *(Figure 32)* and, consequently, an increase in the morbidity rate of hepatic cirrhosis and primary hepatic cancer, which is explained by a high incidence of viral hepatitis B in the past and by a high level of HBsAg carriage status at present.

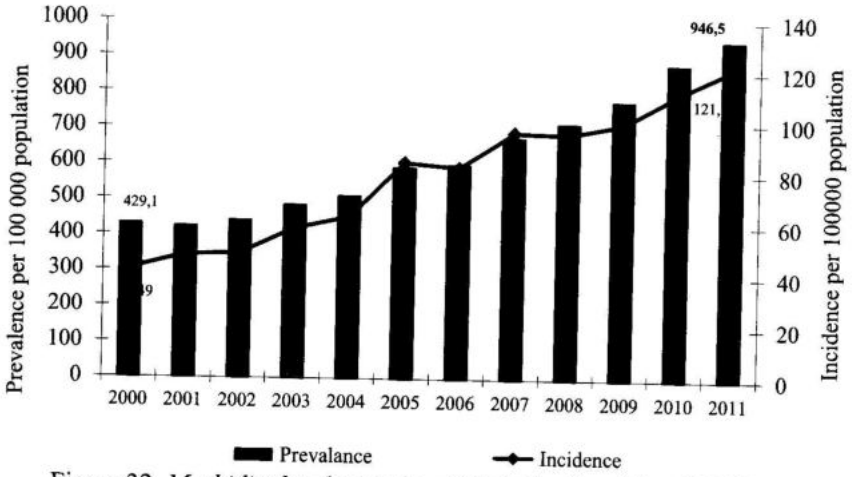

Figure 32. *Morbidity by chronic hepatitis in the Republic of Moldova, in 2000-2011.*

Post-infection immunity is sustainable and is produced in 85-90% of people who have contracted HBV. Seasonality is not characteristic of hepatitis B.

The manifestations of infectious process

Hepatitis B is characterized by two major forms: asymptomatic and symptomatic.

HBV infection can be asymptomatic or subclinical. High levels of liver aminotransferases is reveald in the blood in subclinical infection, which indicates a liver disease, but no symptoms and jaundice are

present. Symptomatic or biochemical abnormalities are not detected in the inapparent infection. Inapparent infections are identified via serological studies.

Symptomatic Hepatitis (manifested by jaundice) basically repeats the same stages of HAV development.

Preicteric period (prodromal), which lasts from 1 to 4 weeks with an average of 1-2 weeks, is characterized by slow onset, loss of appetite, nausea, vomiting 1-2 times per day, low pain in the hypochondrium or in the epigastric region (dyspeptic syndrome), fatigue, headache, general malaise (asthenic syndrome), pain in the joints without functional changes and organic syndrome (arthralgia). One of the early signs of the disease is discolouration of the urine that becomes brownish-gray and grey-colored feces 1-2 days before jaundice.

The icteric period begins with jaundice of sclera, sublingual mucosa, hard and soft palate and skin. At first the skin has a pale yellow color then the color gradually intensifies and reaches its maximum on the second week after onset. The overall condition does not improve, and the clinical signs of general intoxication (anorexia, permanent nausea, repeated vomiting, headache, fatigue, pain intheright hypochondrium) are even more intensified. In severe cases there are bleeding signs (petechiae, nosebleeds, gingivalbleeding, heavy menstruation). Jaundice lasts 3-4 weeks, very rare for 5-6 weeks.

Laboratory diagnosis

HBV diagnosis is based on clinical and epidemiological data and requires the confirmation by laboratory tests.

Biochemical laboratory tests include determining the activity of several enzymes (ALT, AST, F1FA, SDA isoenzymes LDH5, MDH3,4, A3), which appear in the blood and highlight early lesions of liver cell (cytolysis). Serum alanine aminotransferase levels are elevated after an incubation period of about 50 days, which gradually increased whithin several weeks including the jaundice period. In 1-8 days after the increase of alanine aminotransferase, a high level of serum bilirubin is observed. The high level of serum bilirubin is below 10 mg/dl in most patients. Elevated levels suggest a severe infection or signs of cholestatic hepatitis. In convalescents the alanine aminotransferase and bilirubin levels decrease to normal levels.

Tests reveal many specific markers of hepatitis B infection: HBsAg, HBeAg, anti-HBcor IgM and IgG anti-HBcor.

HBsAg is the first marker that appears, followed by HBeAg and anti-IgM HBcor. The natural evolution of HBV HBsAg is already found during the incubation period, 4-8 weeks prior to the clinical onset of the disease. The results of negative HBsAg do not mean the invalidation of HBV diagnosis. In these cases only the detection of anti-HBcor IgM, the only indicator of acute infection, confirms the diagnosis of acute viral hepatitis. HBsAg and HBeAg screening is important not only for the detection of HBV infection but also for the confirmation of blood infectivity (sick carriers) as prognostic tests.

HBV DNA is the most convincing marker of HBV infection. The presence of viral DNA in serum allows a proper evaluation of hepatitis B virus replication.

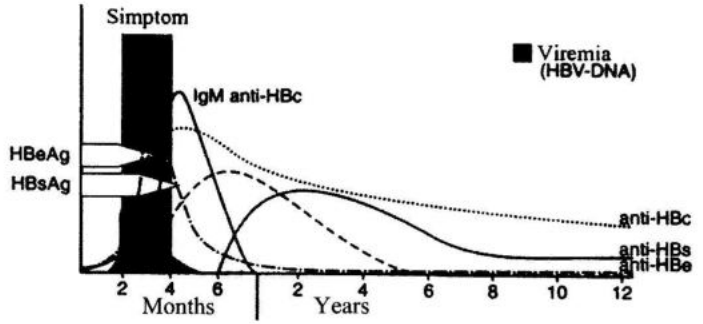

Figure 33. *Acute hepatitis B: the appearance and disappearance of virus markers (D. Cirstina, I. Ciutica, 2002).*

Anti-HBc antibodies are detected shortly after HBsAg and before the appearance of anti-HBs *(Figure 33)*. Initially antibodies IgM anti-HBc predominate. The positive test for IgM anti-HBc is the most sensitive test for identification of acute HBV infection. After an increase of IgM anti-HBc levels, their titer decreases and disappears over 4-8 months after their occurrence. IgM anti-HBc antibodies are replaced by IgG anti-HBc.

The titers of HBsAg decrease over time in acute HBV infection. AgHBs are replaced by anti-HBs (HBs antibodies), which are unique and have an appropriate protective role. Antibodies -HBs remain detectable for several years. HBs -antibodies may eventually be undetectable in rare cases.

Epidemiological surveillance of viral hepatitis B provides the permanent monitoring of the epidemic process, which includes monitoring

of morbidity, population coverage with immunizations, selective serological screening of the population for immune status, screening for the presence of HBsAg in the blood, particularly in risk groups, the assessments of the undertaken measures regarding the situation and its prognosis, taking decisions on the undertaken prophylactic and anti-epidemic measures aimed at reducing morbidity and lethality.

Control measures

Preventive measures

Specific prophylaxis

Viral hepatitis B is included in the list of infections against which preventive vaccination is planned in the national vaccination program. Currently, vaccination is considered the most effective prevention in viral hepatitis B.

HBV vaccines used to prevent HBV are of two types: plasma derived vaccine and genetically recombinant one.

Plasma-derived vaccine consists of particles of HBsAg obtained from the plasma of HBsAg carriers. Then the product is purified, inactivated by formalin or heat, and absorbed on aluminum hydroxide. HBsAg particles are free of nucleic acid, therefore it is not infectious, but induces the production of specific anti-HBs antibodies. Their use is practically limited to the immunization of immunodepresive, hemodialysis and allergic individuals.

Genetically recombinant vaccine is produced by the insertion of AgHBs into yeast cells (Saccaharomyces cereviside). The vaccine contains HBsAg particles absorbed on aluminum hydroxide and preserved with thiomersal. Vaccine production technology ensures the total absence of infectious viral particles or proteins of human origin or any allergic triggers.

Recombinant HepB vaccine is called monovalent vaccine, because it contains a single antigen. HepB vaccine can be used in combination of vaccines, such as DTP pentavaccine - HepB + Hib.

According to the schedule of vaccinations in Moldova, the vaccination is performed as follows:

- within 24 hours after birth - only monovaccin HepB;
- at the age of 2 months - pentavalent vaccine (DTP - HepB + Hib);
- at the age of 4 months - pentavalent vaccine (DTP - HepB + Hib);
- at the age of 6 months - pentavalent vaccine (DTP - HepB + Hib).

According to epidemiological indications, vaccinations against

viral hepatitis B are also administered to other groups of the population at high risk of contracting the infection: medical workers (surgeons, reanimatologists, obstetricians, dentists, hemodialysis staff who work in hematology centers, blood transfusion units, blood products units, analysis laboratories, etc.).

High-risk patients are also vaccinated i.e. hemodialysis patients, haemophiliacs, children or adults from mental asylums, surgical patients before a major surgery (organ transplantation, etc.), HIV infected persons. Other groups vaccinated against HVB include: homosexuals and drug addicts population, immigrants from countries with high endemicity, or people living in countries with high endemicity. The vaccination is indicated to all children and adolescents who have not been vaccinated.

HBV vaccine is administered intramuscularly into the anterolateral thigh in infants and the deltoid muscle in children and adults for maximum protection. The vaccine is not administered subcutaneously, intracutaneously or in the buttock area.

If the vaccination schedule has been discontinued or postponed, the vaccination course does not start from the beginning but only complement the vaccination course up to 3 or 4 inoculations.

The HepB vaccine is one of the most harmless vaccines. Reactions caused by vaccination are very rare and usually mild. Local reactions (sensitivity, slight redness or swelling of the injection site) and general ones (mild fever or headache that persist one or two days after the injection of the vaccine) can occur very rare.

Local reactions occur in 6% of the vaccinees, and the general ones - in 1% of the vaccinees.

Severe adverse allergic reactions (rash on the body, shortness of breath, choking) constitute about 1 case per 600,000 of administered doses. No allergic reaction with a fatal outcome has been reported. However, in case of severe reaction, a health care workers should immediately report to the local Center for PublicHealth. In these cases, HepB vaccinations with monovalent or polyvalent vaccine are discontinued.

Nonspecific measures to prevent hepatitis B include:

- increasing use of disposable medical equipment;
- following the rules of cleaning and sterilization of multiple-purpose medical instruments;

- minimizing the parenteral interventions in medical practice;
- proper management of wastes produced as a result of medical activities;
- protection of medical personnel at risk of contracting the infection by providing sets of personal equipment, depending on the specific character of the professional activity;
- limiting blood transfusions and their derivatives, being used only in cases of strict necessity;
- screening for the presence of HBsAg in blood, organs, tissues and donated spleen;
- testing the medical staff for the HBsAg;
- avoiding accidental contamination during needle punctures; in case if such as accident occurs, it is advisable to wash thoroughly the site with water, apply disinfectants in the form of compresses with 0.05% chlorhexine, 70% ethyl alcohol, 3% hydrogen peroxide for 5 minutes. A person must be tested for anti-HBs, in case of a negative result - post-exposure prophylaxis by immunization with vaccine and specific immunoglobulin against HBV is carried out;
- preventing the common use of personal hygiene items (toothbrush kit, manicure and pedicure items etc.); in specialized institutions (hairdressers, Manicure, pedicure, beauty shops or tattoo parlour etc.) only disposable syringes are to be used;
- avoid sexual relations with unknown and unprotected partners;
- health education of the population about the risk of contracting the HBV infection, preventive measures, symptoms of HBV, and the need to consult a doctor as early as possible.

Anti-epidemic measures

Medical institutions, regardless of the type, inform the Supervisory Service of the Center for PublicHealth about detected cases of acute hepatitis B by notification in the electronic system of epidemiological alert and complete the form no. 058/e.

The patient with acute form is hospitalized in infectious diseases departments. Patients with acute form of disease or chronic carriers of HBsAg are hospitalized according to clinical and epidemiological criteria.

The discharge from the hospital occurs after the clinical recovery and normalization or reduction of bilirubin levels and other biochemical blood indices.

The presence of HBsAg is not a contraindication for discharge. Vaccines, with the exception of anti-tetanus and anti-rabies vaccines, are contraindicated for 6 months. Planned surgery is not recommended during this period. Women are advised to avoid pregnancy for one year after discharge.

All persons who have suffered an acute or chronic form of HBV and HBsAg –carriers people are monitored by an outpatient health center. People who contracted acute HBV are monitored for 6 months after the discharge. Clinical, biochemical and immunological investigations are recommended to be performed in 1, 3 and 6 months.

Clinical and laboratory surveillance of the patient is prolonged if clinical signs persist.

HBs Ag carriers are under the clinical supervision until HBs AgHBs negative results and anti-HBs detection are obtained.

The epidemiological investigation of each case of acute HBV is carried out in the outbreak.

Current disinfection is carried out in acute hepatitis B outbreaks untill the patient is hospitalized; terminal desinfection is performed in chronic viral hepatitis B outbreaks. Current disinfection is carried out by the person who takes care of the sick or carrier, under the guidance of a physician. Decontamination is applied to all personal hygiene items of the patient and other items contaminated with the patient's blood, saliva and other body fluids. Decontamination is performed with disinfectants, which have antiviral action on HBV.

In HBV outbreaks (acute, latent or chronic) terminal disinfection is carried out in case of patient's hospitalization,death, moving to a new place or rehabilitation.

Persons who had contact with patients with acute HBV are supervised for 6 months after the patient's hospitalization. These people are examined by a physician; ALT activity and HBsAg and anti-HBs are to be determined.

People with protective concentrations of anti-HBs antibodies detected in the first investigation, are no longer supervised. In case of patient' home isolation, a continuous surveillance of the otbreak is carried out during the patient' stay.

Emergency prophylaxis of HBV is performed with specific immunoglobulin and vaccine against HepB. Antibodies are produced within 48 hours after the exposure, simultaneously with the first dose

of vaccine. The protection occurs immediatelly, but lasts only within 3-6 months. The following 2 doses of vaccine are to be administered in 1 month and 2 months respectively after the exposure (express- scheme).

1.1.3.2. Hepatitis C

Short history

In 1988, a team of researchers conducted by Houghton and Choo developed the molecular cloning method of hepatic viruses which enabled to detect new types of hepatitis viruses: VHC (1989), VHE (1990), VHG (1995) and VTT (1997), and non-A non-B (HNANB), hepatitis C, G, E and TT produced by the virus.

The pathogenic agent

Hepatitis C Virus (HCV) belongs to the Flaviviridae family. It is a spherical RNA virus with a diameter of 40-60 nm.

The viral envelope comprises a protein and lipid layer and an envelope that surrounds the capsid („core") including the viral nucleic acid.

Genome proteins are used to detect anti-HCV antibodies in diagnosis.

The occurrence of HCV-RNA in the liver and serum may be a marker of an acute HCV infection, preceding an increase in ALT and anti-HCV antibody (anti-HCV) seruma.

HCV RNA is constantly detected during the infection in chronic HCV. Anti- HCV antibodies remain positive in these patients for many years.

HCV is very heterogeneous. There are 6 genotypes, over 100 subtypes and over 50 isolates subtypes.

Genotypes determine the severity of the disease and enable successive infection with multiple viral strains, determining the prognosis of treatment efficacy and duration.

Subtypes differ by 20-25% in the nucleotide sequence and are defined by the lowercase letters of the Latin alphabet: 1a, 1b, etc.

Isolates refer to mutations, which show a variation in sequences ranging between 2 and 15% in patients with the same subtype.

However, high HCV variability makes it difficult to obtain a vaccine.

Circulating HBV genotypes vary in different regions all over the world. Genotype 1b predominates (95.5%) in the Republic of Moldova.

It is considered that HCV is less resistant to external factors compared to HBV. It is sensitive to the action of ultraviolet rays. HCV viability to thermal factors and disinfectants is considered to be similar to those of HBV.

The sources of pathogen are persons with acute and chronic forms of manifested and nonmanifested hepatitis C, and HCV carriers. The HCV infected person has already an epidemiogenic risk during the incubation period (1-2 weeks until clinical signs appear), during the prodromal period and jaundice period, throughout the disease in case of chronic infection. Chronic patients are the primary source (reservoir) of the disease, since the blood RNA is regularly detected throughout the disease. Chronic infection ranges from 50% to 90%, depending on age,and about 60% of chronic HCV hepatitis evolves into cirrhosis.

Factors and transmission routes

In hepatitis C the main mode of contamination is parenteral via blood from patients with acute or chronic HCV or carriers.

Currently, the intravenous drug addicts,who do not take precautions for safe injections are particular danger in the spread of HCV. Injection contamination ranges from 25% to 70%, especially among teenagers aged 15-17 and 18-29.

A very high risk of transmission of HCV is associated with transfusions of untested blood or blood derivatives. It was the primary risk group of patients until the implementation of donor blood testing for the confirmation of HCV. Since the implementation of mandatory testing of donors, the number of contaminations from blood transfusions has been significantly diminished and now it constitutes 1%-2% of the total contamination number.

However, 80-90% of the total transmission of HCV are transmitted by blood transfussion.

Another high risk group of HCV contamination is chronic hemodialysis, oncological and haematological in-patients receiving long-term care, organ transplant patients, and medical workers who contact blood.

The virus can be transmitted from other sources by means of parenteral medical manipulations (dental, surgical, gynecological, angiography, endoscopy, acupuncture etc.) and non-medical (tattoo, manicure, pedicure etc.).

Vertical transmission of HCV from mother to child and through a sexual contact is more rare than in HBV. Low level of HCV in the blood and other body fluids (semen, vaginal secretions, etc.) decreases the risk. The risk of fetus contamination in HIV positive mothers constitutes 2%, which increases up to 7% in HCV-RNA pregnant women and up to 10% in drug addictive pregnant women..

Manifestations of epidemic process

Hepatitis C is a serious public health problem worldwide, although the intensity of spread varies in different parts of the world.

The incidence of infection spreading is assessed by the frequency of detection of anti-HCV, which has become possible since 1989 due to the development of the laboratory-diagnostic test system.

Based on anti-HCV incidence in volunteer donors, 3 endemic regions have been determined: low - up to 2.5%, medium - 2.5-4.9% and high - 5% and more. In the Republic of Moldova, the incidence of antibodies in blood donors is 4.3 to 5.6% (V. Pantea, 2009).

The WHO estimates about 170 million people worldwide, or about 3% of the world population, being infected with HCV, and 3 to 4 million represent new cases recorded annually. The incidence of acute symptomatic HCV is estimated at 1-3 cases per 100,000 population in the world.

Hepatitis C is officially registered in Moldova since 1991. The average annual incidence in that period was 3.5 cases per 100 000 of population.

After a period of increased morbidity, up to 7.60 per 100,000 population (in 1996) due to the implementation of prophylactic measures (donor testing to confirm anti-HCV, wider application of all disposable medical instruments, etc.), a moderate decrease of morbidity up to to 1.80 per 100,000 population followed in 2012 *(Figure 34)*.

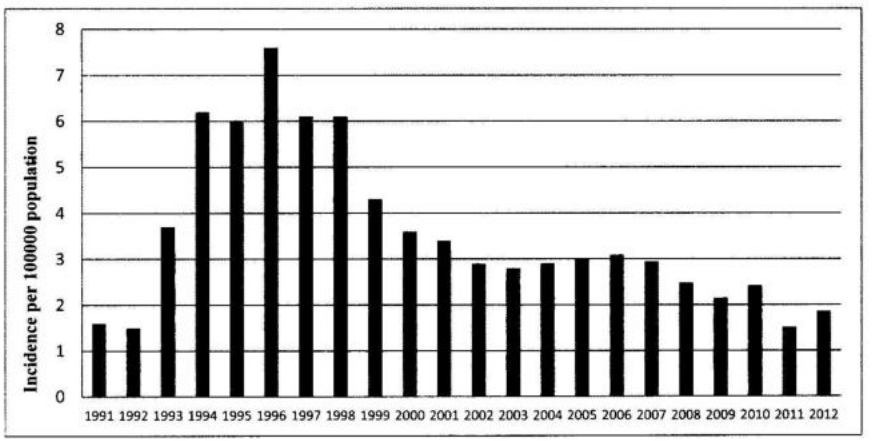

Figure 34. *Dynamics of HCV morbidity in the Republic of Moldova, in the period of 1991-2012.*

However, the overall HCV morbidity rate of viral hepatitis remains high and is still increasing, despite of the fact that the morbidity of HAV and HBV was reduced *(Figure 35)*.

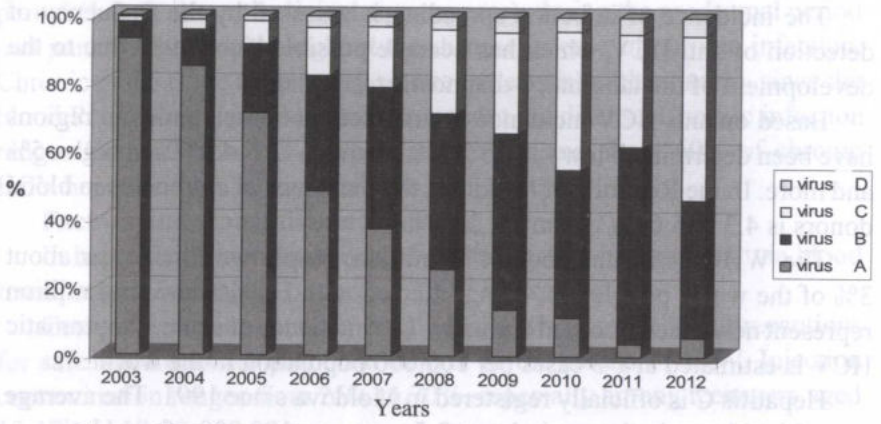

Figure 35. *The incidence rate of acute viral hepatitis A, B, C and D.*

Receptivity to hepatitis C is characteristic of the total population of the world. All persons who have caught the infection, regardless of clinical form, are considered susceptible to HCV infection.

However, the risk groups consist of individuals aged 20 and 50 years. The incidence of HCV is increasing among young people (20-29 years) and adolescents determined by drug addiction epidemic. Children up to 10 years old are less involved in the epidemic. Men are the most affected ones. The share of men in the HCV morbidity is 52.7% compared to 47.3% in females; the share of urban population is 55.0% compared to the rural population - 45.0%.

The prevailing conditions of HBV contamination in young people are the following:various parenteral interventions (50.0%), sexual relations (9.7%), intravenous drug injection (8.3%), tattoos (7.3%); in elderly people parenteral interventions (73.2%) and hemotransfusions (2.4%) prevail. In about 17% of cases the transmission pathway is not identified (V. Pîntea et al., 2009).

Seasonality is not characteristic of hepatitis C.

The manifestations of infectious process

Symptoms of acute hepatitis are similar to those of viral hepatitis B, and cannot be distinguished by this principle. However, acute hepatitis C

differs by a low rate of manifestations (jaundice). Acute hepatitis C often causes few symptoms or remains inapparent and is less severe compared to hepatitis A and B. More than 2/3 of the cases are asymptomatic and anicteric ones. Hepatitis C develops only in transfusion and more than 25% of patients develop acute jaundice.

The incubation period is shorter than in hepatitis B,it varies from 7 to 140 days, and in post-transfusional acute HVC - between 7 and 50 days.

Prodromal period lasts from 3-4 to 10-15 days, but in some cases it does not occur. It is characterized by slow onset with moderate signs of intoxication (abdominal discomfort, asthenia, arthralgia), hepatomegaly, liver sensitive to touch, splenomegaly, dark urine and acholic stools, sometimes increased temperature, pruritus.

The jaundice period is characterized by jaundice, which lasts from a few days to a month, dark urine and acholic stool, symptoms of general intoxication, hepatomegaly, rarely - splenomegaly.

Asymptomatic forms of acute viral hepatitis C can be detected only based on laboratory investigations.

Laboratory diagnosis is based on molecular-biological methods: DNA amplification technique and PCR.

Nucleic acid amplification test is less sensitive and cannot detect low viraemia.

The PCR test reveals HCV-RNA (viremia), confirming infectivity in persons with anti-HCV antibodies that can be detected in blood already in 1-2 weeks after infection.

PCR is indicated in the diagnosis of acute hepatitis in seronegative persons, newborns of HCV infected mothers, in immunosuppressive, hemodialysis, liver transplant recipients, persons suspected of viral infection C or those who received antiviral therapy.

Biochemical diagnosis aims to determine the following indices:

- direct and indirect indices of cytolysis syndrome (ALT, AST, bilirubin, monophosphatefructose, diphosphatefructosaldolase, sorbitol dehydrogenase, prothrombin);
- indices of cholestasis syndrome (alkaline phosphatase, β-lipoproteide, nucleotidase, cholesterol);
- indices of mesenchymal inflammatory syndrome (test with thymol and sublimat, γ-globulin, etc.).

Epidemiological surveillance is carried out under the Supervision Programme of blood hepatitis B, C and D.

Control measures

Preventive measures

Specific prophylaxis of hepatitis C has not been elaborated yet. The obtained vaccine, prepared from glycoproteins proved to be poorly prorective and unstable.

In HCV the nonspecific prophylaxis includes the effective implementation of complex measures aimed at preventing the infection of blood and blood derivatives, preventing parenteral contamination, protecting the medical personnel, intensifying the fight against narcotics, informing population about the ways of transmission, its consequences and preventive measures.

Anti-epidemic measures

Although anti-epidemic measures in outbreak of acute hepatitis C are identical to those in hepatitis B, the measures differ in certain features:

- patients with acute hepatitis C are compulsorily isolated, being hospitalized in departments or hospitals for infectious diseases;
- people who had contact with patients will be examined clinically, biochemically (bilirubin, ALT, AST, prothrombin test thymol), serologically (detecting anti-HCV IgM, anti-HVCsum markers) and instrumentally (ultrasound examination of the abdominal cavity);
- discharge from the hospital is allowed only after the clinical recovery and normalization of liver biochemical tests;
- since 70%-90% of cases of acute HCV progress into a chronic condition at a slower rate than in HBV infection, mandatory fellow-up of the patient are to be carried out after the discharge and they are followed-up by a family physician and infectionist. The patient's follow-up lasts for 12 months. The patient is clinically and biochemically examined in 1, 3, 6, 9 and 12 months, virologically - in 6 and 12 months. If the infectious process is still present after the period of 12 months, medical surveillance will be continued.

1.1.3.3. Acute hepatitis D virus (delta infection)

Short history

The name of hepatitis D orignates from the Greek word „delta", which means „the fourth", when Rezzetto and coauthors, detected the pathogen into the nucleus of hepatocytes of patients with hepatitis B and iniatially they thought that they had discovered the fourth system of antigen-

antibodies HBV. Initially, the pathogen was considered a breach of HBV and later found to be a new single-stranded RNA virus similar to virusoid RNA complexes.

The pathogen agent

Hepatitis D (HDV) or delta virus is a small (31-37 nm), spherical, shell-shaped and nucleocapsid virus. The envelope of HBV is HBsAg and it provides external protection for HDV. The nucleocapsid consists of hepatitis D virus antigen (AgVHD) and RNA genome. AgHDV consists of two proteins: large AgHDV („large") with 214 amino acids and small AgHDV („small") with 195 amino acids. AgVHD consists of two proteins: large (large) AgVHD with 214 amino acids and small AgVHD with 195 amino acids. Small AgVHD occurs in HDV replication, whereas large Ag VHD stops HDV replication.

Hepatitis D virus replication is independent of HBV; the presence of HBV is necessary to form the HDV envelope. Therefore, hepatitis D virus is classified a as defective virus, being dependent on the presence of hepatitis B virus, in order to synthesize all HDV components. Humans may be HDV infected by an already associated HBV (coinfection) or HDV occurs as a superinfection after HBV infection, which is a worsening factor.

There are 3 genotypes of HDV: I, II, III. The first genotype with two subtypes, 1a and 1b is spread all over the world; genotype II - on the islands of Taiwan and the Japanese archipelago; and the IIIrd genotype circulates in South America and Africa. All genotypes are related to the same serotype.

HDV is resistant to high temperatures, acids and UV action. Virus denaturation is obtained by alkaline solutions or protease processing. Multiple freezing and defreezing processes do not affect the activity of the virus.

The source pathogen is manifested in persons who have had contracts with acute or chronic HDV and had contacts with HDV carriers, which are considered to be individuals with HBsAg and anti-HDV. A higher epidemiogenic risk is present in patients with mixed chronic HBV/HVD infection. In case of chronic HBV infection spread, HDV circulation rate also increases.

Both coinfection and the superinfection after contamination result in short-term viremia, and the virus enters into the nucleus of hepatocytes

and specific antibodies appear in the blood. Co-infection commonly occurs after massive blood transfusions or use of injectable narcotics. The period of infectiousness is determined by the duration of HBsAg circulation in the body because HDV is not able to replicate independently in the host organism.

Factors and transmission routes

HDV transmission is similar to that in HBV, with some specific features. Artificial ways of transmission are of great importance since a much higher infective dose is required to contract HDV. The risk of infection is very high for permanent recipients of blood and blood derivatives, persons subjected to frequent parenteral interventions, injecting drug users, people who are in contact with blood. Sexual contact or vertical transmission occur rarely. Sexual transmission is more common in people with a disordered sexual relationships and in homosexuals.

Risk factors: the presence of HBV, hematological pathologies, hemodialysis, intravenous drug use, tattoo practice, promiscuity, etc.

Manifestations of the epidemic process

HDV has a general receptivity. HBV and HDV coinfection occurs in receptive persons who have not had any forms of viral hepatitis D and/ or B, and in whom markers of hepatitis B (HBsAg, anti-IgM and anti HBcor HBcor IgG) have not been detected. People with chronic hepatitis B, HBsAg carriers and acute hepatitis B patients are considered receptive to superinfection of HBV and HDV.

Delta infection is spread worldwide, but with great variations in areas. Delta endemic infection in endemic regions coincides largely with the HBsAg.The Republic of Moldova is considered to be an area of high endemicity for HDV. There were detected anti-VHDsum in blood donors ranging from 6.9% in the northen area to 30.3% in the south of the country (J. Drobeniuc, 1988).

Epidemiological features of viral infection D are largely similar to those of HBV.

The incidence of hepatitis D is officially recorded in Moldova since 1991 *(Figure 36).*

The dynamics of morbidity in the period of 1991-2012 initially showed a wavy character, which increased to 1.9-1.5 cases per 100 thousand population, and since 2002 it decreased to 0.06 in 2012, being primarily determined by a decrease in HBV morbidity.

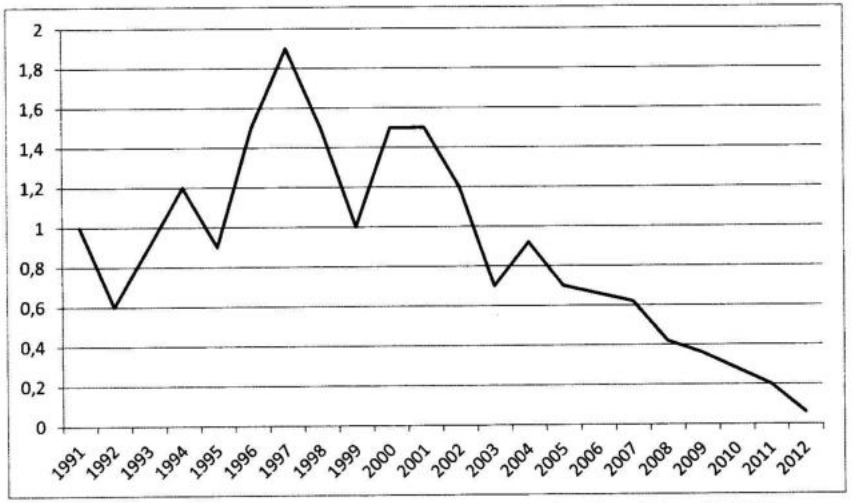

Figure 36. *Dynamics of HVD morbidity rate in the Republic of Moldova, in the period of 1991-2012 years.*

The incidence rate of HDV increases with age. The prevalence of male population constitutes 66.3% compared to 33.7% in females. The incidence is higher in patients with chronic renal failure, hemophiliacs and drug addicts.

It is manifested in sporadic form, but it may also occur in epidemic forms. Major epidemics have been observed in South America, particularly in Venezuela and Colombia.

Seasonality is not characteristic of it.

The manifestations of infectious process

HDV infection is manifested in two forms: coinfection and superinfection.

In coinfection the incubation period coincides with that of HVB 50-180 days.

The prodromal period lasts from 1 to 14 days with an acute onset and 38-39 C° fever. The dyspeptic syndrome, asthenic syndrome, arthralgia syndrome, hepatomegaly, splenomegaly, reveal increased indices since the very onset of the disease, which are important in early diagnosis of the disease. The urine is brown and stool is acholic.

During the jaundice period, that is longer than in HBV (5-6 weeks),

feverish temperature (up to 38°C) persists for 12 days, dyspeptic and asthenic symptoms are more pronounced, vomiting is more frequent and pain in the right hypocondrium is less violent compared with HBV. Arthralgia syndrome intensifies and jaundice increases. One of the features of acute HVD (coinfection) is biphasic, clinical and biochemical aggravation of the disease, more frequently on the 20th - 30th day of the disease, with intensification of clinical symptoms. Severe forms are more common than in HBV and are characterized by the presence of hemorrhagic syndrome, which is manifested by nasal, gastric, intestinal bleeding, edema in the plantar region and the calves.

The convalescence period is longer than in HVB, followed by 80-95% incidence of recovery.

In superinfection the incubation period is 1-2 months.

The prodromal period lasts only 3-5 days and is characterized by acute onset, fever 38-39°C, dyspeptic, asthenic and arthralgia syndromes, hepatomegaly (liver is hard to the touch) and splenomegaly in 100% of cases,which is not characteristic of HBV and coinfection. A clinical particularity of superinfection is the development of multiple complications. The positive evolution is rare, it usually leads to chronicity (70-98%).

Laboratory diagnosis

Biochemical diagnosis is similar to that of HBV.

The HBsAg, HBeAg, AgVHD are determined during the preicteric period. HDV antigen is identified via immunoblot test.

HBsAg, HBeAg, Anti-HBe, anti-IgM HBcor then HBcor anti-IgG, IgM anti-HDV, and IgG anti-HDV are determined during jaundice. HDV RNA is in the blood and liver tissue via molecular hybridization technique or PCR and RT-PCR. The presence of AgHD and HDV RNA in blood means a HDV replication.

The occurrence of these markers is temporary in acute hepatitis, but it is persistent in HDV chronic hepatitis.

Anti-HDV IgM, IgG anti-HDV, HBsAg are used to determine HBsAg carriers. HbsAg, rarely AgHVD, HDV anti-IgM, IgG and then anti-HDV are detected in blood of the patients with chronic HBV. It is possible to determine the various titre of anti-Hbcor, IgM and IgG anti-HBcor.

The diagnosis of acute viral hepatitis D is possible by detection of HDV Ag, HDV RNA, IgM anti-HDV antibodies in serum, and IgG anti-HDV and HDV Ag and HDV RNA in the liver.

HDV epidemiological surveillance is performed in the framework of the HBV surveillance program. In the epidemiological surveillance of *D* virus infection important elements are the diagnosis and illness recording, determination of the risk groups and assessment of the preventive measures.

Preventive measures

VHD prophylactic measures are the same as in viral hepatitis B. Vaccination of healthy individuals against HBV also provides protection against HDV. It is important for patients with chronic HBV to follow the measures aimed at preventing the contamination with HDV and development of superinfection.

Anti-epidemic measures are identical to those of HBV and HCV.

1.1.3.4. Viral hepatitis G

The pathogen agent

Hepatic G virus (HGV) belongs to the Flaviviridae family, which includes HCV. Although there are some sequential analogs (25% homologous sequences), it has been found that HGV is not a HCV genotype, but a novel RNA type virus, which shows independent and similar genomic organization similar to flaviviruses. Structurally, it includes: RNA, a capsid, envelope, and protein envelope. There are 3 HGV genotypes: genotype 1 with subtypes 1a and 1b (West Africa); genotype 2 with subtypes 2a and 2b (North Africa, Europe, North America, India); genotype 3 (China and Japan).

The source of pathogen is HGV infected patients (acute or chronic forms) and HGV carriers. The contagious period begins with the incubation and lasts from several months (acute hepatitis) to 10-16 years (in chronic hepatitis).

Factors and transmission routes

Hepatitis G virus is most commonly transmitted through blood: parenterally, vertically and sexually. Parenteral contamination occurs more frequently after injecting drug use, blood transfusion or blood preparations.

According to previous studies (Nubling C.,1997) the prevalence of HGV infection is 18% in persons with repetitive transfused blood, 18% -in haemolytics, 33%- in drugs users, 3.5-5.5% in dialysis patients.

HGV was found in semen and saliva, and it is assumed that they constitute the routes of transmission.

Intrafamilial cases of hepatitis G have been described. Mixed infection

associated with other types of parenteral transmission viruses like B, C, D is common. In patients with chronic HBV the association with VHG is estimated at 10%, in patients with chronic HCV - 20-30% of patients, with HIV - 9%. HGV as monoinfection is rarely detected.

Manifestations of epidemic process

HGV has a general receptivity, still being considered higher in risk groups, and it is identical to those of HCV and HBV infection. HGV process manifests itself as sporadic, epidemic and endemic.

Endemic manifestation is specific to certain geographical areas. A lower endemicity of HGV is registered in Europe and North America (1.5 to 1.4%) and the highest in Africa (17-25%).

Outbreaks were reported among drug addicts who administer intravenous drugs and hemodialysis patients.

Seasonality is not characteristic of it.

The manifestations of infectious process

VHG infection is characterizied by two evolutionary ways of transmission: acute and persistent, with chronic viral transmission.

The disease starts with an atypical onset, with slight alteration of the overall health condition, presence of moderate dyspepsia and pain in the right hypocondrium, after an incubation period of 14-45 days. Jaundice appears during the period of clinical manifestation, which is less pronounced than in infections caused by other liver viruses. Serum transaminases are slightly elevated or normalized.

An acute HGV infection may develop into a persistent infection (chronic hepatitis or carriage).

Laboratory diagnosis

Diagnosis of HGV infection is based on detection of the viral RNA genome via gene amplification techniques, PCR or RT-PCR, RNA-VHG.

The serological diagnosis is based on the determination of anti-VHG.

The diagnosis is based on the same non-specific biochemical tests as in acute hepatitis C viral infection.

Epidemiological surveillance does not differ essentially from that of other parenteral hepatitis. In HGV epidemic, it is necessary to detect the patients among the persons diagnosed with „viral hepatitis of undetermined etiology" and with chronic liver diseases.

Preventive measures

The prevention of infection is focused on general measures common

to parenterally transmitted hepatitis. In case of incidental infection, which has been reported to the medical staff, the wound is processed, the incident is reported, the source is identified, serological tests for HGV infection are carried aut. If it is necessary, interferon alpha is urgently administred.

Specific Prophylaxis is not developed.

Antiepidemic measures against viral hepatitis G are not developed.

1.1.3.5. Viral hepatitis TTV

Short history

In 1997, T. Nishizawa and his co-authors found a non-A-non-C post-transfusional hepatitis virus in patients, which they called TTV – from the initials of the English words: Transfusion Transmitted Virus.

The pathogen agent

The TTV virus refers to the *Circoviridae* family; it is small in size - 30-50 nm; it contains DNA with a circular genome; no envelope. Two genetic types (1 and 2) are known, which in turn contain several subtypes. The TT virus is thermolabile and it is destroyed under the action of usual decontaminants, including detergents. It's sensitive to interferon.

The source of the pathogen is both the TTV patient with acute (icteric and anicheric) and chronic forms, as well as the TTV carriers.

The TT virus was also found in the blood of farm animals (cattle, sheep, pigs) and domestic animals (dogs, cats), domestic poultry. The zoonotic reservoir of the virus is also possible.

Factors and transmission routes

TTV transmission occurs mainly parenterally, like in other blood hepatitis, both by blood and its derivatives transfusions, and via other parenteral maneuvers with a possibility of transmitting the virus through contaminated blood from TTV-infected persons.

Vertical transmission as well as transmission by sexual intercourse are recognized.

However, the virus has been found in bile, feces and blood that assumes the possibile fecal-oral transmission. A case of acute hepatitis with fecal-oral mechanism associated with TT virus was reported in China. Other hepatotrope viruses were not revealed in that case. Simultaneously, RNA/TTV was detected in the blood of almost all investigated persons, which proved the role of TTV in developing of this infection..

Detection of VTT in the meat of animals used in public catering suggests the idea of a possible transmission of the virus also by food.

Manifestations of epidemic process

The frequency of RNA-TTV detection in European countries is from 1.9% to 16.7%, in Asian countries - from 11.0 to 42.0% in the US - from 1.0 to 10.7%, in Countries of South America - from 19.0 to 83.0%, in African countries - from 44.0 to 83.0% of the surveyed population. TTV detection frequency increases with the age of the investigated persons. The frequency of positive results is reduced in newborns. TTV RNA is detected in 46% of patients with chronic hepatitis of unknown origin, as well as in the chronic form of viral hepatitis B and C.

An increased level of TTV infection is recorded in repetetive blood transfusion persons, hemophiliacs and drug users.

The manifestations of infectious process

The incubation period in post-transfusion hepatitis TT is of 6-8 weeks. The early signs of the disease, are simillar to those of other kind of hepatitis (symptoms of dyspepsia, asthenia, hepato and splenomegaly), accompanied by moderate increase of serum transaminases. Clinical and biochemical improvement, accompanied by the disappearance of viremia, occurs in 16 to 18 weeks since the onset. Viremia is long-lasting in persistent infections. Matsumoto et al. found persistent viremia by detecting of TTV RNA during 22 years. The level of serum transaminase is of medium values, sometimes it is the normal range.

Laboratory diagnosis is based on detection of the viral genome TTV DNA via PCR molecular biological method.

The diagnosis is based on the same non-specific biochemical tests as in acute viral hepatitis C infection.

Epidemiological surveillance and epidemic preventive measures have not been developed yet, but the measures may be applicable to any type of infections caused by hepatitis virus.

1.1.3.6. HIV infection

Short history

Initially, the final manifestation of HIV infection was described, later it was named as Acquired Immunodeficiency Syndrome (AIDS). Since June 5, 1981, the Federal Agency of Epidemiology in Atlanta (USA), Center for Diseases Control (CDC) reported weekly morbidity and mortality rates and presented 5 cases of severe *Pneumocystis Carinii* pneumonia in young gay people in Los Angeles based on severe immune abnormalities.

During the next few weeks, 4 more cases of atypical pneumonia were reported in Los Angeles, 6- in San Francisco and 20 cases - in New York. All patients showed immunodeficiency, which was manifested by severe Pneumocystis pneumonia.

It was also found that all patients were homosexuals, thus the disease was initially called „homosexual compromise syndrome" (Gay-Related Immune Deficiency - GRID).

Shortly after those cases, there were reported cases among drug users of both sexes. At the beginning of 1982 there were discovered signs of the disease in hemophilia patients. At the end of 1982, there were reported AIDS cases in persons who had blood transfusions, which allowed to assume the possibility of the pathogen carriage in healthy persons, including donors.

At that time another issue regarding the new disease was the detection of a large number of people of African origin.

In 1983, Dr. Luc Montagnier (Pasteur Institute, France) reported the isolation of virus from the lymph node of a patient infected with AIDS, which they called LAV (Lymphadenopathy - Associated Virus).

In 1984, Dr. Robert Gallo (National Institute for the Study of Cancer, USA) announced the discovery of the virus that causes AIDS in peripheral blood T cells of a patient with AIDS, calling it HTLV-III (Human T - cell lymphotropic virus III).

The strains of viruses isolated in France and the US were found to be identical according to their morphology and antigenic structure. In 1986, the International Nomenclature of Viruses estabilished that the agent of AIDS is called HIV (Human Immunodeficiency Virus).

Due to the fact that antibodies to HIV are present in the contaminated body long before the appearance the AIDS onset, the disease was termed HIV, which initially determined HIV-infected people without AIDS.

Subsequently, the HIV/AIDS abbreviations, which include both periods of infection development, were accepted for the designation of the infection.

In a relatively short period HIV has achieved pandemic spread, affecting all countries worldwide.

The pathogen agent

The HIV virus is of spherical shape *(Figure 37),* and its size is of 100-140 nm. It belongs to the Retroviridae family, Lantivirus genus.

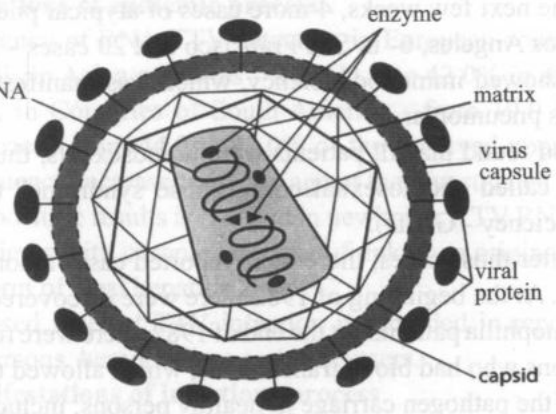

Figure 37. *The structure of human immunodeficiency virus (HIV).*

The structure of the virus contains two capsids: the nucleocapsid and supercapsid. The nucleocapsid contains the viral genome of spiral transcriptase and RNA. The supercapsid contains phospholipids and glycoproteins, whose structure determines the tropism of the virus to a subgroup of blood cells, T-helper lymphocytes (or CD4), and immune regulators. The virus destroys the immune system, by killing CD4 lymphocytes or T-helper cells which leads to the suppression of the immune response.

Another feature of the virus is the ability to integrate into the host cell genome by reverse transcription of viral RNA into DNA, thereby causing the persistence of infection in the host body for a lifetime.

The genome of the virus is presented by two „positive" RNA molecules and contains 9 genes:

• *tat and rev* - participates in transcriptional and post-translational processes;

• *vif* - determines the infectivity;

• *nef* - encodes the viral protein, being able to reduce the replication of the virus and thus facilitating the development of the latent infection;

• *gag* – encodes the nucleotide protein (capsid);

• *pole* - encodes the reversetrasnscriptase synthesis;

• *env* - encodes the glycoproteins envelope (of supercapsid);

• *vpr* - provides the location of the viral genome in the nucleus cell;

• *vpu for HIV-1 and HIV-2 VPX* - ensures the release of the virion from the cell.

According to the structure of the *env* gene fragments, there are identified several subtypes, which are marked with the letters of the Latin alphabet. The subtypes from *A* till *H* are included in the group *M* (major). The global spread is considered to be of subtype *C* with high identification in Africa. Groups *N* (new) and *O* (outlier) are more common in West Africa.

HIV proteins, either natural or synthetic, or engineered genes, are used as antigens in the manufacture of diagnostic preparations.

There are two types of HIV: HIV-1 and HIV-2, which differ in their structural and antigenic characteristics.

Type HIV-1 is the causative agent of primary HIV infection. It is isolated in all of the continents. Type HIV-2 is less widespread, it was initially isolated in Guinea-Bissau in 1985. It is found mainly on the African continent.

HIV is very heterogeneous, with a high genetic variability. The complete life cycle of the virus is very short (1-2 days), but its replication is very fast, up to 1 billion virions daily. The intensity of genetic errors in replication reaches 104-105 per genus / per replication cycle, so no replication virus is repeated. Therefore it is a major impediment in preparing specific HIV infection prevention. For these reasons, so far, HIV prevention measures are based on non-specific prophylaxis.

Beside that, future generations of viruses are not identical to those of the previous viruses in the body over time. They are more aggressive forms of the virus.

The human immunodeficiency virus is not resistant outside the host. However, the virus maintains its infectivity for up to 7 days,when the biological material is dried at room temperature, whereas in the liquid medium – for 2 weeks.

The virus is resistant to low temperatures. The virus is active in frozen serum during several years. Simultaneously, it is very sensitive to high temperatures. It is inactivated at 56°C within 30 minutes and at the temperature of 100°C - 1-5 minutes.

HIV is susceptible to common disinfectants. The following disinfectants are recommended to be used health care institutions: 0.5% lime chloride, 0.005% potassium permanganate, 3% hydrogen peroxide, 70% ethyl

alcohol, Ca and Na hypochlorite 1.0%, 0.05% clorexidine, which ensures the destruction of the virus.

The source of pathogen

According to its structure, HIV-2 is very similar to the lentiviruses of the primates (mangabey and African green monkey) that inhabit the West Africa. The animals are chronic carriers of the virus with no clinical signs of AIDS. They mantain the virus circulation in the primate population.

The origin of HIV-1 remains questionable and till now very controversial, although there are evidences that the virus has spread into the human population from monkeys as well. It is assumed that the natural reservoir is present in chimpanzees that live in West Africa, a region where practically all groups of HIV-1 (*M, N, O*) were detected.

Easy adaptation and reproduction of HIV in the body and human-to-human transmission led to the formation of a new reservoir of virus - the human population, the main sources of pathogen being persons infected with HIV/AIDS in all stages of its development (except for the first 2-3 weeks of exposure) during lifetime, which increases the accumulation of the virus in the human population.

The transmission of the virus by the infected person is higher during the incubation period, and it lasts from 2 to 10-15 years, an average of 6-7 years. During this period the infected person feels good, but can transmit the virus. The first clinical manifestations appears in the AIDS stage, which lasts about 2 years, and at the end of the disease, the amount of virus in the blood is at its highest level.

Factors and transmission routes

Transmission of the pathogen occurs both parenterally, by blood exposure or other HIV- contaminated biological fluids, and transplacentally.

Although HIV has been isolated from blood, semen, vaginal secretions, saliva, breastfeeding, lacrimal fluid, urine, cerebrospinal fluid, alveolar fluid, amniotic fluid, etc., there has been found that there are two factors that play a certain role in HIV transmission - blood and semen, due to a high viral concentration in these biological substances, which is measured by tens and even hundreds of infected doses per milliliter. One milliliter of an infected person's semen contains between 10 and 50 doses of viruses, and one milliliter of blood - up to 3000 doses; milk of the mother and vaginal secretions of HIV infected person contain up to one dose per milliliter and are assessed as being at high risk of contamination. In tears, sweating and

salivary fluids the infective dose is less than one dose, therefore the risk of being contaminated is considered low for these substances. This category includes feces, nasal secretions, urine, vomiting, as long as they do not contain blood.

Hence, the probability of infection with HIV via body fluids that contain blood is considered high. According to the Center for the Disease Control, the contact of the skin and mucosa is considered dangerous with the following factors: blood, amniotic fluid, peritoneal fluid, pleural fluid, synovial fluid, cerebrospinal fluid, vaginal secretions, tissues, any other body fluids contaminated with blood.

The most dangerous way is considered to be HIV transmission via blood transfusions. The risk of infection with HIV via blood transfusion is 90-100%. However, currently, the risk of contamination via blood transfusions and donated tissues and organs is lower because of mandatory HIV screening of donors of these biological products.

Commonly, the viral infection may occur via contaminated syringes and needles in persons administering narcotics intravenously. Drug users are at higher risk of infection since they use narcotic drugs by sharing needles, being exposed to a great number of shots and since they live in high HIV prevalence areas.

According to the studies on assessment of HIV contamination risks in healthcare professionals after accidental blood exposure, it was found that in case of contaminated needle, the risk of HIV transmission is 1:200 cases; when sharing needles - 1:150; after a contact with contaminated medical instruments – 1:300 or 0.3%;and after a scalp lesion -0.29‰o. Simultaneiusly, the risk of contamination varies according to the type and size of the needle, the depth of the lesion, the blood volume, titer of viral load, the duration of contact, the frequency of occupational exposures, the used equipment, decontamination measures and prophylactic treatment.

The HIV sexual transmission is still prevailing worldwide. About 80% the virus was transsmited sexually. The virus is transmitted mainly through unprotected sexual contact. Sexual promiscuity is a major cause of the HIV/AIDS spread.

In many countries the majority of HIV cases are recorded in persons with non-traditional sexual orientation i.e. homo- and bisexual relationships.

HIV infection is characteristic of maternal-fetal transmission of the pathogen, which may occur in 3 ways:

1) transplacental transmission -via the maternal blood during pregnancy. The risk of infection in babies born from HIV positive mothers was assessed between 13.0% and 50%, on average from 30.0 to 40.0%. With the implementation of antiretroviral therapy in HIV-infected pregnant women, the frequency of this type of transmission (transplacentar) was significantly diminished. For example, this index was reduced from 46.0% to 1.0% in Moldova.

2) During labor – by maternal and fetal blood microtransfusions; by contact of the damaged skin and conjunctival mucosa of the newborn with infected maternal blood or maternal secretions; by ingestion of blood or other maternal infected fluids;

3) Postpartum – by breast milk during breastfeeding.

The prevalence of a specific transmission route differs depending on the social, cultural, and ethnic behavior.

Manifestations of epidemic process

The number of people with HIV/AIDS is estimated at about 36 million all over the world.

Over the last decade the number of people infected with HIV has increased because new HIV cases occur every year, and antiretroviral treatment helps to extend the life of those infected, whereas the number of illnesses exceeds the number of AIDS deaths. Annually, about 3 million of HIV and 2 million of people dying of AIDS are recorded all over the world.

The HIV/AIDS pandemic mostly affected the African continent, particularly West Africa.

Two thirds (67%) of all HIV people worldwide live in sub-Saharan Africa. Only in South Africa, about 6 million people live with HIV.

Globally, the proportion of women among people living with HIV remains stable at about 50%. However, in Eastern Europe and Central Asia, there is an increase in the prevalence of women among people living with HIV. There is also an increase in the number of HIV infected children aged up to 15.

During the HIV pandemic evolution, there were found categories of vulnerable population with a high risk of contracting and spreading of infection, such as homosexuals, heterosexuals living in promiscuity, injecting drug users, people providing sexual services, patients with sexually transmitted diseases.

In various regions of the world, the prevalence of HIV spreading ways varies according to the rate of the risk population that may evolve over time.

In Eastern Europe and Central Asia, the epidemic initially developed via sexual intercourse, and then the nasocomial route of viral transmission to the healthcare institutions prevailed. Subsequently, the epidemic had a fast spread among injecting drug users, and ultimately, via sexual intercourse.

In Moldova the HIV situation shows that the incidence increased from 0.04 cases per 100,000 of population in 1987-2000, to 17.06 cases in 2007-2012 *(Figure 38)*.

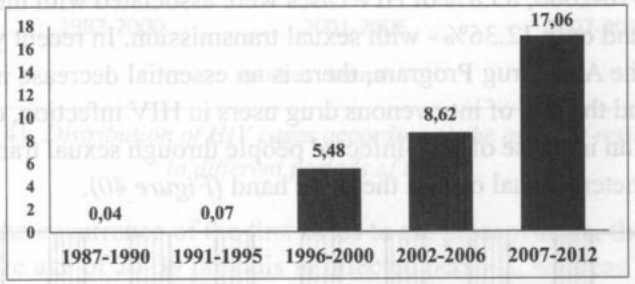

Figure 38. *The HIV incidence rate in the Republic of Moldova in various periods (per 100000 of population).*

The HIV epidemic shows a higher number of HIV infected people is in blood donors *(Figure 39)*.

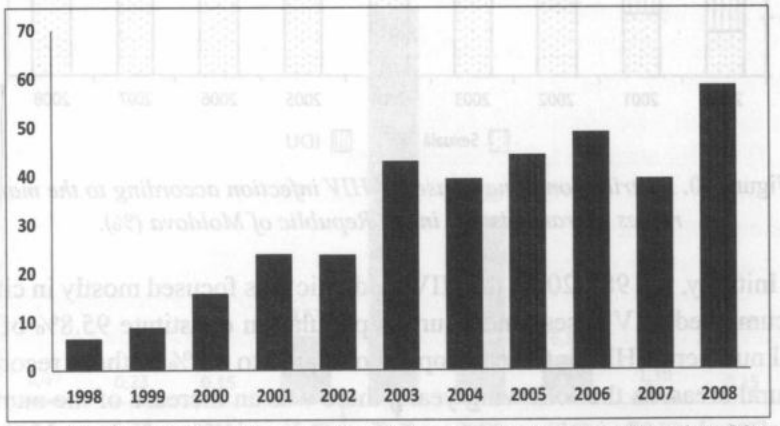

Figure 39. *The HIV incidence in blood donnors in the Republic of Moldova, in the period of 1998-2008, per 100 000 of population (V. Prisacari, L. Guțu, St. Gheorghita, 2009).*

There are two predominant ways of HIV spread - through injecting drugs and through heterosexual way. In Moldova there were recorded 7,000 HIV cases in the period of 1987 - 2012: 54% of them were cases of HIV infection through drug injection; 45.2% - sexual transmission; 1.2% - perinatal transmission; and 0.06% - via blood transfusions.

However, epidemiological research results show a significant change of the major HIV transmission routes in the republic. By 2000, the HIV epidemic was caused by the spread of infection among injecting drug users. During 1996-2000, 85.8% of HIV cases were associated with the injecting drug use and only 12.36% - with sexual transmission. In recent years, as a result of the Anti-Drug Program, there is an essential decrease in both the number and the rate of intravenous drug users in HIV infection, on the one hand, and an increase of HIV-infected people through sexual transmission, usually a heterosexual one on the other hand *(Figure 40)*.

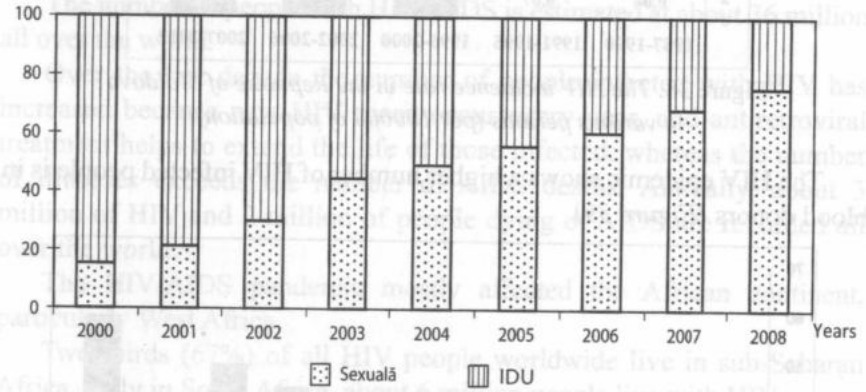

Figure 40. *Distribution of new cases of HIV infection according to the main routes of transmission in the Republic of Moldova (%).*

Initially, in 1987-2000, the HIV epidemic was focused mostly in cities. Documented HIV cases among urban population constitute 95.8% of the total number of HIV infected people, compared to 4.2% of those recorded in rural areas. In the following years, there was an increase of the number of infected people in the rural population, the overall rate being of 55% in 2007-2012 *(Figure 41)*.

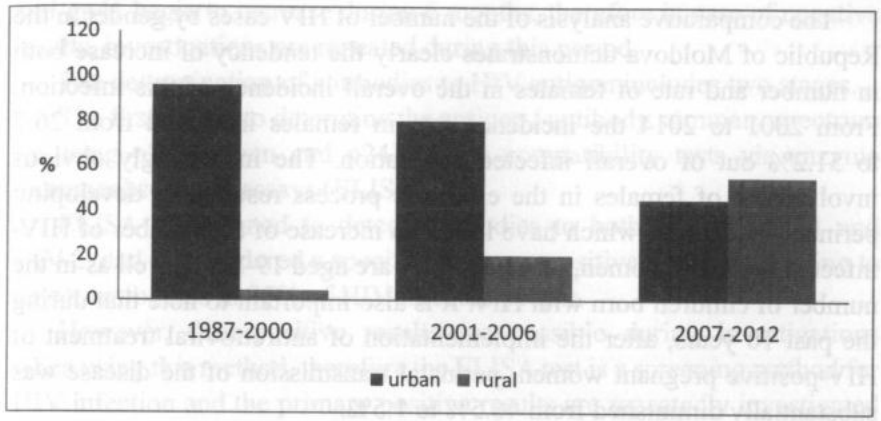

Figure 41. *Distribution of HIV cases according to the areas of residence in different periods of time.*

From the occurrence of the first cases to the present stage, the avarage reproductive age of 20-39 prevails in infected persons, whereas the target group is represented by young people aged 20-29 *(Figure 42)*. However, over the years, an increasing number of infected people from other age groups is also recorded.

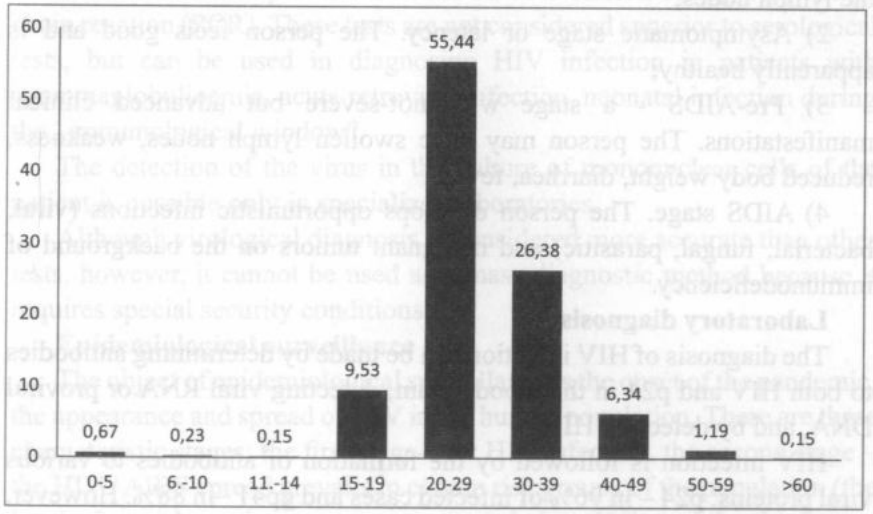

Figure 42. *Distribution of HIV case according to the age group.*

The comparative analysis of the number of HIV cases by gender in the Republic of Moldova demonstrates clearly the tendency of increase both in number and rate of females in the overall incidence of this infection. From 2001 to 2014 the incidence rate in females increased from 26.7 to 51.2% out of overall infected population. The increasingly obvious involvement of females in the epidemic process resulted in developing perinatal infections, which have led to an increase of the number of HIV-infected pregnant women, of whom 75% are aged 19-29; as well as in the number of children born with HIV. It is also important to note that during the past 10 years, after the implementation of antiretroviral treatment of HIV-positive pregnant women, perinatal transmission of the disease was substantially diminished from 46.5% to 1.5%.

Recently, the number of persons with severe form of the infection has increased. The massive increase in the number of HIV- infected people favors the spread of infection in different population groups.

Manifestations of infectious process

The natural course of HIV infection occurs in several stages:

1) Primary HIV infection. During this period the person may show signs of intoxication, mild fever, headache, general weakness, inflammation of the lymph nodes;

2) Asymptomatic stage or latency. The person feels good and is apparently healthy;

3) Pre-AIDS - a stage with not-severe but advanced clinical manifestations. The person may have swollen lymph nodes, weakness, reduced body weight, diarrhea, fever;

4) AIDS stage. The person develops opportunistic infections (viral, bacterial, fungal, parasitic) and malignant tumors on the background of immunodeficiency.

Laboratory diagnosis

The diagnosis of HIV infection can be made by determining antibodies to both HIV and p24 in the blood serum, detecting viral RNA or proviral DNA, and by detecting HIV.

HIV infection is followed by the formation of antibodies to various viral proteins: p24 – in 96% of infected cases and gp41 –in 88%. However, in the first 6-8 weeks antibodies are not determined after the virus entry into the serum („immunological window"), then the titre of anti-p24 and

anti-gp41 begin to increase during 6 months, therefore,in case of negative results, investigations are repeated during this period.

The determination of antibodies to HIV antigen includes two stages.

The first step is to determine the antigen / antibody summary spectrum by using p24 antigen and p24 antigen compatibility tests via enzyme immunoabsorption assays (ELISA).

ELISA is designed to detect antibodies to both viruses HIV-1 and HIV-2, and is considered a specific and very sensitive method, allowing to detect antibodies in 95% of HIV-infected people.

However, false-positive results are possible during investigations when using this method, therefore the ELISA test is a screening method for HIV infection and the primary positive results are repeatedly investigated through the same system tests, preferably of other batches and in other laboratories.

In case of two positive results, the material is investigated by the high-specific immunoblot (Western Blot) method, which allows the final assessment of the HIV positive or negative status (confirmation test).

Therefore, the laboratory test certificate for HIV is issued only based on the positive result in the immunoblot.

Determination of proviral DNA or RNA is carried out by polymerase chain reaction (PCR). These tests are not considered superior to serological tests, but can be used in diagnosing HIV infection in patients with agammaglobulinemia, acute retroviral infection, neonatal infection during the „immunological window".

The detection of the virus in the culture of mononuclear cells of the patient is possible only in specialized laboratories.

Although virological diagnosis is considered more accurate than other tests, however, it cannot be used as a mass diagnostic method because it requires special security conditions.

Epidemiological surveillance

The object of epidemiological surveillance is the onset of the pandemic, the appearance and spread of HIV in the human population. There are three characteristic stages: the first stage- low HIV infection, the second stage – the HIV / AIDS spread prevails in certain risk groups of the population (the level of prevalence in pregnant women is below 1%), and the third stage - the spread of HIV/AIDS in all population groups (level of prevalence

in pregnant women exceeds 1%) and distinctive features of epidemic manifestation under different social environment conditions.

Epidemiological surveillance includes centralized accumulation of systematic information and recording of all HIV/AIDS cases; investigations of HIV cases, including vulnerable groups; the obligatory epidemiological investigation of each HIV case; the study of the consequences of HIV/AIDS; monitoring the movement of genetic HIV strains, which are resistant to antiretroviral preparations.

All information about HIV/AIDS is analyzed epidemiologically according to the age, sex, social status, territory, risk factors, routes of transmission, (to identify the risk groups, planning risk), the causes and conditions of contamination pathways of HIV spread.

In Moldova the epidemiological surveillance of HIV infection is carried out by the Center for Public Health and medical institutions of all types, based on a continuous collection of information, special monitoring of the incidence and prevalence of HIV/AIDS, detection of new cases,and application of serological test control.

Epidemiological surveillance of HIV/AIDS serves as a basis for determining changes in HIV distribution, prediction and epidemic impact assessment, as well as identification of the risk groups, risk assessment, preventive measures, evidence recording, surveillance and providing specific medical treatment of HIV-infected people, planning health programs and justifying the necessary costs.

Laboratory investigations for HIV are planned and conducted in order to: ensure safety of blood transfusion and transplantation; diagnose individual HIV infection; carry out the epidemiological surveillance of infection within the country; and prevent HIV transmission from mother to child.

Preventive measures

Preventive measures for HIV are taken in three main directions:

1. Preventing sexual transmission of HIV through sexual education, preventive measures, preventing casual sex with different partners, educating the sense of mutual fidelity of partners, explaining the importance of condom use, investigation for HIV screening in partners, investigation of some risk groups such as patients with sexual transmitted infections, etc.

2. Preventing HIV transmission through blood and other biological materials.

The screening of all donors is carried on a mandatory basis for detecting antibodies to HIV-1 and HIV-2 and HIV-1 p24 antigen in order to prevent HIV transmission with donor biological materials (blood, sperm, organs, tissues, etc.). However, the presence of „immunological window" makes it practically impossible to exclude completely the risk of HIV transmission through these materials.

Therefore, to minimize the consequences, donors should be selected carefully, excluding people from risk groups; donors should be investigated;virus should be inactivated via different methods, as well as the transfusion of blood and its components should be performed on the basis of vital indications only. A good way is to quarantine the frozen plasma for 180 days with repeated donor investigations.

The prevention of HIV transmission is performed via diagnostic tests and treatment, whereas prevention of work-related contamination among health workers requires an extensive use of disposable tools, strict disinfection, cleaning before sterilization, sterilization of medical instruments and multiple use equipment.

Medical personnel should follow the security measures for the prophylaxis: to use personal protective equipment, to perform safe invasive medical procedures while using sharp instruments and needles, to avoid incidents and other types of blood exposure, or various manipulations that lead to contamination with potentially infected biological products resulting from medical activity etc.

In case of an incident the following prevenive measures are undertaken:

In case of cutting or pricking while working with blood or other organic fluids, first wash hands with soap, then take off the gloves.

If the mucous membrane contacts with blood and other body fluids (buccal mucosa, eye), it is necessary to wash it thoroughly with running water. The medical tools contaminated with blood or other biological products are removed carefully to avoid the contact with the skin, then being disinfected. The surfaces splattered with blood are disinfected twicely with the solution of lime chloride, 3% chloramine solution, 4% H_2O_2 detergent, 70° alcohol, with an interval of 15 min., then rinsed with water.

The chief of the department is immediately informed about the accident. HIV testing is done in 3, 6, 9, and 2 months.

3. Prevention of maternal-fetal transmission of HIV can be achieved by investigating pregnant HIV-positive women with antiretroviral treatment and choosing the tactics for reducing the risk of virus transmission from mother to child during labor and breastfeeding.

4. Prevention of the virus spread among injecting drug users is performed by explaining the danger, and highlighting the ways of innocuous injecting practices. It is important to fight narcotics.

So far, no means for specific prophylaxis in HIV infection have been developed.

1.1.3.7. Epidemic Typhus

Short history

Epidemic typhus was widespread in the past, especially during social cataclysms (during wars or periods of famine), and the mortality rate reached 100%. The term originates from the Greek typhus (typhi - fever, mental confusion, delirium) and it was introduced into medical practice by Hippocrates.

In 1546 Typhus was first described by Fracastoro, who drew attention to its contagious nature. Later, epidemics of typhus were described by several authors, who named it differently, according to some symptoms or geographical spread: *febris pestilens, typhus contagiosus, febris epidemic, Morbus pulicans, febris petechialis, typhus comatosus, febris Militaris, Morbus hungaricus etc.*

The name of „epidemic typhus" was first mentioned by Saugave in 1715 and Gerhard in 1836, separating it from typhoid fever. In 1876 Mociutkovski and Minh demonstrated the contagiousness of the disease by self-inoculation of the blood from typhus patients. In 1901, Weil and Felix developed the serological diagnostic method of typhus, a method that bears their name. In 1908, Gamaleia, reported about the spread of infection tby body lice, which was confirmed by experimental investigations made by Niolle. In 1916, Da-Rocha Lima isolated the pathogen from the intestine of the louse, and suggested to name it Rickettsia prowazekii in memory of Ricketts and Prowazek, who died during laboratory research to prove the etiology of exanthemous typhus.

Tha pathogen agent

The causative agent of epidemic typhus is *Rickettsia prowazeki.* The microorganisms are unstable in the environment, whilst in dry lice feces

they can live from one to several months. The rickettsia can be found in the body of epidemic typhus patient, as well as inside the epithelium cells of the lice intestines and in their feces. The causative agent of Brill's disease (recrudescent typhus) is the same *R. prowazeki* which persists in humans as latent infection for long periods of time and causes recurrence of the disease at various intervals.

The source of pathogen

The only source of infection is a human patient infected with epidemic typhus, who is being contagious during the last days of the incubation period (2-3 days), during the entire fever period, and untill the 7-8th day of the disease after the body temperature normalizes. The overall contagious period lasts 20-21 days. The infection is transmitted by body lice, less frequently by head lice. Body lice deposit their eggs on hair, in pleats and folds of underwear and clothes, while head lice lay them only on hair. Eggs yield larvae which undergo triple sloughing before they grow to mature insects. This period lasts 7-10 days and should be considered in case if the focus has beed identified and while prescribing repeated sanitary treatment. When a louse sucks the blood of a typhus patient it becomes contagious in 4-5 days during which rickettsia multiply in the louse intestinal epithelium. After the epithelial cells of the rickettsia are destroyed, they enter the intestinal lumen and are excreted in great amount with feces. When a louse sucks blood, it excretes a substance that causes itching. As a person scratches the site of a louse bite, he eventualy rubs the faecal mass with rickettsia into the puncture wound of the scratching site. Contamination can occur when a crushed louse is rubbed into the skin. An infected louse lives 20-40 days instead of 45-60 days and dies of the rupture of intestine.

Exanthematic typhus is a typical anthroponosis. The source of the pathogen is the patient with typical or atypical symptoms and contagious for the body lice during the last 1-2 days of incubation (which lasts from 6 to 21-23 days, more frequently 10-12 days); the feverish period lasts for 17 days and during the first 2-3 days of aprehexia, during the circulation of the pathogen in the patient's blood, which does not exceed 20-21 days. The concentration of rickettsii in the patient's blood is higher in the first stage of the disease and even lower in the final stage, being higher in severe form as compared to the mild one.

Manifestations of epidemic process

Exanthematic typhus was widely spread in the past, especially during

wars, being characterized by massive epidemic or even pandemic events. A good example is the epidemic spread of typhus in Russia during the Civil War (1918-1920),, when more than 6 million typhus patients were recorded, with an incidence of 3300 and 3757 cases per 100,000 population in 1919 and 1920, respectively.

A steady increase of the death rate due to typhus disease occurred in the II World War and in the postwar period, including Moldova, where the incidence of this infection reached 240-250 cases per 100 thousand population.

Lately, typhus was eradicated in many countries, while in others, including Moldova it was sporadic. Some countries of Africa, Asia and South America remain endemic.

Persons who work in the service sector (hairdressers, bathrooms, laundry rooms, public transport, healthcare institutions), and prisons, refer to high-risk groups. Seasonality refers to winter-spring periods as a result of room aglomerations, wearing of warm clothes, the lack of hygienic conditions, factors that lead to activation of transmitters.

In case of contracting the infection, it leads to the development of sustainable immunity,while the disease may reccur after several years due to the presence of a causative agent in the body and loss of immunity, as well as after a severe trauma, physical and mental exertion, and old age (Brill-Zinsser disease or repeated typhus). Typhus may spread only in the presence of lice.

The manifestations of infectious process

The disease has an acute onset. It is characterized by high body temperature, headache, chills, malaise, thirst and loss of appetite. Headaches intensify and become debilitating; insomnia develops. Irritability and anxiety during the first days are then followed by excitation. Increasing symptoms, especially weakness and fever, get the patient to his bed on the 2-3rd day of the disease. Hyperaemic and swollen face, hyperaemic conjunctiva, injected and dilated scleral vessels (rabbit eyes) are observed during the first days of the disease. The skin of the neck and the upper trunk is also hyperaemic. The tongue is dry and it is difficult to protrude (Godelier's sign). The skin is dry and hot. Dyspnoea (central), moderate tachycardia and hypotension occur. Petechial haemorrhages can develop within 3 days on the margin of the conjunctiva; haemorrhages also appear on the shoulder or thigh, below the point where a tourniquet is applied.

Specific rash (Plate VII) occurs on the 4-5th day of the disease and the initial period of the disease ends. During the period of the disease the full-blown rash is initially characterized by roseoles which later change to petechia. The rash develops on the flanks, chest, and back, and then covers the whole body. The rash is severe and differs in size i.e. the size ranges from that of a pin-tip to that of a lentil. In moderate cases the rash lasts 12-14 day. As the rash develops, the fever intensifies and becomes continuous or remitting, and persists for 6-8 days. The period of high fever is characterized by moderate neutrophilic leucocytosis (to 9-11 x 109/1), thrombocytopenia and aneosinophilia; ESR is high (18-25 mm/h). The initial symptoms are worsened by skin eruption and new symptoms. The nervous system becomes increasingly involved. The consciousness is clouded; hallucination and delirium develop; the patient is restless and tries to get up from his bed or run. This condition, when high fever is present,is known as *status typhosus*. The following symptoms of meningoencephalitis develop: stiffness of the neck, Kernig and Brudzinski's symptoms, increased tendon reflex, tremor of the extremities, inarticulated speech, throat itching, and difficult swallowing. The heart sounds are dull and the rate is fast, arterial pressure is low and a collapse is possible. The liver and spleen are enlarged. Constipation develops. Severely ill patients can defaecate and urinate involuntarily, or the urine is excreted in small portions while the bladder is overfilled. In 12-14 days of the disease, the body temperature falls and the recovery begins. The fever is caused by an accelerated lysis within 2-3 days; in rare cases the temperature drops critically. Toxaemia decreases and all its symptoms gradually decrease. The rash and consciousness clear up; the patient shows interest to the surroundings; the sleep and appetite improve; urination normalizes; pulse and arterial pressure normalize as well.

Despite a considerable improvement of the patient's general condition, the weakness and pain still persist in the legs and by the course of the nerve trunks for a long time. The central nervous system remains easily excitable. Mild, moderate and severe forms of epidemic typhus have been described. The clinical picture of the disease has considerably changed in recent years (compared to the classic symptoms described earlier). Mild forms of the disease are of greater epidemiologic importance due to the specific fever, rash, toxaemia and less marked nervous and vascular lesions. The body temperature, which is 38-39°C persists for 7-9 days. Roseoles

are prevailing in rash. Pronounced headache and insomnia develop, while other nervous symptoms are either absent or mild. The status typhosus is commonly not present.

Laboratory diagnosis

The diagnosis is based on clinical, epidemiologic and laboratory findings. The serological methods of examination are the most important ones. Blood specimens (3-5 ml) are tested in the laboratory, since the 5-7th day of the disease. Rickettsia agglutination reaction, complement fixation test, indirect haemagglutination, immunofluorescent and allergic tests are among the most sensitive methods. The reaction of rickettsia agglutination with corpuscular rickettsia antigen is considered positive with titres 1:160 and higher. However, the use of these reactions is limited because their results are not always trustworthy and cannot be used for retrospective diagnosis. Complement fixation test is conducted with a corpuscular or soluble rickettsial antigen. The diagnostic titre is 1:160 and higher. The maximum quantity of antibodies is found within 12-20 days of the disease (1:640-1:1280). Indirect haemagglutination tests are the most diagnostically valuable. They are performed on the 3rd-5th day of the disease and the result is considered positive with titers 1:250 and higher. The maximum antibody level is observed within 14-20 days of the disease (1:1000 and higher).

Epidemiological surveillance includes early detection of cases of primary and recurrent exanthematic typhus (Brille disease) and pediculosis (lice) in human population. The morbidity rate is directly proportional to the level of lice population, hygienic conditions in a community, socio-economic and sanitation conditions.

Preventive measures require first of all combating pediculosis, which includes screening of people affected by pediculosis, disinsection of the linen and clothing, health education and hygienic conditions for the population.

In typhus the specific prophylaxis is of secondary importance and is reduced to the use of inactivated or live vaccines based on epidemiological indications.

Anti-epidemic measures include immediate hospitalization of the patient, epidemiologic examination, observation of persons who had contacts with the diseased person, patient's laboratory examinations, disinfection of the focus, and, if necessary, appropriate treatment of

the population in the immediate neighbourhood to the focus. After the hospitalization of the patient, disinsection in the focus should be performed not later than 3 in hours in cities and towns, and in 6 hours in rural areas. The patient should not change his underwear before hospitalization. All diseased patients should be given appropriate treatment in special disinfection units or in common bathrooms, which are transformed into such units. All clothes, linen, underwear, and fomites should be disinsected in steam-formaldehyde or hot air chambers. The rooms and other room spaces should be treated with 0.5% chlorophos, 0.5% methylacetophos, 5% lysol solution, or 10% dilor (10-15 g per square metre). If special disinsection chambers are not available, soft fomites, linen and underwear are boiled in a soda-soap solution or soaked in a 0.15% aqueous emulsion of carbophos (20 minutes), 0.25% aqueous emulsion of dicresyl (20 minutes), 0.5% aqueous emulsion of methylacetophos (30 minutes); the rate of liquid consumption is 4 litres per 1 kg of linen (with subsequent washing). Garments and clothes can be treated by dusting with a 5 p% methylacetophos, 1% neopin, or 10% dilor dust, or by spraying with aqueous emulsions used for soaking the linen. After disinfection, the things should be wrapped tight in bedcloths for 2-3 hours and then well aired. If head lice are detected (from 1 to 10 species, nits included), they should be eliminated mechanically by combing, or by cutting and shaving the hair on the head. The hair should be collected and burned. If necessary, nits are removed from hair by rinsing with 5-10% acetic acid solution with subsequent combing (cotton wool or threads must be passed between comb teeth and wetted well with acetic acid solution). In moderate or severe pediculosis (from 10 lice and more), insecticides should be used. This procedure is however contraindicated in infants under 5, pregnant and nursing women, persons with injured skin, etc. The following insecticides are recommended: 0.15% aqueous emulsion of carbophos (10-50 ml of the emulsion for a person), 20% soap suspension of benzyl benzoate. The persons who were exposed to the danger of infection should be observed during 25 days with obligatory daily thermometry. In order to reveal the source of infection, all persons who had contacts with the diseased patients, as well as the persons who had a disease with fever within the past three months, should be examined by complement fixation and indirect haemagglutination tests. If the tests are positive, special medical follow-up

must be provided with repeated serological testing. If body temperature is increased in those who had contacts with the diseased persons, they should be hospitalized and the diagnosis should be made.

1.1.3.8. Brill disease

Short history

The first records of repeated epidemic typhus in former patients were made by Brill in 1910 and Zinsser in 1934. Since 1935 repeated epidemic typhus has been known as „Brill-Zinsser disease."

The pathogen is the same as in exanthematic typhus.

The source of pathogen is the person who had epidemic typhus 10-40 years ago, as a rule, older people, even in the absence of pediculosis. Patients with Brill disease can contaminate others only in the presence of lice. It is considered that some people infected with typhus can keep the pathogen in the body in the cells of the lymph nodes, liver and other organs. Recurrent cases are caused by immunosuppression.

In Brill disease the infectious period is shorter than in typhus and lasts 5-8 days.

Manifestations of epidemic process

Brill disease is manifested by sporadic cases, especially in areas where typhus outbreaks occurred in the past. Brill disease is observed commonly in old people, generally in urban areas with no presence of lice.

Clinical manifestations

The infection is mild, with a short period of clinical simptoms (during 7-12 days). The symptoms are simillar to those of epidemic typhus but are less pronounced.

Laboratory diagnosis - the same as in typhus.

Prophylactic measures - the same as in typhus.

Anti-epidemic measures - the same as in typhus.

1.2. Zooanthroponosis

1.2.1. Anthrax

Short history

Anthrax is a disease which is known since ancient times. The first studies on the disease refers to the 18th-19th centuries, when the epizootic diseases and epidemics reached the highest incidence rate. In 1752, Moret called this malignant manifestations as „pustule rashes" „and later it was called,,charbon" (anthrax, charbon) because of the black color of the skin.

In 1760 the disease was first described by Furnie in France. Nowdays, anthrax is widely spread in Europe and Siberian regions as well. The Russian doctor Andreevsky contributed significantly to the study of anthrax and demonstrated the identity of animal and human disease by self-inoculating blood from diseased anthrax animals. He studied the disease in 1786-1788 in the Ural region and called it „Siberian ulcer". In 1831, S. Hotovitki studied the geographical spread of anthrax, and in 1863 P. Bogdanov published the paper „About carbunculus sick persons".

In 1876 Koch isolated the pathogen and observed the sporulation phenomena. In 1881 L. Pasteur in collaboration with Chamberland and Roux obtained live anthrax vaccine for animal immunization.

The first authentic reports of the spread of anthrax in Moldova refer to the 19th century, although the infection had been widely spread in this territory long before. During 1895-1897 there were recorded 2,560 cases of anthrax in animals in Bessarabia, whereas, in the period of 1906-1914, there were 5,342 cases of human anthrax that is about 600 cases each year.

In Moldova the anthrax morbidity rate was significantly reduced and became sporadic after the study of the epidemiology of anthrax and implementation of preventive and anti-epidemic measures, including the planned vaccination of animals and humans.

Shleahov E., E. Gruz, V. Prisacaru contributed significantly to the study of anthrax in Moldova.

The pathogenic agent of the disease is Bacillus anthracis, it is a Gram-positive immature aerobic microorganism of 1.0-1.5 μm x 8-10 (мкм) size. There are two forms of B. anthracis: vegetative and sporulated.

The vegetative form is usually found in the living organism (animal and human). As a rule, the vegetative form produces a capsule that has a protective function - it inhibits the humoral bactericidal properties. The vegetative form is sensitive to exogenous factors. It is resistant during heating at a temperarure of 50°C within 40 min, at a temperarure of 60°C - 15 min., at a temperarure of 75°C - 1 min. They can be destroyed easily by usual disinfectants. The vegetative form is formed only in a receptive body. It produces a complex of toxins, consisting of three factors: I factor - inflammatory or edematic, II factor - protector (immunogen) and III factor - lethal. This complex of factors plays an important role in the pathogenesis and immunogenesis of the infection.

The vegetative form is transformed into spores only in the presence of

oxygen or in the external environment. The spore is placed in the center of bacillus and does not distort it. The sporulated form is very resistant to the external environment. For example, it can survive for ten years (even 70-80 years) in the soil, it may also persist on fur, wool and skin of sick animals for several years. It is resistant to boiling during 15 minutes; if it is autoclaved in wet hot air at 110°C, it lasts 40 minutes; it is destroyed in hot dry air at 120°-140°C - in 1.3 hours. It is also very resistant to the action of disinfectants. They can be destroyed with 20% lime chloride or activated desinfectants.

The sources of pathogen

The source of anthrax pathogen can be found in domestic herbivorous animals: cattle, horses and pigs. In other regions it can be camels and reindeer as well. Anthrax occurs in animals with acute septicemia and usually it is results in fatal outcomes. Dogs and cats cannot be infected with anthrax.

Herbivorous animals acquire the infection primarily through the food and water contaminated with B. anthracis, rarely by haematophagous insect bites. The soil may become a reservoir of B. anthracis if it is contaminated during slaughtering or skinning of diseased or dead animals with anthrax. This phenomena forms the telluric focus. It is known that if the soil is contaminated with anthrax bacilli, the natural focus will persist a long period of time and it forms a long-lasting focus of B. anthracis in nature.

The animals catch the infection mostlly in summer. The animal contamination incidence is greater during drought periods due to the damage of the mucous membranes, through which anthrax bacilli easily penetrate. According to the correlation study (V. Prisacari, 1990) it was found that the incidence of anthrax in animals is directly proportional to the annual temperature ($r = 0.657$) and inversely proportional to the amount of annual rainfalls ($r = -0.762$). Animal contamination can occur through flour of meat or other food products contaminated with B. anthracis.

The incubation period of anthrax is 1-3 days in animals, rarely longer. As a rule, they develop septic infection. Symptoms vary according to species of animals. The disease progresses acutely in sheep, it is accompanied by high fever, cyanosis of the mucous membranes, and salivation with blood. The animal falls into convulsions and dies in few minutes or sometimes in several hours. Anthrax develops hemorrhagic gastroenteritis in horses or it is manifested by the appearance of carbuncles on the neck which lasts

1-3 days. There are similar symptoms in cattle, but the disease can develop during 5-7 days. In swines, the disease is manifested by local inflammation of the retropharyngeal lymph nodes and edema in the submaxillary region.

Diseased animals are contagious throughout the disease and eliminate the pathogens with urine, feces, bleeding secretions from the oral cavity and milk. A higher amount of pathogens are eliminated with blood in the external environment during slaughtering or skinning of sick animals.

Pathogen transmission to humans can occur through direct contact with diseased animals when taking care of them, veterinary assistance, particularly during slaughter, cutting, etc., and indirect contact as a result of handling and consumption of products from a diseased animal. Blood, meat, skin, wool, bones, etc. can be transmission factors. The contamination with B. anthracis can occurs through contact with animals, manufacturing and use of clothing of animal origin (coats, hats, collars or sheepskin caps), inhalation of aerosol containing spores through the contact with contaminated soil with spores of B. anthracis, and haematophagous insect bites as well.

The pathogen enters the body, usually through the damaged skin or mucous membrane and by breathing in the dust contaminated with B. anthracis or eating improperly cooked meat products.

Direct transmission from a sick person to a healthy one is possible, but it does not have any epidemiological importance.

Manifestations of epidemic process

Anthrax occurs in all continents, but more frequently in countries where the population raise livestock or have favorable conditions for a long-term persistence of the pathogen in the environment. Anthrax is endemic in Asia, Africa, South America, the Caribbean region, in Eastern Europe and South America, the Russian Federation, and Middle East.

The general incidence of anthrax in animals and human population dropped after obtaining the vaccine and its application in animals. This phenomenon is characteristic of Moldova, when during the period of 1946 - 2012, anthrax morbidity rate was reduced more than 1,000 times in human population *(Figure 43)* and 600 times in animals. Only in 1946, anthrax was recorded in 315 of regions, where 582 people infected with anthrax from farm animals and 432 from humans. In the last 2-3 decades both the epidemic and epizootic processes occur sporadically, although the risk of catching anthrax exists due to the presence of spores in the soil.

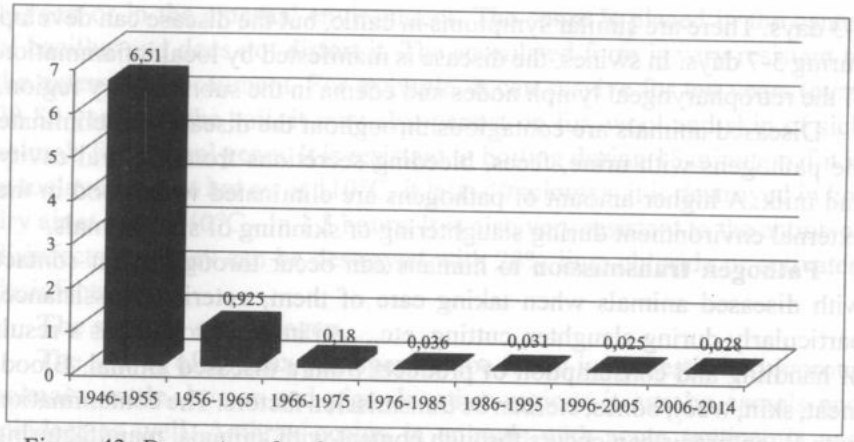

Figure 43. *Dynamics of anthrax incidence rate in the Republic of Moldova.*

Anthrax cases occur predominantly in the Northern part of Moldova, which is characteristic of a typical black soil, which contains humus up to 10-12%. Rare cases of anthrax were reported in the Central part of Moldova, which is characterized by forest soils with high acidity and a 4.5% humus content, and the Eastern Zone, which is rich in carbonate chernozem; poor humus is unfavorable factor for maintaining and multiplying of the pathogen *(Figure 44)*.

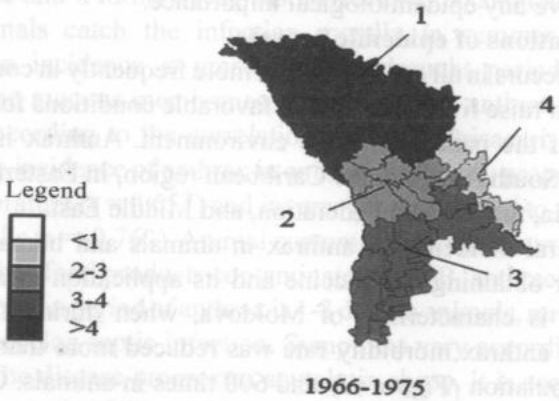

Figure 44. *The intensity of epizootic process of anthrax in the Republic of Moldova (number of cases per 100 km²), in 1966-1975, depending on the teritorial areas.*
1 – North, 2 – Center, 3 – South, 4 – South-Est (V. Prisacaru, 1990).

Clinical manifestations

The pathogen enters the body through injured skin and mucosa. There are two clinical forms of the disease which occur in humans: cutaneous and septicaemic anthrax. The septicaemic form can be both primary and secondary (complicated cutaneous form of the disease). The cutaneous form is more common than the septicaemic anthrax. The skin of the arms, head or other exposed parts of the body are involved. A pustule develops at the portal of the anthrax pathogen entry. It is a serous-haemorrhagic inflammation of the skin and subcutaneous fat with subsequent necrosis, oedema, and regional lymphadenitis. If the body resistance is low, the agent enters the blood and multiplies to cause primary septicaemia. The regional lymphatics are also involved and are accompanied by severe oedema and necrosis. General infection develops, which is manifested by the increase of bacteraemia and toxaemia. Toxaemia is followed by a sudden increase of the body temperature, cardiovascular disorders, and shock that may lead to death.

The incubation period lasts from 2 to 14 days in the cutaneous form and from several hours to 6-8 days in septicaemic anthrax.

Cutaneous anthrax. The disease begins with the development of a pruritic spot at the portal of infection entry. The erythematous spot rapidly develops to a copper-red papule and then a vesicle (pustule) containing cloudy sanguinous fluid. The patient scratches the pustule because of severe itching; sometimes the pustule ruptures spontaneously, and a black eschar is formed which grows in size. The eschar looks like coal (hence the name, anthrax, which means in Greek coal or carbuncle). Secondary pustules develop around the eschar and undergo the same stages of the development. Infiltration develops under the crust. It rises above the intact skin level in a shape of a brown-red ridge. The carbuncle is surrounded by a massive oedema of the skin and subcutaneous fat. The oedema is especially marked on the skin with underlying loose connective tissue. The adjacent skin is specifically painfull except for oedema and the carbuncle itself: even during needle punctures. Lymphadenitis is common. Only one lymph node is usually enlarged. It becomes firm, mobile and painless. During the first hours of the disease the patient complains of headache and malaise. In 2-3 days, the body temperature increases to 39-40°C. The signs of toxicosis develop: headache intensifies, weakness and fatigue becomes more pronounced, the cardiovascular system becomes involved

(the pulse is fast, superficial and sometimes arrhythmic; arterial pressure is low). The liver and the spleen are enlarged. The general symptoms are less pronounced in cutaneous anthrax and the patient can continue performing his routine duties. The condition improves in 5-8 days. The body temperature decreases, oedema gradually subsides. By the end of the 2nd or 4th week, the crust falls off and the ulcer heals rapidly to leave a scar. Cutaneous anthrax can be complicated by septicaemia.

Septicaemic anthrax. The disease begins suddenly with the increase of the temperature to 39°C, chills, weakness, headache, non-productive cough, and dyspnoea. Diffuse abdominal pain is possible. Numerous haemorrhages develop. The temperature rises to 40-41°C.

Severe toxaemia damages the vessels and thus causes the haemorrhagic syndrome in many organs and tissues (the lungs and intestine included). Therefore, pulmonary (inhalation) and intestinal types of septicaemic anthrax are distinguished. Inhalation anthrax is characterized by pneumonia with oedema of the lungs and haemorrhagic pleurisy. Cough intensifies, sputum is first seromucous and then rusty (haemoptysis). The intestinal form of anthrax is manifested by severe stabbing pain in the abdomen, vomiting with bile and blood, and bloody diarrhoea. Symptoms of intestinal obstruction develop later due to paresis of the intestine. Septicaemic anthrax is characterized by euphoria, delirium, convulsions and meningeal syndrome. Pulse is fast and weak (thready); arterial pressure falls. The patient dies of septic shock during the first day (less frequently on the second day) of the disease. Haematologic studies show high leukocyte count (to 20-25 x 109/1) and neutrophilia shifting to the left.

Laboratory diagnosis

In humans the diagnosis of anthrax is based on epizootologic, epidemiologic, clinical and laboratory data. Cutaneous anthrax should be differentiated from furunculosis, carbunculosis, erysipelas, insect bites, ulceroglandular tularaemia, bubonic plague, and glanders.

Excretions and blood should be examined in the laboratory (preferably before treatment begins). Contents of vesicles, carbuncles and ulcers are taken with a syringe, pipette or a sterile tampon. The skin around the carbuncle should be carefully cleaned with alcohol. Blood specimen (1 ml) should preferably be taken during the fever period. Venous blood (1-2 drops) is inoculated into a nutrient medium (agar or Hottinger's broth); two smears are taken for culture and put on glass slides. Samples

of animal material, such as pieces of hide, wool, hairs (20-30 g) should be sent to the laboratory whenever it is necessary. Soil (200 g) and water (at least 1 litre) should be also examined. Materials taken from the patient and animal specimens should be placed into sterile boxes, bottles, or other laboratory glassware. Dry smears are placed in Petri dishes and wrapped in thick paper. The pack must be labelled: „smear is not fixed". The lable must also contain information on the place and time of taking the sample, the name of the material, and the presumptive diagnosis. The name of the patient must be written on the label too. The specimens should be placed in a special sealed box. The box should be handled with care and not overthrown. The luminescent method, thermoprecipitation, and detection of capsule formation are used in the laboratory. The tests are preliminary, and negative results do not exclude anthrax. Bacterioscopy and cultures help isolate and identify the anthrax agent. Inoculation of albino mice, guinea pigs or rabbits (biological tests) is performed simultaneously with inoculation of cultures. The experimental animals are observed for 10 days. Smears are taken from blood and organs of the dead animals; corresponding cultures are also grown. Preliminary conclusion can be formulated in 3-5 hours and the final one is ready in 4 days. The final diagnosis is made only on the basis of the complex studies.

A skin allergic test with dilute immunoglobulin can be done on the first day of the disease. It is positive by the end of the first week in almost all patients.

Prevention and control. Control of anthrax in domestic animals includes veterinary sanitation. It also includes timely revealing, isolation and treatment of diseased animals, their protection from insect bites, and decontamination of feces. Diseased animals should be killed by a bloodless method in order to prevent formation of spores. Killed animals should be burned. Utilization of killed animals or their burial is prohibited. Herds of animals where anthrax cases were detected should be unde the surveillance. It is prohibited to pasture cattle on meadows suspected in infection with anthrax. Cattle may not be allowed to drink water from suspected ponds or other water bodies. Pastures should be given sanation treatment. Animals should be vaccinated with live attenuated vaccines.

Prophylaxis of the disease in humans includes adherence to safety requirements when attending diseased cattle, slaughtering cattle, and when skinning, processing of hides and wool, etc. Hides should be pickled

(treated with 2.5% hydrochloric acid and 15% sodium chloride solution at a temperature of 30°C for 40 hours). Wool should be treated with steam at 100-110°C or with a steam-formaldehyde mixture at 62-65°C for 2 *1/2* hours. Furs should be given similar treatment. Current and final disinfection are required. Preventive disinfection should be done at least twice a year at animal farms, slaughterhouses, at plants processing animal materials, in storehouses, and in vehicles used for transportation of cattle and animal products. Workers should wear protective overalls, they should be provided with disinfectant solutions and means of individual protection: goggles, masks, etc.

If a given batch of animal material is suspected for anthrax, its specimens should be sent to the laboratory for Ascoli's thermoprecipitation test. Specimens of wool, fur, hide, etc. (20-30 g) should be handled in sterile test tubes. Special live vaccine is used in specific prophylaxis of anthrax in the population. Only the persons exposed to the risk of contamination should be vaccinated. Vaccine is given in a single dose, either subcutaneously or by scarification. Revaccination should be repeated each year. The vaccine should preferably be given subcutaneously by jet injections. Contraindications are the same as for other vaccinations.

Anti-epidemic measures in the focus include the hospitalization of the patient. Cutaneous anthrax patients should be discharged after cicatrization of the ulcer and the the crust falling off. Septicaemic anthrax patients can be discharged from hospital after relief of clinical symptoms. Final disinfection should be performed in the focus. Epizootologic and epidemiologic studies are necessary. The contact with animals should be avoided while medical observation is performed within 8 days. The same measures are applied in those persons who had contacts with the diseased animals, pathologic materials, and who used infected meat in food. Anti-anthrax immunoglobulin is recommended in prophylactic purposes. The immunoglobulin is given to persons who had contacts with the diseased animals, those who participated in burying of dead animal, processing meat of diseased animals, or those who used this meat in food. Antibiotics and immunoglobulin can be administrated in emergency prophylaxis of anthrax.

Phenoxymethylpenicillin is to be given in a dose of 1 g twice per day for 5 days, or 0.5 g of tetracycline twice a day for 5 days. Other antibiotics can be also administered: ampicillin (1 g twice a day) or

oxacillin (1 g twice a day). Adults should be administered 20-25 ml of immunoglobulin for prophylactic purposes. Adolescents should be given immunoglobulin in doses less than 12 ml; children should be given from 5 to 8 ml. Immunoglobulin should be warmed before intramuscular injections. Individual sensitivity to horse protein should first be tested by intracutaneous test. Immunoglobulin is useless if more than 10 days have passed since the day of the contact or in more than 5 days after the ingestion of infected meat.

1.2.2. Brucellosis

Short history

The disease was first described by Marston in 1861, during the Crimean War, and it was called *Mediterranean remitting fever*. In 1868, David Bruce isolated the pathogen and called it *micrococcus* due to its small size and demonstrated its infectivity on monkeys. Later the genus was called „Brucella" and „Brucellosis disease", in the name of Bruce.

In 1897, Wriht described serum agglutination test and in 1907, Zammit demonstrated experimentally, the transmission of the disease through milk. Later, the disease was called „fever of Malta", as a result of brucellosis epidemic in the crew of the„ Joshua Nicholson" ship, transporting goats from Malta to New York, whose sailors fell ill after drinking milk from the goats.

The pathogen agent

This infection is caused by microorganisms of six species. Human brucellosis is caused by the following three species: *B. melitensis* (goats), *B. suis* (hogs) and *B. abortus* (cattle). The microorganisms were discovered by the English physician David Bruce in 1886 in preparations of human cadaveric spleen. (Hence the names of the pathogenic microorganism, *Brucella,* and the disease due to these causative agents, brucellosis). The causative agent of brucellosis in goats, *B. melitensis,* is the most pathogenic for humans. Brucella is sufficiently stable in the environment. It survives for 3 months in soil, in milk - 10 days, in sheep cheese - 45 days, and in wool - 3 months. It is sensitive to high temperature and boiling kills it instantaneously; heating to 60°C for 30 minutes is also detrimental. The microorganisms are sensitive to disinfectants (3% lysol solution, 5% solution of lime chloride).

The source of the pathogen agent

Brucellosis is a typical zoonosis disease because domestic animals

are a reservoir of infection. Goats and sheep are the commonest source of infection in humans. Cattle and swine are less important as the source of infection. If healthy and diseased animals of various species are grown together, brucella migrates from goats to cattle and other animals. Outbreaks of the disease in humans are possible as well. Cats, dogs, camels, deer, horses are a secondary reservoir of the infection. A diseased person does not have any risk to the surrounding people. Diseased animals eliminate the pathogenic microorganisms with amniotic fluid, abortus, urine, manure, and milk. Besides, the blood and flesh of the animals contain the microorganisms. The infection is usually transmitted by direct contact with the animal excrements and objects infected by these excrements. Humans get infected when taking care of the animals, during milking, shearing, etc. Another route of infection transmission is through infected foods, such as raw milk, curds, sheep cheese prepared from raw milk, and also through meat of the diseased animal. There is still another, rather rare route of infection transmission - by air-borne droplets. Humans get infected during processing wool of the diseased animals. Since brucella survives for a long time in water, water-borne infection should not be excluded either. Brucellosis is an occupational disease of animal breeders and farm workers. Immunity, that is produced in those who suffered the disease, is unstable. The highest incidence of the disease is during the spring and summer. The first wave of morbidity is recorded in the early spring. The infection is spread by direct contact due to intensive excretion of the organisms with miscarriages, placenta, amniotic fluid, etc. Another wave of seasonal morbidity occurs during the period of maximum lactation in domestic animals. The incidence of brucellosis among the workers of slaughterhouses and meat processing plants coincides with the time of mass-scale slaughter of cattle.

Routes of transmission

Transmission of pathogens from disased animals to humans occurs through alimentary and contact routes. The transmission factors are: unpasteurized dairy products, insufficiently cooked meat, fresh sheep cheese, skin, especially from aborted lamb and soil. Human contamination occurs both by eating infected products from a sick animal and by direct contact with skin and conjunctiva during animal care, wool and skin processing, cleaning etc, transportation, processing and storage of animal products, improper working regime in laboratories related to brucellosis investigations.

Manifestations of epidemic process

Brucellosis is an ubiquitous infection. Outbreaks of brucellosis have been reported on all the continents. Morbidity in humans depends on the diseases in cattle, sheep and goats. Brucellosis is recorded mainly in regions,where livestock is grown such as in the Mediterranean Basin, Latin America and Asia.

Brucellosis occurred in Moldova during the period of 1947-1955. It was triggered by the collectivization process of the cattle farms while cattle was imported from unfavorable territories affected by brucellosis. There were found 9,000 animals (cattles) and 280 persons infected with brucellosis in that period. Due to active programs, brucellosis has been eradicated in the territory of Moldova. In the last four decades cases of outbreaks of brucellosis have been decreased, mainly because of some reduced import of animals.

Annually, the WHO reports about 500,000 of new cases. The true incidence rate is probably higher due to the polymorphism and clinical diagnostic difficulties.

The receptivity of the human population to brucellosis is a general one, although the infection more commonly occurs in people who are at an increased risk of contamination,depending on their type of activity (veterinarians, shepherds, millers, slaughterhouse workers, etc.). The epidemic process is more often manifested in the rural population (≈75%), mainly in males (≈65%).

Seasonality is characteristic ofr brucellosis, particularly in winter-spring period of calving in sheep and goats. The incidence of brucellosis increases in the summer months and extens until October-November, being determined by the lactation period in large cattle. Contraction of infection occurs mainly through contaminated milk and other dairy products.

The manifestations of infectious process

After an incubation period of 1-3 weeks (sometimes up to 2 months), the disease develops slowly, rarely acutely. The acute period lasts from a few days to a few weeks. Acute brucellosis shows signs of acute septic infection with remitting fever, profuse sweating, predominantly nocturnal, asthenia, anorexia, micropolyadenopathy, musculoskeletal impairment in 10-15% of cases, genital and peripheral nervous system disorders.

Subacute forms are characterized by relapses, polyorganic disorders, allergic reactions (exanthema, dermatitis, etc.). Typical musculoskeletal

disorders (arthritis, polyarthritis, synovitis, bursitis, etc.), genital imparment (orchitis and epididymitis in men, menstrual cycle disorder, endometritis and abortions in women), nervous system disorders (plexitis, sciatic neuralgia).

Chronic forms of brucellosis are characterized by reccurent infectious processes. Various organs and systems, and complex organ damage are affected predominantly. Chronic brucellosis may last 2-3 years, and even more in some cases.

Both organ and system damages, as well as complex organ impairments prevail. Chronic brucellosis may last for 2-3 years, and in case of reinfection even more.

Laboratory diagnosis

The laboratory investigations are very important in the diagnosis of brucellosis, since it commonly does not have any clinical simptoms, while symptomatic forms show a polymorphic nature.

The laboratory diagnosis ensures the identification of bacteria in the blood and tissues, but the results may be false-positive in 10% of acute cases. An intradermal skin test can be applied only on the 3rd week of the disease.

Epidemiological surveillance in brucellosis provides both prevention of the pathogen import through diseased animals and control of the epizootological situation among animals via investigations.

Preventive measures

Prophylaxis of brucellosis in humans is based on the prevention and eradication of morbidity in animals, avoiding habitual and industrial contamination of people and immunization of the risk groups.

The first group measures are achieved through:

a) Protection of the areas (livestock farms) from the import of the infection;

b) Carrying out regular sero-allergic tests (planned and based on epizootological indications) in livestock, aimed at detecting and removing the infected animals;

c) Vaccination of animals: cattle- with live attenuated vaccine of B. abortus strain 19; and sheep and goats- with B. melitensis strain Reu1.

The prevention of contracting the infection by humans is based on the education of the population about the risk of consuming raw or unpasteurized milk or dairy products from uncontrolled brucellosis farms,

and vaccination of people at risk of catching the infection in unfavorable areas.

For the purpose of prophylaxis, professionals who work in livestock areas or processing units which present risks of contamination with brucellosis, may be admitted to the job only if they are:

a) investigated for brucellosis;

b) vaccinated against brucellosis;

c) show sero-positive allergic reaction to brucellosis;

d) provided with protective equipment and means of disinfection;

e) instructed on the measures to prevent the infection.

In order to prevent food contamination, milk from farms is subjected to thermal processing by boiling or pasteurization. Meat also is heat-processed. A veterinary surveillance is established on food markets to ensure the safety of animal products and prevent brucellosis.

Anti-epidemic measures

It is mandatory to report suspected cases of brucellosis. The patient is hospitalized. Admission to the hospital is determined by the difficulties of treatment and not of infectiousness.

Epidemiological investigation is carried out concurrently with the epizootic one as it allows detection of animal sources and achieving measures. It is necessary to intensify the decontamination of food and personal hygiene compliance in outbreaks of brucellosis.

The epidemiological survey is carried out simultaneously with the epizootiological one, as it allows the detection of animal sources and achieving the epizootic measures. It is recommended to increase the measures for food decontamination and personal hygiene in the outbreaks of brucellosis.

1.2.3. Salmonellosis

Short history

In 1884 Salmon and Smith, US, isolated from pigs a germ which was initially called *Bacillus choleraesuis* and in 1988 Gartner, from Germany, isolated another germ from both animals and patients suffering from food poisoning with contaminated meat of those animals. The germ was called *Bacillus enteritidis*. In 1900, the Salmonella genus was introduced into the taxonomy of microorganisms, and the described microorganisms were

introduced into this genus type. Over the years, the Salmonella genus has proven to be very varied in species and subspecies.

The pathogen agent

Salmonellosis is caused by a series of microorganisms belonging to the Salmonella genus of the Enterobacteriaceae family, which comprises 0.6 / 2.4 μm gram-negative bacilli, with mobile cilia peritrichia (except Gallinorum / Pullorum serum), which ferment glucose and produce gas. The antigenic structure of Salmonella species contains over 2500 species. Salmonella genus consists of two species: *Salmonella enterica*, which includes six subspecies: *enterica (the largest), salamae, arizonae, diarizonae, houtenae indicates, and Salmonella bong.*

All salmonellae are divided into serological groups (A, B, C, D et al.), according to Kauffmann-White scheme; and within the group (A, B, C, D et al.) – there are more than 2500 serological variants. In Moldova, there were detected more than 100 variants of serological Salmonella, including S. Typhimurium, S. enteritidis, S. virchov, S. java, S. newport, S. anatum and others.

Salmonella refers to microorganisms with increased resistance to the external environment, which easily supports drying and low temperatures. Salmonella can survive in soil up to 4.5 months, in frozen meat, in eggs - up to one year, in water, cheese, butter - up to 4 months, in milk - 10 days, in sausage - 75-80 days. At the same time, it dies at 70°C in 5-10 minutes, but it resists to boiling in a piece of meat measuring 10-11 cm. When boiling eggs the pathogen is destroyed in 4 minutes. A high resistance to the external environment, antibiotics and disinfectants is characteristic of hospital strains of Salmonella.

In food Salmonella can not only survive for a long time, but in optimal conditions (28°-35°C), it can multiply and accumulate in large amounts without changing the appearance or taste of the product.

The sources of pathogens

The natural reservoir of Salmonella involves both farm animals (poultry, pigs, cattle) and wild ones. They have a higher epidemiogenic significance. In most cases, infection develops in animals in the form of intestinal tract infections. Salmonella penetrates the intestinal tract and eneters the tissues and organs of the animal as a result of impairing the body's protective functions (chronic disease, exhaustion, stress, etc.), which causes septic salmonellosis. Namely, the meat from these

salmonella-bearing animals, which are slaughtered, present an extremely high epidemiological risk.

Noncompliance with following of hygiene rules during cutting, transportation, storage and processing of meat may cause Salmonella contamination of meat.

Chickens, ducks, geese and turkeysare the main reservoir of Salmonella, commonly in S. enteritidis. The transovarian transmission is possible in birds. Contamination of eggs can occur within some hours after the laying when Salmonella is absorbed through contaminated air.

There have been identified many other species of animals and birds as a reservoir of salmonella (dogs, cats, foxes, bears, deer, rodents, pigeons, sparrows, starlings, gulls, fish, turtles, crayfish from the river, lake or sea, crabs, snakes etc.).

The man also can serve as a source of Salmonella, although in rare cases. Salmonella carriers play a greater epidemiogical role, especially when these people are involved in the manufacturing or marketing of food products.

Routes and factors of transmission

The main Salmonellosis transmission route is the fecal-oral one. Human contamination occurs mainly via food. The main factors of transmission is meat, meat products, eggs, creamy foods. Bird eggs represent a particular risk. Salmonella diseases have been described as a result of contamination of cheese, smoked fish, seafood, etc.

Water is the secondary important factor of transmission in salmonellosis. At the same time, waters in open basins polluted by leakage of municipal or industrial waste (mincemeat, poultry or livestock items) show a real epidemioglogical risk.

Transmission by a habitual contact through household items or contaminated hands is also possible. Habitual contact transmission is more characteristic of nosocomial salmonellosis.

Manifestations of epidemic process

Salmonellosis is spread everywhere, mainly in aconomically developed countries, thus some authors refer it to the diseases of civilization.

In different regions Salmonellosis morbidity, depends on both social and environmental factors. These factors include changes related to food production and consumption by the human population, increased

urbanization and migration processes of the population, intensive pollution of the environment etc.

The incidence of salmonellosis is recorded mainly in the urban population. For example, in the Russian Federation 80% of cases of salmonellosis are related to the urban population. The same applies to Moldova.

The multiannual morbidity dynamics has two periods: the first period refers to 1961-1989, showing an increasing morbidity tendency, associated with livestock in livestock farm; and the second period is marked by a decreasing morbidity rate associated with a reduced number of animals and, conversely, the increasing number of animals grown in the private farm *(Figure 45)*.

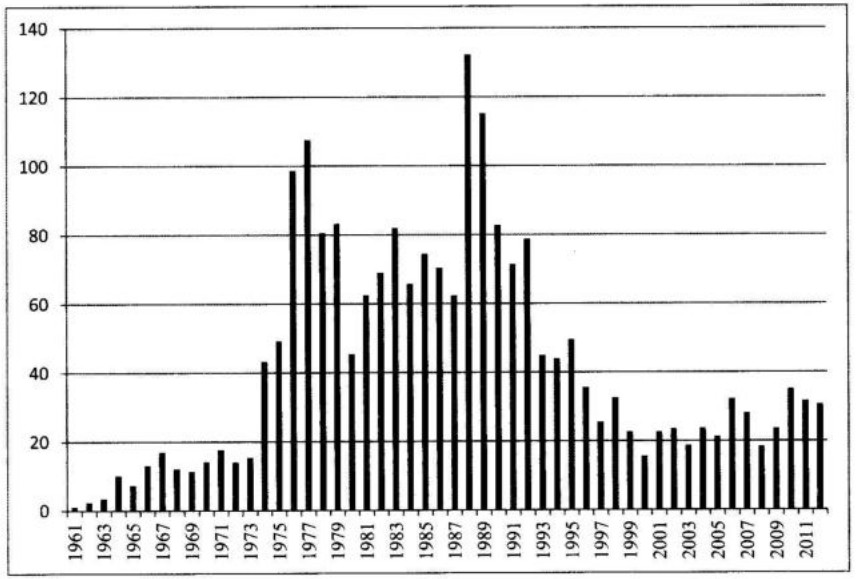

Figure 45. *Dynamics and tendency of the salmonelosis morbidity in the Republic of Moldova, in the period of 1961-2012.*

The rate of salmonella in the diarrheal disease morbidity structure is about 10% and that of salmonella food poisoning in the etiological structure - 35-40%. Children are most commonly affected by salmonellosis, especially the younger ones aged under 2, followed by the age groups of 3-6 and 7-17 *(Figure 46)*.

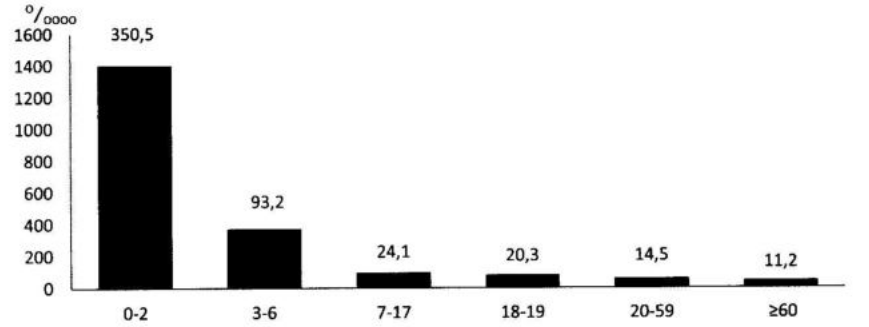

Figure 46. *The incidence of salmonelosis according to the age groups.*

Salmonellosis are recorded throughout the year, but the annual morbidity rate growth is seasonal in nature *(Figure 47)*.

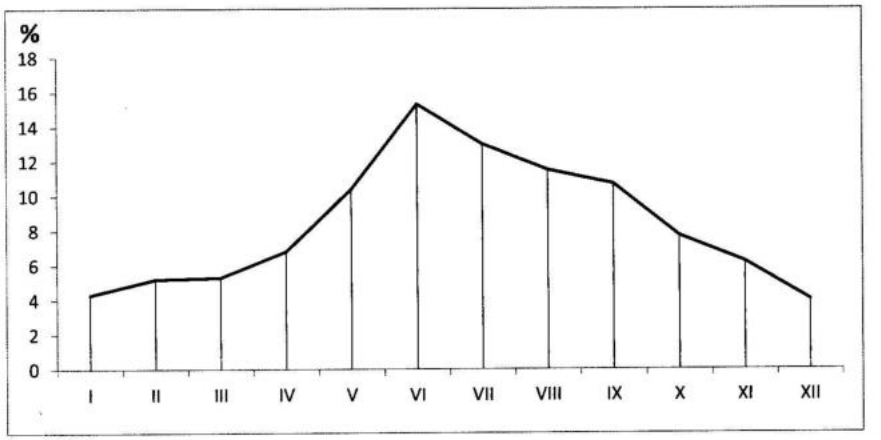

Figure 47. *Annual dynamics of salmonelosis.*

The incidence of salmonellosis increases during the warm periods, from May to September. It accounts for 60% of cases.

Meat, meat products (44%) and eggs (29%) are prevailing factors in transmission of salmonellosis.

The manifestations of infectious process

Salmonellosis may have a varried clinical picture: inapparent and pronounced forms;localized or generalized ones (septic forms). Gastrointestinal forms predominate and are accompanied by acute onset

and intoxication symptoms (nausea, vomiting, fever, headache). The patient complains of diffuse abdominal pain, diarrhea characterized by liquid, abundant, frothy, greenish stools, usually with mucus.

Septic form is common for in-hospital salmonellosis. The clinical sepsis is similar to that of other etiology. Foci may be located in the lungs, pleura, pericardium, brain, meninges, blood, liver, kidneys, and other organs.

Laboratory diagnosis

The Bacteriological method is the basic one. It provides isolation and identification of Salmonella in culture media and standard biochemical tests. The feces, vomiting, stomach washings and, if necessary, urine, blood, bile, pus and other affected organs discharges should be sampled from the patient.

The serological method provides the detection of antibodies in the serum of patients by indirect hemagglutination reaction. The minimum diagnostic titer is of 1 : 200, in children under 12 months - 1 : 100. The reaction confirms the diagnosis, if the antibody titres increase 4 times and more in dynamics.

Epidemiological surveillance provides a systematic study of the evolution of the epidemic process in dynamics and space; monitoring of biological structure and causative agents isolated from patients, animals, foodstuffs, environment; assessment and prognosis of the situation; developing programs of prevention and control.

Control measures

Preventive measures

Salmonellosis prevention is based on three groups of measures:

- measures undertaken within the livestock farm, manufacturing units of food products of animal origin, food trade, catering and food markets;
- preventive means of salmonellosis under habitual conditions;
- measures to prevent in-hospital salmonellosis

The disease prevention is based primarily on the implementation of veterinary measures aimed at preventing illnesses in agricultural animals and birds, followed by a vet expertise ensuring a sanitary-hygienic regimen suitable for abattoirs, meat and dairy product manufacturing.

The sanitary-hygienic rules must be respected during the transportation, storage, preparation and food trading. Deratization and pest control are important measure during storage and food processing.

At the stages of production, it is necessary to determine the critical high risk levels with systematic bacteriological investigations to prevent or avoid the risk of product contamination.

Food workers should be investigated for the presence of Salmonella. The detected carriers are temporarily removed from the workplace and subjected to sanitation.

The habitual prophylaxis includes informing the people about the risk factors and preventive means.

In-patient prevention of Salmonellosis involves the strict compliance with the sanitary and anti-epidemic regime applied to medical institutions, especially within the pediatric ones.

Anti-epidemic measures

All illness or carriage cases are reported to the Center for Public Health by the medical institutions within the deadlines.

Hospitalization of diseased or suspected cases of salmonellosis and carriers is performed according to clinical and epidemiological indications. Compulsory hospitalization of food industry workers, children's and medical institutions should be carried out. The patient is isolated and transferred to the infectious disease unit, if salmonellosis case is detected within the hospital. Hospitalization of Salmonella carriers shall be based on epidemiological indications.

The discharge from the hospital occurs after clinical recovery and two negative results of bacteriological investigations for risk group of adults and children under the age of 2 attending preschool institutions. The first investigation is carried out at least 24 hours after the treatment is complete, and the second - in 1-2 days. Those treated at home undergo the same examination.

All patients are admitted to collectives and their activities after bacteriological investigations which show negative results.

All persons in the focus and those who are at risk of contamination undergo medical surveillance during 7 days, including: questioning, examination, temperature measurement, etc.

Current disinfection is carried out before the hospitalisation and in the course of the home therapy. The following items are subjected to desinfection: bed linen, care items, toiletries, plumbing, toys, room surfaces, excretions and dishes for excretions, by using chloramine solution or 1% lime chloride, 0.5% calcium hypochlorite, 3% hydrogen peroxide, 0.1% dichloroisocyanuric acid salts and other approved disinfectants.

1.2.4. Leptospirosis

Short history

In 1886 Adolf Weil and in 1888 A.K. Vasiliev described the clinical picture of the disease, today known as the icterohemoragic leptospirosis (Weil's disease, Weil-Vasiliev disease).

In November 1914, Japanese researchers J. R. Inada and Ido, isolated the leptospira icterohaemorrhagiae pathogen from the liver of guinea pigs infected with the blood of a sick person.

In 1916 Ido et al., isolated L. icterohaemorrhagiae from the gray rat kidney and urine (Rattus norvegicus) caught in coal mines.

Lately, there appeared reports on the discovery of similar morphological leptospheres, but they are different according to serological properties and country: L. hebdomadis (Ido et. Al., 1918), L. bataviae (Walen, 1926), L. grippotyphosa (Tarasov, 1928), L. canicola (Klarenbeek, Schiifnar, 1933), L. pomona (Clayton et al., 1937), L. tarassovi (Ananin, Kiktenko, 1941), which caused more benign forms of leptospirosis compared with L. icterohaemorrhagiae.

The pathogen agent

Leptospires belongs to the Leptospira genus, the Spirochetaceae family, which includes Borrelia and Treponema genus as well. The Leptospira genus (Leptos - thin; speira - spiral) includes two types: pathogenic - *Interrogans* and nonpathogenic - *Biflex*. The distinction between pathogenic and non-pathogenic forms (saprophytic) of leptospire is based on cultural, biochemical and serological properties.

The antigenic structure of leptospire is very complex. Two major antigenic components are known: the surface antigen, antigen (P), such as polysaccharide that confer the type specificity, whilst the second component is the somatic antigen (S antigen), such as lipopolysaccharide, conferring group specificity.

According to the antigenic characteristics of Leptospira *Interrogans,* it contains more than 250 serovariants combined in 25 serological groups, which are pathogenic to both animals and humans.

The etiological structure of leptospirosis varies within different geographical areas.

In Moldova has been identified the circulation of 13 serogroups of leptospire: *Pomona, Tarassovi, grippotyphosa, Hebdomadis, icterohaemorrhagiae, Bataviae, Australis, Javan, Cynopteri, autumnalis, canicola, Ballum pyrogenes.*

Leptospires are grown aerobically, on a rabbit serum-containing culture media, at an optimal 7.2-7.4 pH level, at 27-30°C temperature and in darkness.

The virulence varies in different types. Leptospires are very fragile and are easily destroyed in unfavorable environments. They do not stand dryness, intense acid or alkaline environment. The pathogen survives in rivers up to 30 days, in moist soil - up to 279 days, in food - from a few hours to a few days. Both high and low temperatures easily destroy them. It dies at a temperature of 50-60°C within 30-60 minutes, at 65°C - in a few minutes, at boiling - instantly. It easily dies under the sunlight exposure. Bile, gastric juice, acid urine acts destructively on leptospires. Common disinfectants used in digestive infections are also harmful to leptospires.

The sources of pathogen

In leptospirosis the natural reservoir of the pathogen is represented by different species of rodents (house and field rat, mouse, water rat, and others). After contamination, leptospires circulate in the blood of rodents for 5-10 days, then locate in the kidneys and are excreted with urine. The reservoir of leptospirosis is presented by domestic animals like cattle, swine, sheep, dogs, rarely horses. These animals show sometimes severe, even lethal, clinical forms. The lethality reaches 10% in cattle. Dissemination of leptospires is also done through urine, for a long time, since these animal species tend to develop chronic infections - up to 120 days in cattle, up to 700 days in rabbits, up to 700 days in pigs. During acute infection in cattle and swine,, leptospires are disseminated to all organs, connective tissues, especially in the blood. Silver foxes, nutrias and other animals may be sources of leptospire. Carriage of leptospire is found in more than 130 species of mammals with not apparent infection.

There is a correlation between different species of leptospires and different animal species. Each type of leptospire may have a preferred host, such as *L.pomona* and *L.tarassovi* - pigs, *L.hebdomadis* - cattle, *L.grippotyphosa, L. bataviae* - microtine, *L.icterohaemorrhagiae* - gray rats, *L.Dog* - dogs, *L. autumnalis* and *L. australis* - hedgehogs, *L.sejroe* - mice. This phenomenon was observed in Moldova *(Table 3).*

Table 3

The rate of serological groups of leptospirosis detected in natural host in the Republic of Moldova (V. Prisacari, 1993)

Serologic group	Animals		Rodents	Grey rats
	swine	catlle		
Pomona	49,9	10,5	1,8	6,8
Tarassovi	18,3	10,0	1,2	0,0
Grippotyphosa	0,9	1,6	68,9	9,0
Hebdomadis	0,5	62,9	6,0	0,0
Icterohaemorrhagiae	1,1	0,8	1,5	68,2
Bataviae	0,6	0,3	2,1	2,2
Australis	0,0	0,0	5,7	2,2
Javanica	0,0	0,0	5,7	2,2
Cynopteri	0,0	0,1	1,2	2,2
Autumnalis	0,0	0,0	4,2	4,6
Canicola	0,1	0,1	1,0	2,2
Ballum	0,0	0,01	1,0	0,0
Pyrogenes	0,0	0,01	4,2	0,0

However, the same species of animals can be carriers of several types of leptospire, so cross-contamination can occur. The man may contract various species of leptospire.

In the Republic of Moldova, L. grippotyphosa, L. pomona, L. hebdomadis, L. icterohaemorrhagiae, L. australis and L. tarassovi predominate in human leptospirosis *(Table 4)*.

Table 4

Etiological structure of leptospirosis in humans in Moldova (in 1963-2010)

Serologic group	%
L. grippotyphosa	37,42
L. pomona	29,24
L. hebdomadis	9,40
L. icterohaemorrhagiae	8,58
L. australis	6,13
L. tarassovi	2,86
others	8,89

Humans who suffered leptospirosis usually are not a source of pathogen, so they are not contagious.

Routes and means of transmission

Pathogenic leptospires usually enter the human body parenterally through the mucosa or damaged skin. Thus, leptospires contraction mainly occurs during bathing in water-contaminated ponds, polluted by livestock or waste of xenanthrope animals and cattle farms; during the care of animals, especially of the sick ones; by rodent contaminated soil, during animals slaughtering, meat cutting and processing. Transmission can also occur by water, by using drinking water from unsuitable sources, and by contaminated food, especially the one infected with L. icterohaemorrhagiae.

Manifestations of epidemic process

Leptospirosis occurs in different geographical areas with different prevalence of serogroups of leptospire.

In Moldova (Slobodzia) first cases of leptospirosis at bovine were recorded in 1950 then it spread throughout the country. The most affected domestic animals were pigs (73.2%), cattle (26.3%), sheep (0.37%), horses (0.01%) and dogs (0.03%).

In humans leptospirosis was recorded for the first time in Moldova, in 1963. Subsequently, cases of leptospirosis in humans were reported annually. The dynamics of the morbidity is characterized by ascending and decreasing cycles *(Figure 48)*.

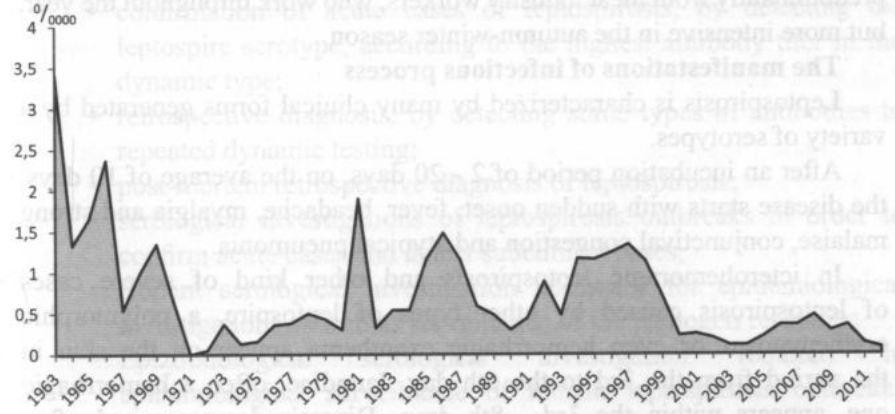

Figure 48. *Dynamics of morbidity rate in human leptospirosis in the Republic of Moldova (in1963-2012, per 100,000 population).*

Cases of leptospirosis are recorded throughout the year, but a significant increase of incidence occurs during the warm season of the year

(Figure 49). Only within three months (July, August, September) 70% of annual leptospirosis morbidity were recorded.

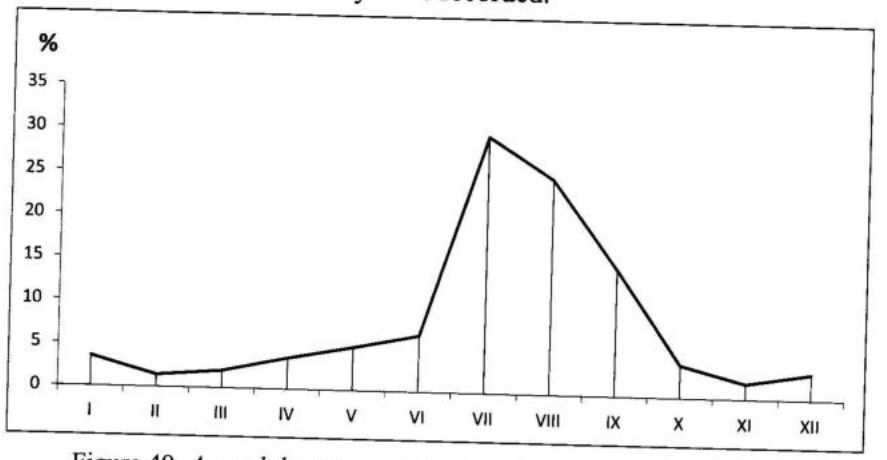

Figure 49. *Annual dynamics of human leptospirosis morbidity in the Republic of Moldova.*

Summer seasonality is typical for the rural population and it is less pronounced in the urban population, where leptospirosis is contracted predominantly from meat industry workers, who work throughout the year, but more intensive in the autumn-winter season.

The manifestations of infectious process

Leptospirosis is characterized by many clinical forms generated by a variety of serotypes.

After an incubation period of 2 - 20 days, on the average of 10 days, the disease starts with sudden onset, fever, headache, myalgia and strong malaise, conjunctival congestion and atypical pneumonia.

In icterohemoragic leptospirosis and other kind of severe cases of leptospirosis caused by other types of leptospire, a polymorphic erythematosus or even hemorrhagic exanthema appear on the skin in the period from the 3rd to the 6th day. Jaundice, often a hemorrhagic one, appears within the 3rd - 8th days. Diuresis decreases and often anuria phenomena develops. The recovery is slow. The severe forms of leptospirosis may be associated with meningitis, encephalitis or neuritis.

Laboratory diagnosis

In leptospirosis bacteriological investigations may be performed only

at the reference laboratories and are necessary in cases of its serological confirmation. Blood is examined in the first week of the illness and urine analysis is made at the end of the first week of the illness. In special cases, such as serous meningitis, the cerebrospinal fluid can be examined; in acute abdomen surgery – the peritoneal exudate is checked; and post-mortem – the liver, renal cortex, lungs, adrenal glands samples are examined. The samples should be collected in sterile containers, stored and transported at ambient temperature within a few hours of sampling.

The methods of molecular biology is carried out by PCR analysis of bacterial DNA.

Serological diagnosis, regardless of the clinical form, is made by confirmation test - the microscopic agglutination test (MAT) - with living type antigens prepared from pathogenic strains from an international reference laboratory.

It is possible to make rapid diagnosis of sero-orientation by microscopic agglutination test (MAT) with antigen from Patoc genus in microbiology laboratories of the health institutions. The positive samples are sent to the reference laboratory for confirmation.

Serological reaction of microscopic agglutination test (MAT) is indicated in:

- confirmation of acute cases of leptospirosis, by detecting the leptospire serotype, according to the highest antibody titer in the dynamic type;
- retrospective diagnosis, by detecting some types of antibodies to repeated dynamic testing;
- post-mortem retrospective diagnosis of leptospirosis;
- serological investigations of leptospirosis outbreaks in order to confirm acute cases and detect subclinical cases;
- Rodent serological investigation necessary for epidemiological investigations as well as surveillance of the pathogen reservoir;
- Epizootiological serological investigation required in epidemiological surveillance of human leptospirosis outbreaks caused by domesticated leptospirosis animals.

Epidemiological surveillance

Epidemiological surveillance is necessary to determine the circulation of leptospires in animals, the etiological structure and development rate of epizootic process.

It is also important to explore the natural outbreaks of leptospirosis; study the peculiarities of sources of infection; reveal their possible role in the events; specify their deployment; and carry out the mapping, dynamic supervision and zoning of the territory.

Epidemiological surveillance should provide the assessment of the density and composition of rodent species in order to determine their concentration territories, as well as the dynamic surveillance and prognosis.

It is important to monitor their density dynamics in the outbreaks where livestock is kept, especially on cattle and swine farms, as well as their concentration in certain areas and participation in ecological expertises that justify the need for buildings for animals. The epidemiologist should have data on leptospirosis morbidity in farm animals.

It is important to monitor meteorological values. These factors have a prognostic significance.

It is mandatory to monitor the evolution of the etiological structure in the anthropological outbreaks. The immunization of the farm animals may result in a commonly subclinical form, which leads to a latent maintenance of an epizootic process in outbreaks.

The epidemiological surveillance of both types of leptospirosis outbreaks is very important, thus the monitoring of leptospirosis morbidity in human population, as well as the determination of risk groups, etiological structures, and conditions of contracting the infection are performed. Detection of leptospirosis in humans is often a significant indicator of natural and human outbreaks.

Control measures

Preventive measures

The prophylaxis is aimed at natural and humans outbreaks.

It is highly important to carry out regular deratization in human and animal settlements, zoological and serological confirmation of natural outbreaks, and vaccination of agricultural animals (cattle, pigs) in anthropocorous outbreaks. It should be noted that vaccination prevents the illness, but not the formation of the carriage status.

Measures to prevent the catching of the infection by humans include: avoiding swimming in pools contaminated with leptospire from domestic and xenantropic animals or polluted by cattle or swine wastes; avoiding the consumption of poluated drinking water located in the fields; ensuring sanitary conditions for livestock workers, livestock processing companies

and croppers working in the meadows; providing them with protective equipment (shoes and rubber gloves, overalls, goggles), lavatories, disinfectants, antiseptic such as iodine tincture for the treatment of skin lesions; protecting food from rodents access; public health education regarding the risks of contamination, clinical manifestations and prevention. According to epidemiological indications, vaccination of risk-bearing groups is performed with polyvalent inactivated corpuscular vaccine containing the antigen of the most common serologic leptospire groups: *Icterohaemorrhagiae, Grippotyphosa, Pomona, Sejroe.*

Measures in outbreaks of leptospirosis

All suspected cases of leptospirosis detected in humans are reported to the Center for Public Health. The Veterinary Service must be informed as well.

Suspected leptospirosis patients are hospitalized in infectious diseases units.

Epidemiological investigation of the outbreak is carried out by specialists from CPH and veterinary service. The investigation is based on the study of the conditions of contamination, presence of animals carriage of leptospire, finding natural outbreaks of leptospirosis, and the possible link of the patient with the professional activity, etc.

Both persons suspected of contracting the infection and patients infected under the same conditions are supervised for 14 days. People presented with fever and those who have contracted infectious diseases within the last 3-4 weeks are to be detected. These people should undergo laboratory investigations to confirm leptospirosis.

The decision on emergency prophylaxis is taken by the epidemiologist, based on specific conditions. Doxycycline is recommended: in a dose of 0.1g once a day during 5 days.

People who have been in contact with leptospirosis-diseased patients are not isolated.

Animals with Leptospirosis are subjected to the treatment. Disinfection is carried out in case of slaughtering a diseased or disease-bearing animal. Meat and milk from diseased or leptospire-bearing animals can be used in food only after heat treatment.

It is mandatory to carry out the deratization of leptospirosis outbreaks, especially of the icterohemoragic ones.

1.2.5. Plague

Short history

Plague is of Asian origin. The invasion of Europe by the black rats (Rattus rattus) following the crusades, caused large epidemics in the 14th century, which killed 50-60% of the European population. Numerous other epidemic waves took place in the 18th century. At the end of the 19th century, there was recorded a pandemic with millions of victims, infected by rats transported from one geographical area to another by the sea. Only in India plague caused 6 million casualties. In the early 20th century, about 170,000 deaths were reported annually worldwide. The incidence decreased after 1960 with hundreds or at most 3,000-4,000 cases per year. In this period, the quarantine was introduced in Italy, which then generalized in other countries. Although plague, currently occurs in sporadic cases and small epidemic outbreaks, it remains a conventional infection.

The pathogen agent is Yersinia pestis, a gram-negative coccobacillus, of the Enterobacteriaceae family. It is aerobic, optionally anaerobic and immobile. It develops a capsule at 37°C in enriched media.

There are three biotypes of Yersinia pestis that differ by fermentation of glycerol and nitrate reduction and which do not correlate with the virulence. *Orientalis* biotype was responsible for the third pandemic, which spread throughout the Western part of the world; the *medievalis* biotype was responsable for the great medieval epidemic in the Caspian Sea region; the *antigua* biotype is spread in the southeastern part of Russia, Central Asia and Africa territories.

The resistance of Yersinia pestis lasts 7 months, in buried cadavers - up to one year, in the pus from bubo - up to 20-30 days,in the rodent corpses - up to 60 days, in the flea body - up to 400 days and in ticks - over 500 days. It easily susurvives at low temperatures and freezing. Direct sunlight destroys the microbe within 2-3 hours.

The sources of pathogen

Rodents are the main reservoir of Yersinia pestis. Plague is found in over 200 species of rodents, rabbits, squirrels and other animals (camels, elephants, buffalo, bears, goats, prairie dogs, cats, etc.). Birds, reptiles, amphibians are not susceptible to plague. Rodents are the main carriers of plague in natural outbreaks (marmots, hamsters, etc.). The epizootic process can involve synanthropic rodents (rats, mice). In human outbreaks the main reservoir is the black rat, especially rats from the ships, but also those of home types, like the Egyptian black rat, and gray rat.

The pathogen (Y. pestis) is transmitted from a sick animal to a healthy one by fleas (the main vectors) in natural outbreaks and some species of ticks, which maintain the epizootic process, but it can be transmitted to the synanthropic rodents, camels and dogs as well. The flea can transmit the pathogen within 3-5 days after catching the disease from the sick animal and can be contagious up to one year and more. The epizootic process ceases, when the number of rodents decrease significantly.

Humans usually catch the infection in natural outbreaks. Nowdays, the risk of contamination is very high during the transportation of containers by ships. The importing of infection is increased by rats, however the infection can be transmitted from a plague-diseased person,as well.

Routes and means of transmission

Y.pestis is transmitted from animals to humans by bites of infected fleas (Xenopsylla cheopis). The flea is infected while ingesting blood of a bacteraemic animal. As the bacillus multiplies in the intestinal tract of the flea, a gel is formed at the entrance of the stomach that prevents the passage of subsequent meals. As a result the bacilli are regurgitated when the infected flea attempts to ingest another portion of blood. The flea remains hungry and its activity increases. In the absence of rats, the flea attacks humans.

If a person hunts in the focus of plague he may get contaminated by direct contacts with marmots, hares or other infected animals, either dead or captured. If an individual damages the skin when removing the pelt, or touches the mucosa with the contaminated hands, he gets infected. People also get infected during funeral ceremonies because the fluid issuing from the mouth or nose of the dead contains the plague agent. Ingestion of contaminated food also leads to the penetration of the plague bacillus into the gastrointestinal mucosa. The susceptibility of humans to plague is extremely high. When infected from an animal, the patient usually develops the bubonic form. Bubonic plague is characterized by slowly increasing incidence. In primary or secondary pneumonic plague the infection is transmitted from person to person by airborne route which is a great epidemiologic danger, because the disease can spread widely within a short period of time. Pulmonic plague usually follows the bubonic form and very soon it becomes the main clinical form. The immunity developed in the plague patient is rather stable and repeated infections are rare.

Manifestations of epidemic process

The epidemic process is manifested by the outbreaks or sporadic cases in the human population. Usually, it occurs in geographical areas with natural active foci of plague (Central Africa and South America, the US, Central Asia, India, China, Southeast Asia, Eastern Europe). In 1970-1995, 341 cases, transmitted by bites of the fleas (78%), via direct contact with infected animals (20%) and via inhalation (7.2%) were reported in the USA. A patient with uncomplicated bubonic plague is not contagious, but there is a risk of haematophagous infection. The transmission of pulmonary plague from the sick is possible through droplets eliminated during expiration.

Being a zooanthroponosis disease, the eruptions in humans, are usually, preceded by an epizootic process. Epizootic mortality can reach 85-99% depending on the species. However, rodents play a major role in the epidemic transmission through various types of transport means used for long distances shipping.

Ciclicity and seasonality of epizootic process also differ depending on the particular natural focus. Increased morbidity rate is observed more frequently at intervals of 3-5 years. The number of cases has diminished worldwide recently. In the 90s of the last century, around 850-2000 people were infected with plague around the world annually, lethality being of 10%. In the last decade sporadic cases have been recorded in about 50 countries of the world, with an incidence of 150-300 cases per year. At the same time, Y.pestis can be used as a biological weapon and has a high risk in all the world.

Manifestations of infectious process

The incubation period lasts 2-6 days. It is shorter in the pulmonic form. In the vaccinated people it can lasts 8-10 days. The plague has a sudden onset, with a severe chills and rapid increase of the temperature till 39°C and higher. Toxaemia rapidly develops in all clinical forms. It is manifested by severe headaches and vertigo, insomnia, myalgia, weakness, nausea and vomiting. The patient is excited at first. His face and conjunctiva are hyperaemic, the tongue is white and swollen and speech is inarticulated speech. All these symptoms (unsteady gait including) resemble those of alcoholic intoxication. Circulatory disorder is marked; tachycardia develops (120-160 beats per minute); arterial pressure falls; arrhythmia occurs in severe cases. Severe cases are also characterized by cyanosis, pointed facial features (expression of fright on the face of

some patients), delirium and hallucinations. Neutrophilic leucocytosis with a shift to the left and accelerated ESR are reveald in the blood. The diuresis decreases; the urine contains protein, granular and hyaline casts and red blood cells. In addition to the symptoms that are common to all forms of plague, each particular form is also characterized by its specific symptoms. Depending on the route of infection transmission, the patient may develop either localized form of plague, such as cutaneous, bubonic, cutaneous-bubonic, or tonsillar (pharyngeal), or generalized form, such as primary septicaemic, secondary septicaemic, primary pulmonic, secondary pulmonic or intestinal plague.

In the cutaneous-bubonic form, a spot is first seen at the portal of entry, which is then converted into a papule, a vesicle, a pustule, and an ulcer. The ulcer is surrounded by a red zone, later it becomes covered with a dark crust and does not heal for a long time. Compared to anthrax, a plague carbuncle is painful. The regional lymph nodes are almost always involved. Lymphadenitis (plague bubo) develops on the first or second day of the bubonic form. The bubo is painfull not only on movement but also at rest. The patient is therefore motionless. The pain makes him take a forced posture. If the bubo is in the inguinal area, the patient flexes his leg. In the presence of an axillary bubo, the patient lies on his back with the arm set apart from the trunk. The bubo fuses with the subcutaneous cellular tissue; the overlying skin is tense and cyanotic. The bubo either disappears spontaneously or purulates and scleroses.

Cutaneous-bubonic forms can be complicated by secondary buboes, secondary pulmonic and secondary septicaemic plague. The tonsillar (pharyngeal) plague lasts 2-3 days. Toxaemia is weak, the body temperature rises to 38°C, the submandibular and neck lymph nodes are enlarged.

The primary septicaemic form is characterized by delirious hyperactivity or complete adynamia, dyspnoea, rapid and weak pulse. Haemorrhagic rash and haemorrhages into the skin and mucosa develop, haematemesis and bleeding can occur. Untreated patients die during the first days of the disease.

The intestinal form is characterized by high body temperature, extreme weakness, loss of appetite, nausea, recurrent vomiting, ample liquid stools with streaks of blood and mucus, severe abdominal pain during defaecation.

Primary pulmonic plague is characterized by a fulminating course with dyspnoea (40-60 breaths a minute), severe chest pain, cough with

discharge of liquid blood-stained foaming sputum. Cardiovascular failure develops on the very first days of the disease. In the pre-antibiotic era, pulmonic plague transformed into its secondary septicaemic form in 1-2 days and the patient died. The prognosis is more favourable today.

Laboratory diagnosis

The diagnosis is based on clinical, epidemiologic and laboratory investigations. Special precautions must be followed during the collection of the infected material, its transportation and further handling in the laboratory. The following specimens are taken: bubo contents, spontaneously draining exudate from ruptured buboes, vesicles, pustules, carbuncles and ulcers; sputum is taken from patients with the pulmonic form; if sputum is absent, faucial mucus is taken. Feces should be taken from patients with intestinal lesions. Blood specimens of patients with all forms of the disease are studied. The dead should be autopsied and pieces of the buboes, cutaneous lesions, lymph nodes and the parenchymatous organs (spleen, liver,lung), as well as blood from the heart or large vessels should be examined in the laboratory.

Exudates from buboes, vesicles, or pustules should be taken by a sterile syringe. Since the amount of the material is small, about 0.5 ml of a sterile broth is taken in the same syringe and the contents are transferred to a test tube. Sputum should be collected into wide-mouth bottles with ground-in stoppers. Blood (10 ml) is taken from the cubital vein. Several smears (4-5) are taken at the patient's bedside. A 5-ml portion of blood is inoculated in a vial containing 50 ml of broth, while the remaining blood is placed in a sterile test tube. If the laboratory is remote, the blood is placed in two 5-ml test tubes and examined in the laboratory not later than 5-6 hours (in the absence of a refrigerator). In the laboratory, the smears are stained with Gram's stain and methylene blue (Loeffler). The serologic luminescence analysis should be made if a luminescent microscope is available. Hottinger's or Martin's culture media containing sodium sulphite and gentian violet are inoculated. The remaining material is used for infection of guinea pigs and albino mice. The serologic method is used for retrospective diagnosis in those who sufferd plague, in patients to where treated with antibiotics. Indirect haemagglutination and indirect agglutination inhibition tests are commonly used. The latter test with antigen diagnosticum is used to control specificity of the positive indirect haemagglutination test. Serologic reactions should be carried out on the 5th

day of the disease and then at 5-day intervals till the patient is discharged from the hospital. The reaction of fluorescent antibodies can be used to detect the plague agent within 2 hours. Fleas and rodents, and also dead animals, especially camels, should be examined bacteriologically in the focus of infection.

Control measures

The prophylactic measures are targeted at enzootic areas to prevent the import of diseases. Measures to prevent the import of plague are governed by International Health Regulations (2005), which specifies plague as a conventional disease.

The quarantine is necessary in plague. The presence of natural foci in various countries and reports of plague cases in them indicate possible export of the disease to other countries. The anti-plague measures should be taken at the airports, sea ports, and railway border posts in accordance with the international requirements. Persons with plague should be detected and isolated. Those suspected of plague should be also isolated and observed. All persons who had contacts with plague patients should be under surveillance. Objects suspected of contamination should be examined bacteriologically. Vaccination is necessary. Current and final disinfection, disinsection, deratization and quarantine measures are necessary. Special anti-plague institutions should be involved in prevention and anti-epidemic measures in natural focci of plague. The complex of antiplague preventive measures includes the following: (1) epidemiologic surveillance; (2) rodent control (deratization) and destruction of the flea vector (disinsection); (3) vaccination of population against plague; (4) health education of population. Eradication of natural nidi located mostly in remote locations is an expensive measure. In this connection the species structure and populations of rodents and their ectoparasites should be systematically controlled in enzootic areas. Laboratory examinations are necessary for timely detection of epizootics, extermination of rodents and ectoparasites in populated places and the surrounding areas. Deratization and disinsection measures should be taken on the territories of the epizootic zone. Dry live vaccine is used for specific immunization of people. Vaccination should be done according to the epidemiologic indications.

Antiepidemic measures.

The medical institutions inform the Ministry of Health, National Center for Public Health, local Centres for Public Health within 2 hours

(emergency) about the detection or suspected cases of plague and within 24 hours the Ministry of Health informs the checkpoint of WHO.

If plague is detected or suspected, measures should be taken to localize and eliminate the focus. These measures include: (1) revealing and hospitalization of patients, revealing and isolation of persons who had contacts with patients, the dead, or with infected materials; (2) revealing and burial of the dead; (3) disinfection, disinsection and deratization in the focus, in populated places and in fields; (4) observation of population in the focus; provisional hospitalization of all patients with fever, lymphadenitis, tonsillitis, and pharyngitis; (5) implementationof quarantines or restriction in migration of population; (6) epizootiologic examination of the focus and the adjacent areas; (7) vaccination of population according to epidemiological indications. The epidemiologic studies should be conducted by a special group with personnel of the anti-plague institutions should be included.

Plague patients and people suspected of plague are admitted to special hospitals. Any room is suitable for preliminary isolation. All persons must be removed from the room where the patient with plague was present. The plague case should immediately be reported to higher medical authorities. Each hospitalized patient must be placed in a separate room or at least screened from the other patients in the room. The hospital must be guarded. The personnel must wear special anti-plague overalls.

The discharge of a patient with plague is allowed no earlier than one month after the disappearance of clinical signs and three negative results of bacteriological analysis of bubonic puncturing, sputum or blood tests done on the second, fourth and sixth day after the treatment.

Persons who had contacts with plague patients should be isolated for 6 days. Persons who had contacts with pulmonic plague patients should be isolated in individual rooms. All persons who had contacts with patients or the dead (with pediculosis) must have their body temperature measured at least twice a day and must be given preventive treatment with doxycycline (0.2 g once a day, intramuscularly) or tetracycline (0.5 g three times a day) for 5 days. The dead must be buried in coffins (or without coffins) at a depth of 1.5-2 metres. Dry chlorinated lime should be placed on the bottom of the grave. The dead can be burned. If hospitalization of all contacts is impossible in view of their multitude, observation is especially important.

People should be observed at home with obligatory thermometry. Patients with fever should be examined by the physician (who must make the preliminary diagnosis) and sent to the corresponding hospital. Whenever necessary, observation should be combined with vaccination and health education of the population.

Vaccination is performed with killed vaccines, given in two parenteral administrations or single administration of live attenuated vaccine (strain EV EV7b non-pathogenic after repeated passages strain of murine Tjiwidej).

Vaccination is recommended to laboratory personnel, travelers and troops arriving at enzootic areas. Y. pestis killed vaccine is administered intramuscularly in 3 doses, every 6 months and revaccination every 6 months. Post-vaccination reactions are benign, but in revaccinations erythema, induration, local pain, fever, headache, lymphadenopathy, malaise, lasting 48 hours may occur.

Current disinfection in the focus should be conducted when taking care of patients, during evacuation of patients and persons who had contacts with them. Final disinfection should be carried out in residential houses after evacuation of the patients and contacts, and also after burying the dead. Disinsection and deratization should also be carried out.

1.2.6. Tularemia

Short history

In 1910, an epidemic that resembled bubonic plague occured in San Francisco. In the same year D.McCoy, after studies made in the region of Tulare Lake, California, found an illness (injury) similar to the plague in marmots. Shortly, McCoy and Chapin (1912) found that the infection in rodent is caused by gram-negative microorganism, which was originally called *Bacterium tularense*. Later (1914) it was established that the man is susceptible to this infection. It was called „tularemia" on the proposal of Edward Francis and then the causative agent Francisella tularensis was named in honor of E. Francis, who studied thoroughly this infection in humans.

The pathogen agent

Francisella tularensis belongs to the *Francisella* genus, of the *Francisellaceae* family. *Francisella tularensis* is divided into four subtypes: 1. *Francisella tularensis subsp. tularensis (F. tularensis subsp.*

neartica or biovar A) circulates predominantly in North America, and is the most virulent for humans with a mortality rate of 5-20% with an infective dose of 10-15 microorganisms; 2. *F. tularensis subsp. holartica subsp. medioasiatica, biogroups polaeartica japonica*, is isolated in Europe, Asia, Japan, North America and it is less virulent for humans; 3. *Francisella novicida* is isolated in the US and rarely involves humans; 4. *Francisella philomiragia* is considered less virulent than *F.tularensis* and causes necrotic pneumonia, bacteremia, meningitis in immunocompromised individuals.

The Genus *Francisella* are gram-negative, strictly aerobic and small coccobacilli all together (0.7 to 1 μm/03 μm), which grow in cysteine-rich environments.

The microorganisms contain the somatic antigen (O) and the surface antigene (VI), which determine the virulence and the immunogenic properties. The main pathogenic factor is endotoxin. The microbe is resistant to the external environment, especially at low temperatures. It is resistant in open water sources at 1°C for 9 months and at -5°C - up to 10.5 months. In the moist soil at 4°C, it resists 4 months and at 23-25°C - up to 2.5 months; in grain and hay at 0°C - up to 6 months, at 8-12° - up to 2 months, at 20-30° - up to 3 weeks. The pathogen survives on rodents skin died of tularemia at 8 to 30°C from one month to one week and in contaminated and frozen meat - up to 3 years. It is resistant to drying, but is destroyed under UV light in 0.5 hour. It is sensitive to high temperatures and disinfectants.

The reservoir and sources of pathogen

The reservoir of *F. tularensis* is presented by more than 100 species of wild animals (especially hares, rodents, muskrats, hamsters, house mice, other rodents), and livestock (cattle, sheep, pigs, dogs, cats). A greater significance is paid to synanthropic and xenanthrope rodents (house mice, muskrats, hamsters), which can contract the infection by very low doses of tularemia pathogen, even unique; they develop acute (sepsis) forms and usually die after getting the infection.

The pathogen is transmitted to animals parenterally by haematophagous insects (mosquitoes, ticks, gadflies) and alimentary (through contaminated food and water). It was confirmed that the pathogen is present in ticks lifelong and is transmited from generation to generation.

A diseased person has no epidemiological significance.

Mechanisms, factors and routes of transmission

The pathogen is transmitted by haematophagous insect bites (ticks, mosquitoes, gadflies) from animals to humans, through direct contact with infected animals or their bodies (rabbits, rodents, pets), or indirectly during the processing of agricultural products, livestock, by eating food (bread, biscuits, crackers etc.) or through contaminated water (wells, springs) by inhaling aerosols during cereals proccesing in the fields, while working with contaminated hay or straw where the pathogen resists weeks or months.

Manifestations of epidemic process

Tularemia is spread in the Northern Hemisphere - North America, Japan, Norway, Sweden, Austria, Germany, the Russian Federation etc.

It is characterized by general responsiveness. All the exposed persons are at risk of catching tularemia during the outbreaks, regardless of race, sex or age. Suffering the infection leads to the formation of a solid immunity, which is usually lifelong.

The epidemic occurs more frequently in rural areas through both sporadic and epidemic cases. Adults prevail in general morbidity rate.

The risk groups include farmers, hunters, fishermen, tourists, shepherds, people exposed to the pathogen during laboratory work etc. Tularemia cases may occur throughout the year, but more frequently in summer and autumn (80%). Habitual contamination is posible by consuming food or water from a rodent-contaminated well.

Occupational morbidity is conditioned by agricultural, hunting and fishing activities, as well as during grain growing and processing.

Manifestations of infectious process

The clinical picture of the disease depends on the route by which the agent invades the individual. The incubation period lasts from 2 to 8 days. All clinical forms of tularaemia are characterized by some common symptoms. The disease starts suddenly: a short-lasting chill is followed by elevation of temperature to 38.5-40°C. The patient complains of headache, muscular and lumbar pains, weakness, hyperhydrosis, and poor appetite. Examination reveals hyperaemic face and conjunctivitis. The spleen and liver are enlarged by the end of the first week. Leucopenia, moderate shift to the left, relative lympho- and monocytosis are noted; ESR is high. Fever can last from 5 to 30 days. Remittent and intermittent fevers are common. The mentioned general symptoms are supplemented by specific signs

of the disease that depend on the gate of infection entry. The following clinical forms of tularaemia are differentiated: bubonic, ulceroglandular, oculoglandular, angioglandular, gastrointestinal, pulmonic and primary septic (generalized) forms.

In bubonic form, the infection penetrates through the skin and mucosa to the regional lymph nodes, where it causes lymphadenitis (bubo). The location of a bubo depends on the route of infection. Ulnar and axillary buboes usually occur in persons who were infected occupationally by direct contact with infected animals. Submandibular and neck lymph nodes are involved in water- and food-borne infections. The size of a bubo varies from the size of a nut to that of an egg, and greater. A group of lymph nodes is often involved. The nodes do not fuse between themselves or with the surrounding cellular tissue. The nodes are only slightly painful. As the body temperature falls, the buboes disappears slowly. If the treatment is untimely, an abscess can be formed that ruptures and drains spontaneously with the release of thick cream-like pus.

In ulceroglandular tularaemia, a spot at the site of the agent entrance a spot transforms gradually to a papule within 6-8 days, then to a vesicle, pustule, and finally to an ulcer with simultaneously developing processes in the nearest lymph node (bubo). Primary lesion of the skin is common for the transmissive form of tularaemia.

In oculoglandular tularaemia, the agent enters through the eye tunics with development of follicular proliferations over the conjunctiva and simultaneous enlargement of the lymph nodes (parotid, anterior neck, submandibular nodes, etc.). The eyelids become swollen, papules and ulcers can appear on the eye tunic.

The angioglandular form is characterized by hyperplasia of the tonsils with the subsequent formation of a greyish-white necrotic coat, and formation of deep slowly healing ulcers. Unilateral involvement is more common. Since the tularaemia agent invades the regional lymph nodes, submandibular, neck and other buboes develop.

The angioglandular form is common in water-borne outbreaks.

The gastrointestinal form of tularaemia develops on ingestion of the tularaemia agent with food. This form is characterized (in addition to the general symptoms) by severe abdominal pain (which is due to the involvement of the mesenteric lymph nodes), nausea, vomiting and diarrhoea. The diagnosis of the gastrointestinal form is difficult.

In pulmonic tularaemia primary inflammation occurs in the lungs. The disease is complicated by development of focal pneumonia with a flaccid and long-standing course. X-ray studies and skin-allergic tests are decisive diagnostically.

The septic form of tularaemia is characterized by the development of general symptoms without primary local and regional reaction at the gate of infection entry of the infection. Clinically this form has a more pronounced picture of toxaemia. Polymorphous erythematous rash is more common in this form of the disease. During the recovery stage, specific complications can develop: secondary pneumonia, nervous and cardiovascular diseases (vegetative neurosis, degenerative changes in the myocardium, etc.). Working capacity and appetite are restored very slowly in convalescents.

Tularaemia relapses and conversion of the disease into the protracted form (lasting 2-3 months) are possible.

Laboratory diagnosis

Tularaemia is diagnosed on the basis of clinical, epidemiologic and (in the pulmonic form) X-ray examinations. Laboratory studies (allergic, biologic and serologic tests) are diagnostically important. Skin-allergic tests can be doned on the 3rd-5th day of the disease: 0.1 ml of tularin (diagnosticum) is injected intracutaneously in the upper third of the palmar surface of the forearm. If a hyperaemic infiltration of at least 0.5 cm in diameter appears at the site of injection, the test is considered positive. The result can be assessed in 24, 36 and 48 hours. Abrasion cutireaction can be used instead of the intracutaneous test. The biologic tests are done on albino mice and guinea pigs. The following inoculating material is necessary for subcutaneous or intra-abdominal infection of the laboratory animals: bubonic exudate taken before the 14th day of the disease; pustular contents; exudate from the ulcer bottom taken before the 8-12th day of the disease (this should be mixed with isotonic sodium chloride solution before inoculating the animals); conjunctival secretion taken before the 15-17th day of the disease; blood (5-6 ml) taken before the 6th day of the disease. The animals are followed up for 15-20 days. Infected animals die in 3-4 days. The microbe is identified by the tularaemia agglutinating serum after the death of the animal. A 2-3 ml blood specimen taken on the 7-10th day of the disease is sent to the laboratory for the agglutination reaction. This reaction is repeated 2-3 times at 4-5 day intervals in order to follow the progress in the titre values. The agglutination reaction is

considered positive with serum dilutions of 1:100 and higher. Indirect haemagglutination test is more sensitive and the result is ready earlier (red cells sensitized with tularaemia antigen are used as the diagnosticum).

Epidemiological surveillance

Epidemiological surveillance provides the monitoring of tularemia in natural foci, which include:

- monitoring the density of rodents and vectors of transmission;
- timely detection of disease in wild animals;
- differentiation of territories from epizootic natural foci to determine prophylactic measures;
- determination of pathogen movement in rodents and the environment;
- developing prognosis.

Natural foci are considered active in the areas where there are recorded local cases of diseases in humans, isolated cases of pathogen, or tularemia antigen is regularly detected in bird or rodent excrements. Natural foci are considered to be weakly active in the territories where diseases in humans and isolation of pathogen are not recorded, but there are irregular detection of tularemia antigen on environmental objects.

Control measures

Preventive measures

Non-specific prophylactic measures include:

- deratization and desinfection to reduce the population density of carriers and transmitters of the pathogen;
- protection of water sources, food and agricultural products from the rodents access;
- timely outdoor agrotechnical works;
- prevention of tick or mosquito bites by applying repellent substances;
- education of population on the risks of contamination and protection, especially in territories with natural foci.

Prophylaxis with administration of corpuscular live attenuated vaccine is performed according to the specific epidemiological indications, usually in the territories with the presence of natural foci. Vaccination is carried out in children the age of 7 without contraindications. Revaccination is recommended every 5 - 10 years.

Anti-epidemic measures

Medical institutions inform the Center for Public Health about the detection of suspected cases of tularemia.

Outbreak investigation is conducted by the epidemiologist.

The hospitalization is performed according to the clinical indications because the patient is not contagious.

Persons under the same risk conditions as the patients are subjected to serological and allergic investigations (cutaneous tularin test). Isolation of people who had a contact with the patient is not required. Antibiotic medication is also not recommended, however persons who where incidently contaminated in laboratory are administrated gentamicin or streptomycin for 5-7 days.

Deratization and disinfection is performed in the focus. If necessary, vaccination of the population is carried out.

1.2.7. Lyme disease (Borreliosis)

Short history

In 1909, the Swedish dermatologist Arvid Afzelius described migratory erythema for the first time. By 1921 he also reported six cases, suggesting the idea that the disease was due to the tick bite.

Associated cutaneous manifestations of erythema migrans in Russia were described by Nikolski (1896), Mescerski (1898) and Pisemski (1902).

In 1913, in Austria, B. Lipschuter introduced the term *chronic erythema migrans* and established the clinical diagnostic criteria, underlining the role of ticks in the spread of the disease.

In 1922 Ch. Gariu and Ch. Boujadoux described neurological manifestations (radicular pain, limb paralysis, changes in CSF) associated with erythema migrans, developed after a tick bite.

In 1975-1976, in the town of Old Lyme (Connecticut, USA), an outbreak of atypical arthritis affecting mainly children occured in 51 cases,; in some cases the disease onset was characterized by cutaneous erythema migrans and the tick bite was detected. Epidemiological and clinical investigations outlined the existence of a new disease - Lyme arthritis.

Lyme arthritis in association with skin and neurological symptoms, leads to the definition of new infectious disease in human pathology, transmitted by a Lyme bearing tick,described by A. Steere et al.

In 1982 Willy Burgdorferi identified *spirohete* in *Ixodes Damini* ticks collected on Shelter Island, New York, by immunofluorescence reaction, using antibodies from patients with Lyme disease, and in 1984 Johnson et al. confirmed their identity with those isolated from patients. Spirochete

was included in the genus *Borrelia* and named *Borrelia Burgdorferi* in 1984.

In 1985, at the 2nd International Conference on *Borrelia* (Vienna, Austria), the term Lyme disease was replaced by the term Lyme borreliosis.

In Moldova, several researches on Lyme borreliosis and description of clinical and epidemiological features of the disease were conducted by Dr. George Muşet, Elena Manole and Stela Gheorghita regarding.

Borrelia burgdorferi pathogen belongs to the Spirochaetaceae taxonomic family, genus Borrelia, which includes other 20 species responsible for human and animal diseases.

In 1991 The use of molecular biology methods for the isolation and identification of DNA by polymerase chain reaction (PCR) allowed to distinguish two distinct classes of Borrelia burgdorferi, spread across North America, and strains with common features in Europe and Asia. These two classes display the differences in typical manifestations of Lyme borreliosis in North America and Europe.

Borrelia Burgdorferi is a gram-negative germ, of 4-30 μm/0.2 - 0.3 mm size, with 7-11 flagella at each end, mobile, microaerophilic, catalaze-negative, with slow multiplication speed at 30-37°C. It has a protoplasmic cylinder surrounded by the cytoplasmic membrane. The latter has a trilobal structure and is very fluid. The microorganism may have the form of uncoiled filaments, L-shaped forms without a cell wall, and cystic or granular forms.

Nowdays, there are about 80 polypeptides (antigenic determinants), of which 12 are immunodominant, responsible for the formation of specific antibodies in the serum of patients with Lyme borreliosis. *Borrelia* groups have the following antigens: surface antigens (Osp A, Osp B, Osp P Osp E, F Osp - the location of their genetic plasmids being linear, Osp C – with circular plasmids) and flagellar ones.

The reservoir of the pathogen

The natural hosts of Lyme borreliosis involves birds, mammals and specific vectors like ticks of the *Ixodes* genus.

The Ixodes genus includes a large number of species, but not all of them are infected with *B. Burgdorferi*. The species of ticks that can transmit *B. Burgdorferi* to humans, causing characteristic clinical manifestations of Lyme borreliosis are *I. ricinus* and *I. persulcatus* in Europe and Asia; *I. pacificus* in the west of the US and *I. dammini* (*I. scapularis*) in the

North eastern and southeastern part of the United States. *Ixodes* tick is most commonly found in wet areas, woodland and grassland. The presence of the tick in nature is assotiated with the presence of both large wild mammals (deer, wild boars, foxes) or domestic (sheep, cattle, dogs) and smaller ones (rodents).

The geographical distribution of both hosts and vectors varies and depends on weather conditions. Mature ticks feed blood of wild animals and immature stages of ticks (larvae and nymphs) parasitize on various species of small animals. In European countries, including Moldova, immature ticks commonly host on rodents *Clethrionomys glareolus* and *Apodemus sylvaticus*, these being the main reservoir of *borreliae*.

Ixodes ticks pass through a three-stage cycle (larva, nymph, adult). The tick may feeding blood only once at any stage of its life cycle then the pathogen transmssion may occur at the next stage. Therefore, contamination occurs at one stage and the pathogen transmission take place at the next stage. The trans-stagial transmission (larva-nymph and nymph-adult) helps to maintain the infectious cycle; transmission of adult female eggs (Transovarian transmission) occurs rarely. Nymphs feed, usually in the spring, infecting rodents that will become a source of infection for larvae. Larva feed in summer and autumn.

An infected tick can transmit the pathogen at all stages of its development, but the maximum infectivity level is characteristic of the nymph stage. Humans acquire *B. burgdorferi* from nymphs especially in late spring and early summer and less frequently from adult ticks that feed human blood in autumn and winter, less in early spring.

The mechanism, factors and routes of transmission to humans

Lyme borreliosis pathogen transmission to humans occurs usually by ticks of the genus Ixodes - *I. ricinus, I. persulcatus, I. dammini (scapularis), I. pacificus* in various stages of the development: the nymph and adult, rarely in the larval stage.

The small size of larva and nymph, causing painless skin lesion, make it unnoticed compared to adult tick bite, which is larger and easier to observe and less time is necessary to attach to the skin. Iin the transmission of the pathogen an important factor is the time of attachement to the wound. It was found in animal experiments that the risk of contamination is low within 24 hours, in 48 hours the risk is 50% and in 72 hours it is at maximum.

Pathogen is transmitted by saliva and/or regurgitated contents during

feeding. Ticks with systemic infection can transmit the bacteria during the first feeding. The ticks infected with *B. burgdorferi* which is found only in the intestine can transmit bacteria within several days when the bacteria will get into the salivary glands. Several cases of vertical transmission in pregnancy have been described, but these cases are not confirmed by isolation of the pathogen. Transfusion transmission is possible, but such transmission has not been reported.

Manifestations of epidemic process

Lyme borreliosis is widespread on all continents, except Antarctica, with a varied territorial manifestations of the epidemic from one region to another, and with pronounced endemic areas. The territorial spread of the disease is directly dependent on the area of the main vector - *Ixodes ticks*, which ensures the preservation and transmission of the pathogen in humans. The most active outbreaks are related to woodlands or forest-steppe areas.

According to the WHO data, the last two decades there has been a trend of increasing morbidity of Lyme borreliosis endemic in all territories. In 1991 in the US, for example, where Lyme disease is considered the most common infectious disease transmitted by arthropods, according to the Center for Disease Control and Prevention (CDC, Atlanta), there were reported 10 354 nationwide cases, in 1996-16461 cases, and in 2011-2012 - 46 358 cases of Lyme borreliosis. Although the disease has been reported in 45 states, 95% of cases were recorded in eight states in the northeast. The same trend is observed in countries of Europe and Asia (Russia, Ukraine, Romania, Germany, Italy, France, Poland, the Netherlands, Austria, Belgium, Britain, China, Japan, etc.), where the disease has also an endemic spread.

For example, in the Russian Federation, the incidence of Lyme borreliosis increased from 0.7 cases per 100,000 population in 1991 to 5.42 cases in 2008 *(Figure 50)*.

The highest incidence was recorded in Tomsk region, where the morbidity index had exceeded 8 times the national average.

Romania and Arad county, which appears on the entomological maps with a high density of ticks, also has a higher prevalence of Lyme borreliosis both in risk groups and control population of the area.

In Germany, 31.4% of forest workers were found to have antibodies against B. burgdorferi. In Sweden, the incidence of the disease is 69 cases per 100 thousand inhabitants. Most cases of borreliosis originate

in the South-East region, where the prevalence of ticks infected with B. burgdorferi is of 30%. Seroprevalence ranges around 20-25% in the areas where endemic disease develops and 1-2% in the non-endemic areas. In Spain, the number of Lyme borreliosis decreases from north to south and is related to the distribution of I. ricinus ticks.

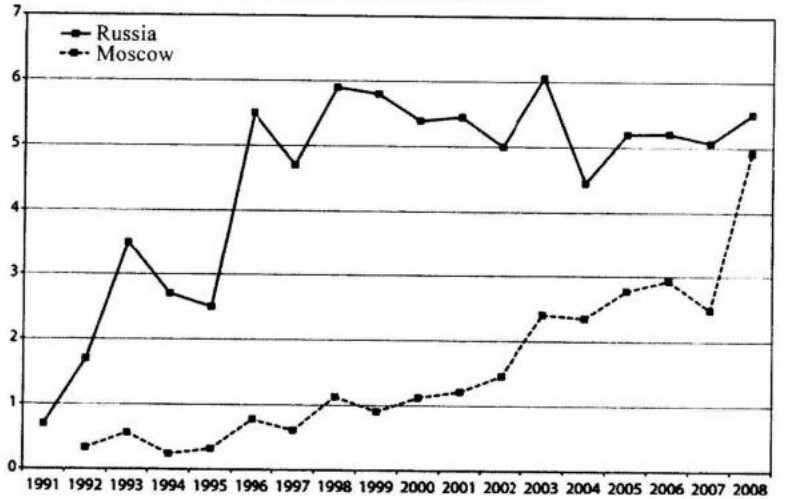

Figure 50. *The incidence of Lyme borreliosis in Russian Federation population and in Moscow, in 1991-2008 (N.I. Briko et al., 2013).*

Taking into account the epidemiological particularities of the disease, the risk groups of Lyme borreliosis include forest workers, hunters, veterinarians and farmers, persons who run the household or hold recreational activities in forested areas. In France, Lyme disease is considered as a professional disease of farmers. In Belgium the highest seroprevalence of anti-*B. burgdorferi* antibodies was found in Forestry workers - 28% compared to 5% in the control group. In Italy, the seroprevalence of anti-B burgdorferi antibodies in risk groups (forestry workers, farmers, hunters) elevates to 27.2%, while in general population it is from 0 to 5.7%.

Moldova is an area with a temperate climate, where the geographical area and the presence of parasitic system components create favorable conditions for the circulation of Lyme borreliosis pathogen in the population of *Ixodes genus* animals and arthropods.

The first information on the existence of Lyme borreliosis outbreaks affecting the human population in the Republic of Moldova, dates back to

1990-1992. The first cases of borreliosis began in 2000, with a significant increase in the incidence rate were reported in 2000 *(Figure 51)*. In 2000 - 2013, 815 cases of Lyme borreliosis were reported in humans, with an obvious predominance in Chisinau.

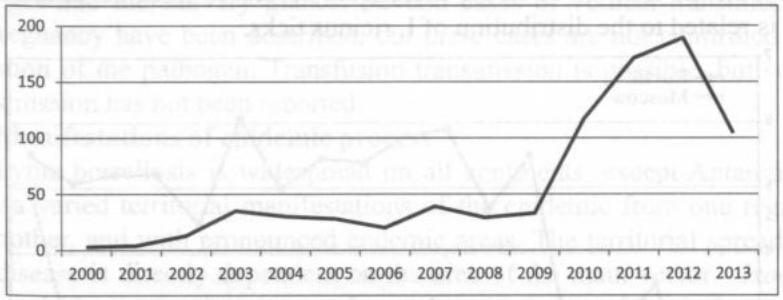

Figure 51. *Multiannual dynamics of Lyme borreliosis morbidity in the Republic of Moldova, (2000-2013).*

In this period, Lyme Borreliosis cases were recorded and reported in 24 administrative rural territories regions and 4 municipalities, including all three zones: North, Central and South, with obvious predominance in the center of the Republic (Orhei, Straseni, Calarasi, Ialoveni, Anenii-Noi), which can be classified as high-risk areas *(Figure 52)*.

Figure 52. *Distribution of Lyme Borreliosis morbidity in the Republic of Moldova, according to its administrative territories (2000-2013).*

Lyme Borreliosis illnesses were recorded during the whole year *(Figure 53)*, with a maximum incidence in the spring-summer months. More than 75% of borreliosis Lyme cases occur in May-August. Seasonality correlates directly to the active period of arthropods and outdoors activities of the population.

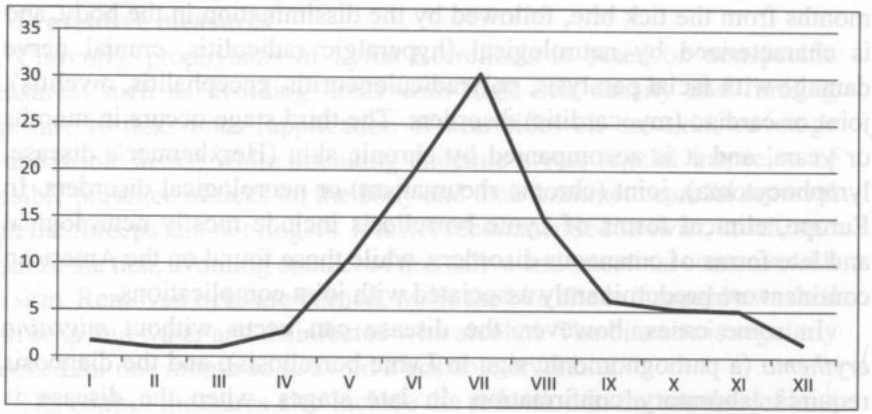

Figure 53. *The seasonality of Lyme Borreliosis in the Republic of Moldova.*

Responsiveness to Lime disease is general. The disease affects persons of all ages with a higher frequency in children under the age the age of 14 (18%) and adults aged 31 - 60, which constitutes about 50% of general morbidity rate.

According to the data in Moldova, the morbidity prevails in females - 65.9% compared to 34.1% in men. The infection is conditioned by: tick bites during the rest time in the forests, parks or recreational areas - 35.0%; living near the forests, where there is high risk of being infected by ticks - 16.3%; agricultural activities - 9.3%; vacations abroad - 1.2%.

tick bites occured most commonly on the legs - 54.2%, abdomen - 12.8%, head and neck - 8.38%, thorax - 7.74%, and on buttocks, axillary, groin or pubic fossa in 3.8% of cases.

In Moldova the risk groups of contracting the infection inlude: the unployed- 34.1%, pensioners - 15.4%, children under the age of 14 - 17.9%, foresters, farmers - 14.6%, servants - 12.7%, disabled people - 4.5%, and students - 2.0%.

The manifestations of infectious process

Lyme disease is characterized by various clinical manifestations

(dermatological, neurological, joint and heart), evolving in three stages. The stage of the disease is manifested by skin lesions presented by erythema migrans, which occurs after 1-3 weeks and is located in the region of tick bite accompanied by the manifestations similar to those of influenza and meningitis. The second stage of infection occurs in a few weeks or even months from the tick bite, followed by the dissimination in the body, and is characterized by neurological (hyperalgic radiculitis, cranial nerve damage with facial paralysis, polyradiculoneuritis, encephalitis, myelitis), joint or cardiac (myocarditis) disorders. The third stage occurs in months or years, and it is accompanied by chronic skin (Herxheimer's disease, lymphocytoma), joint (chronic rheumatism) or neurological disorders. In Europe, clinical forms of Lyme borreliosis include mostly neurological and late forms of cutaneous disorders, while those found on the American continent are predominantly associated with joint complications.

In some cases, however, the disease can occur without *migraine erythema* (a pathognomonic sign in Lyme borreliosis), and the diagnosis requires laboratory confirmation. In late stages, when the disease is manifested by joint, neurological, cardiological and skin disorders and the signs that are specific to other diseases as well, it is necessary laboratory tests ired to confirm the diagnosis.

Laboratory diagnosis includes:

- The isolation of the pathogen (*B. burgdorferi*) from the pathological samples (blood or skin samples - in the initial stage, whilst cerebrospinal, sinovial fluids, and blood – in the second or third stages). At the same time, the reduced time of dissemination of the pathogen in biological fluids restricts the use of bacteriological methods in the diagnosis of borreliosis.
- Serological diagnosis detects specific IgM and IgG antibodies in the serum or cerebrospinal fluid. It is recommended to use two tests: enzyme-linked immunoassay (ELISA) or indirect immuno-fluorescence reaction (RIF), followed by confirmation by Western blot.

In Lyme Borreliosis **epidemiological surveillance** has become more actual, especially in endemic territories and includes: monitoring the incidence and prevalence of diseases; estabilishing the time, territories and risk groups; conducting zoological and microbiological research on the area of distribution; the density and level of infestation by arthropods

- vectors and hosts; studying the seroprevalence in human population in different territories and groups; determining the level of awareness of the population about the epidemiology and means of prevention; evaluating the means; forecasting the epidemiological situation; developing programs for preventing and fighting against the disease.

Preventive measures

Currently, prophylaxis of Lyme Borreliosis is based on nonspecific measures, such as avoiding areas with high tick density and limiting exposure to ticks bites (application of acaricides on the skin; coverage of accessible areas) while attending endemic areas; regular inspecion of possible presence of ticks on the body and their imminent careful removal with the forceps and not fingers. It is not recommended to twist, crush, and squeeze the tick, avoiding contact of tick saliva and intestinal contents with the skin. Removed ticks are burned, while the bite site is throughly washed with soap and water and disinfected with alcohol. The bite site is regularly checked for one month so to avoid a possible erythema.

The control measures also include the reduction in the number of host animals (in America the deer density is reduced to 2 specimens per 240 ha, which undoubtedly lowers the risks of transmitting the infection) and the use of disinfectants to reduce the tick density.

An effective prophylactic measure is to inform the population about the risk of catching the disease, especially within a professional environment.

1.2.8. Rabies (Rabies, hydrophobia)

Short history

Rabies was reported since the early history, about 5 thousand years ago (dog bite-related fatalities). It was described by Democritus 2500 years ago, and by Celsius (in the 1st century AD), who called it hydrophobia. In the Middle Ages there were described epizootic spread in wolves, and in the eighteenth and twentieth centuries in wolves, foxes and dogs. In 1804, the German scientist Tzuka first determined that rabies is transmitted by saliva into blood.

In 1879, Victor Galtier used domestic rabbits as experimental animals, which facilitated further studies conducted by Louis Pasteur, Emile Roux and Joseph Chambarland, who prepared the first rabies vaccine taken from the spinal cord of an infected rabbit. In 1906, in Odessa, the first Pasteurian vaccination station against rabies was founded. In the 19th and 20th centuries, V. Babes and A. Negri described the histopathological

eosinophils formations in neurons of animals that died as a result of madness (Babes-Negri corpuscles). However, the virotic nature of rabies was proved by P. Remlinger only in 1903.

The pathogen agent is rabies virus that belongs to the Rhabdoviridae family, the genus Lyssavirus. The viruses are cylindrical in shape, of 180x75 nm size, a rounded on the one side and flat on the other side. They resemble a cartridge in shape. The nucleocapsid has a helical symmetry, and the RNA genome is a single-stranded, linear and negative one. The bilipidic viral envelope derives from the cell membrane and contains the glycoprotein G-factor of neurovirulence, which induces protective antibodies.

The rabies virus is considered to have a single antigenic type and has been divided into four main serotypes:

- serotype 1 - prototype rabies virus, including mammals and bat isolates (*classical rabies virus, wild, street virus*) and *fixed* rabies virus;
- serotype 2 - virus of *Lagos* bat;
- serotype 3 - virus of *MoKola* rodent;
- serotype 4 - Duvenhage virus isolated from humans and bats.

It is a typical neurotropic virus. Its presence in the central nervous system is evidenced by Babes-Negri inclusions (corpuscles), pathognomonic eosinophilic intracellular formations, which are more commonly found in Ammon's horn, in the marrow and spinal ganglia.

The rabies virus resists to + 4°C for several weeks in the nervous substance and salivary glands. It loses its infectivity at 45°C in a few hours, at 56°C- in one hour and at 100°C- in a minute. It is resistant to -190°C (it lasts over 3 months in the rabbit brain). Rapid and controlled dissection (lyophilization) maintains its viability, whereas the slow desiccation at 22°C reduces the virulence.

It resists for a longer period in the bats feces. However, it is also quckly inactivated by ultraviolet rays, sunlight, X-rays, lipid solvents (ether, desoxicolat sodium), trypsin, disinfectants - 2% lime chloride, lysol, and carbolic acid.

The *fixed virus* obtained by L. Pasteur et al. through multiple intracranial inoculations of the wild virus in rabbits differs from the first one by the following:

- it is not pathogenic for humans;

- it is not eliminated by saliva;
- the incubation period is shorter – 7 days.

Since both versions are similar according to their antigenic structure, vaccination with the strains of the fixed virus also leads to the protection against the *street virus* a well.

The reservoir and sources of pathogen

The rabies virus is pathogenic to a wide range of hosts: wild animals (wolves, foxes, raccoons, coyotes, jackals, wild boars, badgers, martens, squirrels, bats, rodents, etc.), domestic animals (dogs, cats, cattle, sheep, horses, pigs etc.), and humans. Almost all warm-blooded animals, including birds are susceptible and may transmit the virus to humans.

The prevalent hosts, however, are the carnivorous mammals and hematophagous bats. Within a biocoenosis, the reservoir may consist of several species, but not all are primary hosts for maintaining the virus in nature. Typically, enzootitis is found in sensitive species within both natural and humans foci, in the local fauna, as well as social and economic conditions.

The dog, for example, is the main source of rabies virus in Asia, Latin America and Africa; the fox - in Central and Eastern Europe, Canada, Arctic areas (Arctic fox); the raccoon dog - in Eastern Europe and the western US; the coyotes - in Latin America and the western US; the skunk - in the northern US and Canada; the mongoose - in South Africa and India. Hemophagocytic bat is an important source in the South and Central America.

The source of pathogen is, as a rule, the diseased animal or healthy carriers. In enzootic areas, up to 15% of the bats colonies are carriers of the virus, which persist during hibernation. A particular feature of rabies in bats is that the virus can also be eliminated through feces, where it retains its viability for a long time even on dry land.

The virus is eliminated from the host with saliva of the diseased animals 2-3 days before the onset of the disease and is transmitted among animals through bites.

Rabies is widely spread among fox population, especially in the first quarter of the year and close to the winter season.

There are two main clinical forms of rabies among animals: the furious one, with an evolution of 2-5 days and the other is paralytic, which lasts 5-10 days.

There was recorded an increasing number of rabies among animals in Moldova *(Figure 54)*, which confirms the intensification of rabies epizootic process.

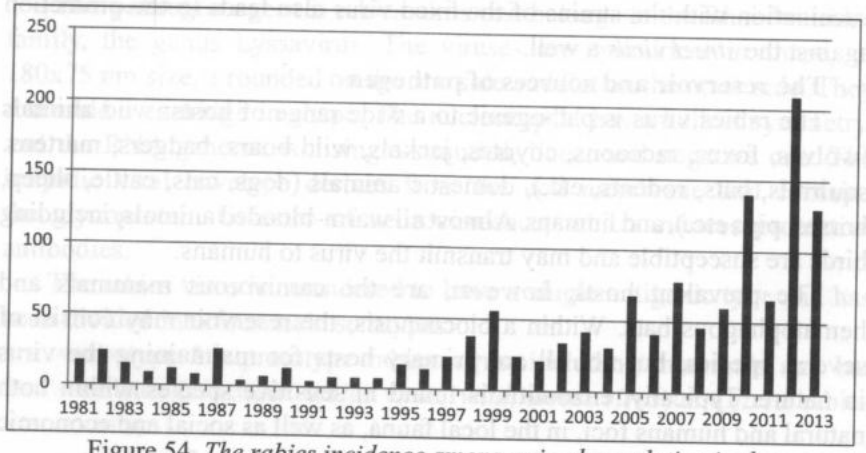

Figure 54. *The rabies incidence among animal population in the Republic of Moldova, in 1981-2013.*

41.8% of confirmed cases are in foxes and other wild animals, 18.1% - in dogs, 10.2% - in cats, 29.0% - 0.9% in cattle, horses and pigs.

Both humans and farm animals, contracting rabies, do not serve as a reservoir of the virus in nature. There were no cases of human-to-human or farm-borne disease. Moreover, human and animal diseases serve as indicators of epizootic disease evolution within the territory. The disease has an exclusively fatal outcome. The diagnosis of rabies in animals is confirmed by laboratory examination: the presence of Babes-Negri corpuscles and immunofluorescence microscopy.

Ways and means of transmission

The rabies virus is transmitted from animals to humans, usually through direct contact, when the saliva enters the body through bites or scratches injury from a diseased animal. The virus does not penetrate the intact skin. Other epidemiologically important routes of transmission are the mucosal contamination with infective material, infected corneal transplantation (so far 8 such cases have been described), through aerogenic ways or aerosols, and in laboratories. The transmission to humans is neither oral nor transplanted. Therefore, the virus transmission is carried by the bite of a diseased animal. The transmission is facilitated by the severity of injuries.

Bite severity depends on the depth, multiple injuries and lesion site. The most serious bites are considered to be on the head, face, upper extremities - open areas and rich innervated endings, favoring a quick access from traumatized nerves and shortening the pathway to the CNS. Bites through clothing are less serious. The duration of the incubation period depends on the severity and bite site and is much shorter (8-14 days) in the multiple bites on the head or upper extremities.

Manifestations of epidemic process

Rabies is widespread across the globe with the exception of some territories (England, Norway, Malta, Japan, Hong Kong, Singapore, Malaysia, New Zealand, Hawaii), where rabies was eradicated due to the management programs and preventive measures. Millions of exposures are reported annually, with about 55,000 of deaths worldwide, of which 60% did not require medical assistance.

The territory of the Republic of Moldova is considered unfavorable to rabies, since the main reservoir and sources include different species of wild and domestic animals in different time periods.

Three periods are distinguished in the evolution of rabies in Moldova:

1. In the 19th century - the first half of the 20th century, wolves are considered to be the main reservoir of rabies virus in nature due to their high density in the territory of Moldova.

2. The postwar period (1940s-50s of the 20th century)is presented with an essential predominance of dogs as reservoir of the rabies virus, driven by a decreasing number of wolf population and the increasing number of homeless dogs.

3. The contemporary period, starting from the 1960s is represented by the predominance of foxes as rabies virus reservoir.

There are two types of rabies outbreaks detected in Moldova:

- natural nidi, where the virus circulates predominantly in wild animal population;
- human outbreaks (urban-type), where the rabies virus circulates predominantly among domestic animals.

The manifestations of infectious process

In humans, the rabies manifestations occur dramatically, following a variable incubation period, on average 2-3 months from the bite, scratch, or wound contamination. There are also rare cases when, due to multiple and deep bites in the head region, the incubation period may last for 10-14 days, and sometimes it may extend up to 6 months, whereas in

cases of superficial bites on the body and the lower limbs,or bites through clothing it may last for a year.

The prodromal period, which lasts 2-4 days, is characterized by nonspecific symptoms: mild fever, loss of appetite, headache, nausea, sore throat, tingling in the area of the bite, nervousness, and anxiety.

The disease is manifested by high fever (40-41°C), shortness of breath, increased salivation, intense excitation, painful spasms, changes in voice, a fear of water - hydrophobia (the patient refuses to drink), aerophobia (fear of air movements) and photophobia (fear of light), tendency to bite everything around. Encephalitis occurs in the late stage, which can quickly turn into a comatose state. Death occurs within 4-5 days as a result of the respiratory disorders.

Laboratory diagnosis

It is possible to detect the virus from the patient's saliva or cerebrospinal fluid as well as through direct immunofluorescence of fingerprints during the cornea and skin biopsy. In practice, these investigations are difficult to perform, therefore the diagnosis is usually based on clinical signs (hydrophobia, aerophobia, photophobia, hypersalivation, intensive excitement). The post-mortem diagnosis is based on detecting Babes-Negri bodies taken from the areas with their high density (Amon's horn, cortex, medulla, Gasser's lymph node) and ELISA - detection of rabies antigen in the brain tissue.

The samples (of brain, spinal cord, brainstem, cerebellum, skin fragments) are transported in frozen state or in saline solution with 50% of glycerol.

Virological investigations can be carried out on baby mice by the intracerebral administration of pathological material with subsequent surveillance for 28 days. If the animal dies in 6-7 days, it allows to suggest the rabies virus.

Epidemiological surveillance include:

- supervision of animal populations - main hosts of the rabies virus, which is highly flexible and easily accommodates to high density of canine population;
- surveillance of the situation and epizootological natural and human outbreaks;
- Epidemiological analysis of morbidity due to rabies;
- monitoring the movement of the pathogen;
- prognosis the epizootological and epidemiological situations.

Control measures

Preventive measures

Preventive measures in natural enzootic foci include:

- adjusting the number of wild carnivores, especially the fox (main reservoir of rabies in nature) to the ecological balance (0.1-0.2 individuals for 1 km2). This level prevents the spread of rabies and contributes to maintainance of species;
- oral vaccination of wild carnivorous animals with live attenuated vaccine. The vaccine can be distributed among animals by appropriate food baits (5-10 ha). In some countries the vaccination with live attenuated rabies virus has resulted in the eradication of rabies.

Preventive measures in human enzootic foci:

- record, evidence and vaccination of pets (dogs, cats). Mandatory use of the animal leash outside;
- reducing the number of homeless animals by surgical or chemical castration, keeping them in asylums with the subsequent mandatory vaccination;
- quarantine of pets imported from abroad.

Prophylactic measures - before the exposure include vaccination of practitioners at risk of being infected with rabies (doctors, veterinarians, veterinary laboratories workers, hunters, people working in enzootic areas or residing in these areas etc). The vaccine is prepared from human diploid cell cultures. It is administrated intramuscularly, on the 0, 7, 14, 28 days. Serologic testing is recommended every 6 months within 2 years.

Prophylactic measures - after the exposure to human infection (hydrophobia).

The lethality rate is absolute if the illness develops. The prevention of illness in people exposed to rabies virus contamination is crucial and includes the following measures:

- coniderable reduction of the risk of contracting the virus immediately after the accident (bite, scratch, wound or mucosal contamination with the diseased animal's saliva) by local non-specific treatment including washing with soap or detergent, abundant rinsing of the wound with water, wound disinfection with 70% ethyl alcohol, aqueous iodine solution, or oxidizing substances (hydrogen peroxide, potassium permanganate);
- emergency visit to family doctor or any nearby located medical institution.

Primary care provides:

- Wound processing and disinfection. Wounds are sutured not earlier than 3 days. If the suture is recommended, it will be done only after local infiltration with anti-rabies serum;
- specific therapy involves the administration of human immunoglobulin or equine on the lesion as soon as possible;
- emergency tetanus prophylaxis;
- examination of the victim by a traumatologist;
- informing the veterinarian service.

Specialized medical care in trauma or surgery units includes:

✓ wound treatment;

✓ indication of specific conditional and unconditional prophylactic treatment, see *Table 5* bellow.

Table 5

The scheme of emergency prophylaxis with antirabies vaccine and specific immunoglobulin

Character of contamination	Animal status		Prophylactic treatment
	At the moment of contamination	During 10 days of supervision	
Contamination of undamaged or damaged skin with animal saliva; isolated superficial bites or scratches on the body, upper or lower limbs (except for the palm and fingers).	Healthy	Healthy	It is not indicated
	Healthy	Disease, death, disappearance	Initiate the treatment since the appearance of the first signs of disease or disappearance of an animal: 1ml of vaccine- on the 1st, 3, 7, 14, 30, 90 days.
	Suspected of rabies	Healthy	Vaccine treatment should be initiated immediately- 1 ml on the 1, 3, 7, 14, 30, 90 days; the treatment should be stopped on the 10 th day if the animal remains healthy.
	It has been proved to be infected with rabies; it disappeared; it was killed; the diagnosis is not known.	-	Treatment should be initiated immediately, unconditionally: vaccine -1ml on the 1st day, 3, 7, 14, 30, 90.

Continue

Contamination of mucous membranes with saliva of the animal; bites or damage of the head, face, neck, hands or fingers of upper or lower limbs; Multiple and severe bites with damages in any location of the body, isolated deep bites.	Healthy	Healthy	Treatment should be initiated immediately, unconditionally, with rabies immunoglobulin – at a dose of 40 IU/kg on the 1st day + rabies vaccine with 1ml on the 1st, 3, 7, 14, 30, 90 days.
	Healthy or suspected of rabies	With rabies	Prophylactic treatment should be initiated immediately, unconditionally, with rabies immunoglobulin – at a dose of 40 IU / kg, on the 1st day + 1 ml of vaccine on the 1st, 3, 7,14, 30, 90 days.
	It disappeared or died, the diagnosis is unknown	-	
Skin contamination with saliva or mucous of any location, scratches, damage caused by bites of wild carnivorous animals, bats and rodents	-	-	Unconditional treatment should be initiated immediately: rabies globulin -dose of 40 UI/ kg on the 1st day + 1ml rabies vaccine on the 1st day, 3, 7, 14, 30, 90.

Antirabies vaccination is the only effective way of preventing the disease. The vaccination failure is determined by high virus concentration in the bite, short incubation period, delayed vaccination, inappropriate behavior of the victim (alcohol use, cooling, overdosage). It was found that in 70% of hydrophobia cases, recorded in the Republic of Moldova, with a period of more than 45 years, the victims did not seek treatment, 13% of cases presented to have the treatment, but they were not adiministered prophylactic measures and 17% did not follow the treatment regimen.

Anti-epidemic measures

Medical workers of all medical institutions are obliged to identify people with bites or scratches of animals. Medical workers are obliged to consider these persons as victims at risk of contracting the rabies virus.

The medical institution notifies the territory CPH by phone and fills in the form No.058/s for each case of rabies within 12 hours. The veterinary service is informed as well. Epidemiologic and epizootic investigations are performed for each case.

People with bites are hospitalized if they are:

- persons who are residents of rural areas;
- persons with neurological, immunological, allergological social status;
- persons with multiple and deep bites, especially on the head and extremities;
- persons subjected to revaccination.

The sick or suspected hydrophobic person is hospitalized urgently to the infectious disease department. The patient is to be isolated throughout the disease. The patient's room, is to undergo a current and terminal disinfection.

It is important to avoid contact with the patient's salivary secretions. It is mandatory to wear protective equipment (gloves, aprons, goggles, etc.).

Epidemiologico and epizootic investigation aims to discover the source of the virus, time and conditions of contamination, people who were in contact with the sick or are at risk of contamination under the same conditions as the patient did, will undergo prophylactic rabies treatment and clinical surveillance.

Epizootic measures:

- animals with aggressive behavior, will be reported to the sanitary-veterinary authorities for isolation and surveillance.
- apparently healthy animals, who have bitten a person, are isolated and monitored for 10 clinical days. Suspicious signs of the disease arise within 4-7 days.
- wild animals suspected of rabies or which have bitten a person are euthanized. The head is transported frozen (dry ice) to a veterinary lab.
- unvaccinated domestic animals, bitten by a rabid animal are euthanized or can be supervised by a veterinarian for at least 6 months. When the restriction is suspended, the animal is vaccinated 30 days before being released. If the animal has been vaccinated against rabies, a follow-up surveillance is required during 45 days.

1.2.9. Yellow fever

Pathogen agent

Amaril virus belongs to the *Flaviviridae family*, the *flavivirus genus*, it is the group of *mosquito-transmitted* viruses (vector classification).

The virus is spherical, having a size of 40-50 nm. The single-stranded RNA genome is located inside the capsid, forming an icosahedral symmetry. The bilipidic viral envelope contains the outward-oriented glycoprotein E, responsible for neutralizing and hemagglutinating activity,and being the major protective antigen and inducing neutralizing antibodies.

The reservoir of infection

The main natural reservoir is presented by various species of monkeys and sometimes marsupials and rodents from the areas of sylvatic jungle. Mosquitoes are both vectors and a reservoir of virus. The infection is transmitted transovarially among mosquitoes, which explains the persistence of the virus in the dry season.

Transmission is carried by infected mosquitoes of *Aedes* species. Mosquitoes of the species *Sabethes Haemagogus* from South America forests can be involved in the spreading as well. In Africa, the main vector is Aedes africanus, involved in the transmission of the virus from monkeys to humans.

Although *Aedes albopictus* is inefficient in transmission, it continues to expand as a potential vector in the urban and sylvatic circulation.

The patient's blood is infectious before a febrile onset and within the first 3-5 days of the illness. The disease is easily transmitted in the presence of the virus. It is not transmitted by contact or objects. The incubation (in the mosquito body) is 9-12 days in tropical areas. The optimal activity is temperature-dependent, being 30° -35°C in case of *Aedes* females. Mosquitoes remain infected lifelong.

Manifestations of epidemic process

Yellow fever is present in Central America, South America and Africa, where it develops sporadically - endemic, epidemic and enzootic.

Central America, South America, from Mexico to Argentina and in Africa - Angola, Zaire, Zambia, Tanzania, Uganda, Kenya, Ethiopia, Somalia, Sudan are found to be endemic areas. The incidence ranges from a few hundred to ten thousand of cases annually.

According to the viral reservoir, the epidemiological process may have two aspects:

- *urban yellow fever*, with the following cycle: human → mosquito *Aedes aegypti* →human;
- *jungle yellow fever* from sylvatic, rural, ecological cycles: monkey → *Haemagogus mosquito* → monkey.

Yellow fever has been reported in Asia.

Seasonality is dependent on weather conditions, which influence the biology of the virus vector. Epidemics of yellow fever reach the peak during the rainy season in jungle. In areas with isothermal limits of +25°C, the disease develops endemico-epidemically throughout the year. The territorial and localized outbreaks occur in geographic areas with mean temperature + 25°C.

The manifestations of infectious process

The clinical spectrum is varied, from inapparent, subtle, and mild forms to very severe and even fulminant types. The incubation period lasts 3-6 days.

The *common clinical form* starts suddenly with high fever, chills, headache, generalized muscle pain, prostration, nausea and vomiting. There are the following successive stages:

The *initial* (hyperaemic) phase lasts 3-4 days. The onset is acute: the patient develops severe headache, chill, vertigo, lumbar and extremities pain. The body temperature rises rapidly to 39-40°C and higher. Thirst, nausea and recurrent vomiting with mucus are characteristic. The face, neck and the upper chest are markedly hyperaemic and swollen from the first days of the disease. The scleral and conjunctival vessels are injected (rabbit eyes). The patient complains of insomnia. The pulse rate is 100-130. On the second or third day, the patient's condition worsens, he develops cyanosis and then slight jaundice of the skin and visible mucosa. Blood studies reveal hyperbilirubinaemia and high transaminase (mostly AsAT) activity. The liver and spleen are slightly enlarged and painful to touch. Tachycardia is followed by bradycardia. Epistaxis and gingival bleeding are frequent. Blood is present in the vomitus („black vomit").

The remission stage starts in 4-5 days. It lasts from several hours to one day. The body temperature drops to normal or subfebrile. The patient's condition improves, vomiting ceases, pain abates. In case of a mild form of the disease, the recovery begins with the fall of temperature.

In moderately severe and severe cases, the phase of remission is followed by the phase of *reaction* or *venous stasis* (toxaemia) that lasts 3-4 days. Remission can be absent and the initial period can be followed immediately by the reaction phase. The patient's condition worsens rapidly. The temperature rises again, and jaundice intensifies. The skin is pallid; haemorrhagic rash (petechiae, purpura, ecchymoses) develops on

the trunk and the extremities. Gingival bleeding, haematemesis, nasal and uterine bleeding leading to miscarriage, develop. Arterial pressure falls. Oliguria or anuria associated with azotaemia develops. Haematologic changes: leucopenia, neutropenia, lymphocytopenia, high globulin and colour index, and accelerated ESR; blood coagulation is delayed.

The fever period lasts 8-9 days, and then the phase of recovery begins with a slow restoration of the disordered functions of the organs and tissues.

A fulminating form of the disease leads to death in 3-4 days.

Laboratory diagnostic

Diagnostic procedures are performed according to the standard precautions recommended by the level 4 biohazard.

Pathological matters: blood, cerebrospinal fluid, liver, brain, and kidneys samples.

- *The direct examination* is conducted to detect viral antigens through immunohistochemical staining, immunofluorescence of infected tissues or ELISA in blood, and viral RNA by RT - PCR.
- *Virus isolation* is obtained after inoculation of mice, mosquitoes (Toxorynchites) or tissue cultures of mosquito,and rapid identification by RT - PCR isolates from plants or animals,or the ELISA and EF tests with monoclonal antibody.
- *Serological diagnosis* based on IHA, CFR, neutralization tests, the radial haemolysis and indirect EF, ELISA, RIA tests, showing a 4-time increase in the specific IgM antibody titer (convalescent serum / serum) or recent infection.

Control methods

Preventive measures

Active immunization with live attenuated vaccine *amaril 17 D*:

- ✓ involves all persons at risk, starting with children aged 9 months or older, those with job-related activities in endemic areas, residents or traveling. A single dose of live attenuated *amaril 17D s*train vaccine is effective in 95% of cases. The antibodies appear in the 2nd week and persist for at least 35 years. The International Regulations provide for revaccination every 10 years when traveling outside the endemic areas.
- ✓ In Africa, in endemic and epidemic areas, active immunization was introduced due to the expanded vaccination program (WHO). The vaccine can be administered at the age of 6 months.

Contraindications:

- During the first 4 months of age (encephalitis associated with vaccination), it is administered with caution – at the age of 4-9 months, when the risk of exposure exceeds the risk of encephalitis associated with vaccination;
- in pregnant and lactating women;
- it is not recommended for people with HIV symptoms or immunosuppressive people
- The side effects are not severe, as a rule; 10% of vaccinees suffer from headache, fever and myalgia. Severe reactions are observed in advanced ages and thymic disorders. Meningoencephalitis is very rare, and occurs in 6 months after the vaccination. It develops without sequelae.

Active immunization and vector control/ eradication is recommended in *urban fever.*

Sylvatic fever is controlled by vaccination of rural residents who enter the forest areas. It is recommended to wear personal protective equipment (appropriate clothing, repellents).

Anti-epidemic measures

Control of patient, contacts and environment:

- Reporting to local Center for Public Health. The reported cases are assessed at the national level in order to be notified to WHO.
- Isolation: following the precautions related to blood and body fluids. Mosquitoes are prevented from the access to the insulator for at least 5 days from the onset. Disinfection is performed daily.
- Desinsection of patient's house and neighborhoods.
- Immunization of family and neighborhood contacts, if they have not been immunized.
- Epidemiological investigation. Investigation of the contacts, source, and patient's visits during the period of 3-6 days prior to the onset, in the endemic-epidemic area or in forest areas
- *Investigation* to the presence of mosquitoes in the area of residence or workplace.
- Investigation of mild febrile illness and unexplained deaths.
- There is no quarantine.
- There is no specific treatment.

Epidemic measures

In urban yellow fever the immunization is carried out, starting with people at risk and those living in *Aedes aegypti* -infested areas and who were vaccinated more than 10 years ago:

The immunization is carried out to all residents and those who enter the forest areas in sylvatic yellow fever. Non-immunized people will avoid entering those zones for 7-10 days after vaccination.

The presence of infection in Africa is hardly attested in nature, because in monkeys only asymptomatic infection develops and death rarely occurs. Compared to Central and South America,where monkey die after the disease and therefore the isolation, identification and confirmation by histopathological examination is recommended.

In natural disasters, mass vaccination is recommended to prevent outbreaks.

- International measures:
  - ✓ International Health Regulation (2005) does not provide the reporting to WHO, unless yellow fever is a public health emergency, which is to be reported within 24 hours, according to the following criteria:
    - Particular impact on public health.
    - unusual and unexpected event.
    - The risk of international disputes.
    - obvious risk, which may result in trade and travel restrictions.
  - ✓ International Health Regulation no longer provides applicable measures to vessels, aircraft, other transport.
  - ✓ Quarantine for primates during 12 weeks and in case of farmed primates for only 30 days.
  - ✓ Many countries require a proof of vaccination – the certificate of vaccination for travelers entering or leaving the endemic-epidemic areas of Africa and South America. In the absence thereof, they are to be quarantined for a maximum of 6 days. The international vaccination certificate is valid for 10 years from the date of vaccination or revaccination.

1.2.10. Dengue hemorrhagic fever

Pathogen agent

The Dengue fever virus belongs to the *Flaviviridae family, genus flavivirus.* The virion is spherical in shape, of 40-50 nm, with positive,

single-stranded RNA. It has a bilipidic coating with included E glycoprotein inside.

There are 4 serotypes known to induce the disease, types 2, 3, 4, and 1. The serotypes 2 and 4 induce hemorrhagic forms in recurrent infection and types 1 and 3 - in the primary infection.

The reservoir

Humans and monkeys are the natural hosts.

The virus is transmitted according to the following cycle:

Human→Aedes aegypti → Human, in tropical urban areas, or

Monkey→mosquito → Human, within the forests of SouthEast Asia or West Africa.

The man is contagious during the maximum viremia, before the febrile period and until the fever drops.

Mosquitoes become infective after 8-12 days of infected blood feeding.

The transmission

The transmission of the disease is carried out by mosquitoes, *Aedes aegypti* mainly. The mosquito bites in the daytime, with a maximum activity after the sunrise (2 hours) and several hours before the sunset. In West Africa, it is likely an epizootic transmission from monkeys.

Manifestations of epidemic process

Dengue fever virus is widely spread in different geographical areas: Australia, Japan, Central and Latin America, the Caribbean, Southeast Asia, Greece. There are 4 serotypes of the *Virus Dengue* that are endemic in Africa.

At the begining of this century, *Dengue virus* was reported in Chile, Easter Island, Puerto Rico. In 1998, there were reported 1.2 million cases in 56 countries.

About 3 million cases of *Hemorrhagic Dengue fever* have been estimated, mostlly in the Asian territory.

The epidemic is dependent on the biology of the mosquito, being endemic where *Aedes* aegypti lives in optimal conditions throughout the year. The epidemics occur anywhere where the virus is imported or present either in rural or in urban areas (*urban Denga*).

The susceptibility is general. In children clinical forms are milder in Asia, whereas *Dengue Haemorrhagic fever* being a childhood illness. The immune protection is lifelong, but only against the infecting serotype. The disease severity and possible occurrence of *Dengue hemorrhagic fever* form can not be predicted.

The epidemics occur during summer in temperate zones.

The manifestations of infectious process

The WHO definition of the clinical case implies:

- ✓ recent fever or a 2-7 - day fever;
- ✓ at least one of the following haemorrhagic manifestations: petechiae, ecchymosis, purpura, haematemesis, melaena, other hemorrhages (positive tourniquet test);
- ✓ thrombocytopenia (/100.000/mm3 units);
- ✓ plasmorrhagia evidence, at least one of the following: increased PCV by 20%, pleural effusion, ascites, hypoproteinemia.

There is shock, rapid pulse, weakened heartbeat, hypotension, clammy skin, clonic contractions of the hand In additon to all of the above symptoms.

After a 5-8 - day incubation period, *Haemorrhagic Dengue fever* starts with high fever (40°C), chills, retroorbital pain, myalgia, back pain, and 24-48 hours of a transient macular rash. In contrast to the common form of Denga's disease, which is characterized by such symptoms as lost of appetite, vomiting, skin hyperalgesia, arthralgia during 2 - 7 days and which evolves into healing (before feverish fever, „biphasic sadleback fever"), haemorrhagic Dengue exacerbates the patient's general condition within 2-5 days after its onset. There is a profuse, cutaneous-mucosal hemorrhagic syndrome, manifested by skin petechiae and ecchymosis, blood clotting disorders, increased vascular permeability, vasomotor disorders, tendency to hypovolemic shock and collapse. Death is the result of polyvisceral failure, profuse bleeding, hemorrhagic shock and hypovolemia.

The death rate is of 40-50% in undiagnosed and untreated cases, and 1-2% in diagnosed and treated cases.

The differential diagnosis of common form includes measles, rubella, influenza, chikungunya disease, leptospirosis, typhoid fever, typhus, and other systemic febrile diseases manifested by rash.

Laboratory diagnostic

Diagnostic procedures are performed with the precautions recommended in the 4th level of biohazard.

Pathological samples. Antemortem – blood and cerebrospinal fluid, collected as close as possible to the onset of the disease. *Postmortem* – brain, spinal cord, liver, kidney, heart can be collected at autopsy.

- Direct examination highlights:

- ✓ viral antigens by immunohistochemistry staining, isolated from cerebrospinal fluid and blood and by immunofluorescence as well.

✓ viral RNA detected by RT - PCR (sensitive and specific Dengue fever).

- *Isolation on cell culture* of mosquito tissue (*Aedes, Culex*), which is very sensitive to Dengue fever or mosquito inoculation of blood samples taken during the first days of the disease, identified by RT – PCR test.
- *Serological diagnosis* is carried out with IHA, CFR, ELISA, RIA tests, and the positivity is identified by a 4-time increase of antibody titres in the serum collected during the onset and the recovering period. Recent infection is detected by ELISA for IgM antibodies.

Serum antibodies are detected in 6-7 days after the onset of the infection.

Control Methods

Preventive measures:

- Destruction of mosquito larval habitats (all kinds of water sources that serve for multiplication of mosqutoes).
- Use of larvicides.
- Personal protection against bites (clothing, repellents).
- Active immunization in all sylvatic areas and their periphery.

Anti-epidemic measures:

- reporting to the Local Public Health Authority (about all cases and outbreaks).
- Compulsory hospital isolation and universal precautions when handling the blood samples, use of insecticides in case of febrile patients.
- Disinfection.
- The quarantine and disinfection are not recommended.
- The contacts are not immunized.
- Epidemiological investigation (source, not investigated cases etc.).

The nonspecific treatment, especially that of hypovolemic shock includes: oral and parenteral hydration (i.v. saline, Ringer's solutions), 10 to 20 ml/h/ kg. The transfusion of blood, fresh plasma or other colloids, fibrinogen and platelet concentrates are used in severe hemorrhage. Aspirin is not administered (hemorrhagic potential, Reye syndrome).

Epidemic measures:

- The destruction of larval habitats; draining of mosquito breeding areas; disinsection inside the dwellings.

- Use of repellents and protective equipment.

1.2.11. Haemorrhagic fever Kyasanur

Short history

The disease is reported to occur in humans in the forests of Kyasanur-Karnataka, India, with a zoonotic outbreak among monkeys (monkeys fever, monkey disease) since 1957.

The pathogen agent

The Haemorrhagic fever virus belongs to the *Flaviviridae family, the Kyasanur flavivirus genus*, having morphostructural features similar to other members of the genus: it is spherical in shape,40-50 nm, icosahedral symmetry nucleocapsid, positive, single-stranded RNA genome. The coating contains glycoprotein E and it is related to the complex of tick encephalitis viruses.

Virus reservoir: monkey, small rodents, probably bats. It i possible that sheep, goats or cattle, do not provide effective transmission due to the low viremic level.

Transmission: by *Haemophysalis spirugena* tick bite in the nymph stage.

Manifestations of epidemic process

The disease develops during the dry season in Kyasanur forests in India. There were 104 cases of death of the 1142 cases reported in 1983. The annual incidence is 100-500 cases with a mortality rate of 3-5%.

The susceptibility is general. The risk of infection is associated with the entrance of humans into the forest.

The epidemic process is manifested as an epizootic one among monkeys.

The manifestations of infectious process

The disease starts with high fever, chills, headache, epistaxis, vomiting, diarrhea and prostration after 3-8 days of incubation period. In 4-5 days, a vesiculopapular enanthema, lymphadenopathy, hemorrhagic conjunctivitis, bradycardia and hypotension develop. It is accompanied by bleeding of the digestive, respiratory and urinary tracts, resulting in hemorrhagic shock and death in severe cases. Biphasic disease can develop after a period of remission, when meningoencephalitis phenomena develop, and recovery without sequelae occur. A long period of convalescence may be associated with debility and fatigue.

Laboratory diagnosis

Pathological samples: blood and cerebrospinal fluid. They are processed according to the precautions required to level 4 biohazard.

- *Virus isolation* is carried out in cell cultures by inoculation into newborn mice and identification by hemagglutination, ELISA or IF tests.
- *Serological diagnosis* reveals the presence of specific antibodies by seroneutralization, haemagglutinin inhibition and complement fixation.

Control Methods

Preventive measures

It is important to detect the factors involved in the epizootic process:

- Knowledge of the ecosystems and the species that constitute the reservoir and vectors.
- Knowledge of human / animal disease transmission modes.
- Knowledge of climatic features that influence the evolution of vector-host cycles.

Individual protection measures, especially for forest workers are as following:

- The use of repellents and insecticides by spraying them on the clothes.
- After a 3-4 - hour activity in the infected areas, the entire body surface ist o be checked for ticks, which are to be removed with the forceps, being particularly careful when removing the parts of the tick's cephalic extremity. During this operation, hands are to be protected by gloves.
- Forestry personnel cabins are to be installed on deforested, burned and phenol-treated land.
- Persons at risk are given a high-protection vaccine, inoculated in cell cultures and inactivated by formalin (1992).

Anti-epidemic measures

- Epidemiological investigation is required only if the contact took place outside the known habitat of the hosts/vectors.
- The patient is hospitalized without being isolated. The quarantine is not imposed.
- There is no specific treatment.

- Vectors combating is difficult. Disinsection measures are apllied in the natural habitat of ticks via insecticide treatment of the woodland.
- Rodents that are hosts for tick larvae are to be combated. Lands and pastures hosting the rodents and ticks are to be deforested and cultivated if it is possible.

1.2.12. Omsk haemorrhagic fever

Short history

The disease is similar to Kyasanur haemorrhagic fever. It was discovered in 1947 in Omsk, in western Siberia.

The pathogen agent

The Omsk haemorrhagic fever virus belongs to the *Flaviviridae family, genus flavivirus,* characterized by morphostructural features of the genus. It is related to the tick encephalitis virus.

The reservoir of the virus - rodents, rats (Muskrat) and ticks.

Transmission: the pathogen is transmitted by the *Dermacentor reticulatus and Dermacentor marginatus ticks* bite, but also by a direct rat-human contact via infected blood and tissues, goat milk and infected sheep. The virus survives in water, thus being transmitted through the contaminated water.

Manifestations of epidemic process

Omsk haemorrhagic fever is recorded in Omsk, Novosibirsk and Tyumen. It is an evolving epidemic or sporadic process. The incidence is 100-150 cases annually. The risk of infection is increased among the residents of rural areas, who deal with hunting or forestry activities.

The manifestations of infectious process

The incubation period is 3-8 days and it resembles the symptoms of Kyasanur disease. Hemorrhagic diathesis is present, which involves practically all the organs, along with hypotension, dehydration, and thrombocytopenia. Profuse bleeding can lead to hypovolemic shock and even death. The symptoms reccur after a remission of about 3 weeks, accompanied by encephalitis. There is a long convalescence, marked by fatigue, neurological and psychological disorders. The mortality rate increases up to 1-10% of cases.

Laboratory diagnostic

The methodology is similar to that in Kyasanur disease.

Control Methods

They are identical to those applied in Kyasanur disease. Attempts are being made to obtain an effective anti-viral vaccine.

1.2.13. Crimean-Congo haemorrhagic fever

Short history

Crimean-Congo haemorrhagic fever was described as a distinct nosological entity, first in Russia (1944), then in Congo and Uganda. But in 1960 after the virus isolation, it was demonstrated that, although geographically separate, the diseases were caused by the same virus.

The pathogen agent

The Crimea-Congo fever virus belongs to the *family Bunyaviridae*, genus Nairovirus, which brings together all animals' pathogens. The genus comprises more than 30 viruses, classified in 7 serogroups (serological and genetic), of which only the Crimean and Congo hemorrhagic fever viruses are pathogenic to humans.

Virions are spherical or pleomorphic, with the size of 80-100 nm. The nucleocapsid is helical and forms three separate units. The single-stranded, negative-sense RNA genome is composed of three circular segments. The virus is covered. The glycoproteins G1 and G2 are embedded in a bilipidic envelope, which are dominant antigens, inducing neutralizing antibodies specific to the subgroup and type.

The reservoir of the virus

There are various species of *Hyaloma* ticks that participate in the transovarian transmission of the virus at various stages of the development of arthropods. These ticks parasitize on a wide range of wild and domestic mammals (cattle, horses, goats, sheep) or birds, thus becoming main infection reservoir. The infection has a viremic phase, when the maintenance of natural cycles (tick → various vertebrate hosts) develop.

Transmission: by tick bite or through direct human-to-human contact. Nosocomial infections were revealed among health care personnel exposed to blood, aerosols, secretions and excretions of the patient.

Manifestations of the epidemic process

Crimean haemorrhagic fever can evolve into outbreaks or sporadic cases, on an endemic basis. Isolation and identification of viruses in *Hyaloma* ticks and seroprevalence studies demonstrated the virus circulation in South Africa, Central and Eastern Europe and China.

The susceptibility is general. People exposed to the tick bite (farmers and veterinarians) are at major risk.

The manifestations of infectious process

The incubation period lasts from 3 to 5 days with variations to 14 days. A human patient is contagious during the first 5 days of the disease. The disease begins after an incubation period of 3-12 days with fever, headache, photophobia, nausea and vomiting, followed by bleeding with various localizations (petechiae, epistaxis, hemoptysis, hematemesis and melena). Gastrointestinal bleeding can mimic an „acute abdomen". Thrombocytopenia, leucopenia, erythrocytopenia and low hemoglobin value are present. It can be associated with hepatomegaly, jaundice and changes in the normal values of serum transaminases. Death occurs in renal failure and hemorrhagic shock in 10-50% of patients.

Laboratory diagnostic

Pathological samples: blood, rarely samples of tissue biopsy, and *post-mortem* - samples from the liver, kidney, heart.

- Direct examination. It detect the viral antigens in tissues by immunohistochemistry.
- *Virus isolation* in cell culture (VERO, BHK). Isolates are identified by virus neutralization (reduction in cell cultures), IHA, CFR, ELISA. There is no experimental animal model suitable to produce human disease. The mouse is susceptible to the virus; the receptivity is age-related, fatal infection occurring after subcutaneous and intracerebral inoculation of the virus in adult mice.
- *Serological diagnosis* detect specific antibodies in serum neutralization, hemagglutinine inhibation, ELISA.

Control methods

Preventive measures

They are the same as in all human tick-transmitted infections. Mous-brain-derived inactivated vaccines are not effective.

Anti-epidemic measures

The ticks are removed from the patient, who is isolated in hospital for infectious diseases.

The treatment is symptomatic, in order to combat shock and bleeding.

Reporting to the Public Health Authority is required after the epidemiological anamnesis, clinical diagnosis and laboratory confirmation.

1.2.14. Haemorrhagic fever with Renal Syndrome

The pathogen agent

The severity of *haemorrhagic Hantaviruses* is associated with the *Hantaan and Dobrava-Belgrade* viruses, a rodent etiologic agents transmitted by rodents belongs to the *Bunyaviridae family, the Hantavirus genus.*

These are spherical or pleomorphic viruses, 80-120 nm in size, with helical, nucleocapsid and single-stranded, negative-sense RNA genomes consisting of three circular segments. The bilipidic viral particle is coated with integrated glycoproteins G1 and G2, inducing protective immune responses.

There are 8 groups of hantaviruses, comprising more than 30 viruses, which correspond to a particular rodent species in a particular geographical area.

There are 8 main hantaviruses that cause diseases in humans. The haemorrhagic manifestations are characteristic of: *Hantaan, Seoul and Dobrava-Belgrade viruses* and the *Puumala virus* leads predominantly to renal disorders.

The reservoir: Wild and field rodents, *Apodemus agrarius* species are the reservoir for the *Hantaan virus* in Asia and *Apodemus flavicolis* for the Dobrava virus in the Balkans - Serbia and Montenegro. *Clethrionomys glareolus* species are the reservoir for the *Puumala virus* in Europe, whereas *Rattus rattus* and and *Rattus norvegicus* species- for the Seuol virus all over the world. The human is an accidental host.

The transmission is via airborne mode, through aerosols, from rodent excrements, which is an experimentally proven fact, that,however, does not explain the transmission of all cases among human and rodents. The virus is excreted with urine, feces and saliva of rodents. The highest concentration is found in the lungs of rodents. The cases of nosocomial transmission have rarely been reported. The rodents' ectoparasites do not transmit the virus.

Manifestations of the epidemic process

The Hantavirus is spread across the globe, being reported annually 150000-200000 cases of Hemorrhagic Fever with Renal Syndrome, 50% of which ocurr in China, then Japan, Korea, Russia, Finland, Sweden, Bulgaria, Greece, Hungary, France and the former Yugoslavia.

The outbreaks have a maximum incidence during November-December months, whereas in the rural area - in August.

The susceptibility is general. The risk of infection is higher among farm workers, who come into contact with rodents and their excrements and the laboratory personnel handling the viruses. Inapparent infections were recorded as well.

Its seasonality is related to the density and distribution of rodent population and frequency of contact with humans. Furthermore,the ecological changes that influence the dynamics of rodent species also matter during the periods of increased agricultural activity (ex. In Asia, the spring-summer months).

The transmission from person to person is rarely possible.

The manifestations of infectious process

After 2-42 days of incubation Hemorrhagic fever associated with renal syndrome,, presents a fulminant onset with fever, chills, headache and malaise.

There are 5 consecutive or simultaneous phases in severe disease:

- The *febrile phase of onset* is present with nausea, vomiting, back pain, flushing, conjunctival hemorrhage and petechiae on the face, neck and torso. Proteinuria occurs at the end of the phase with decreased platelet and increased hematocrit values. The fever drops suddenly on the 5th - 7th days.
- The *hypotensive phase* follows is characterized by hypotension, oliguria and shock. Blood platelet count decreases, while the bleeding time and hematocrit values increase. Proteinuria reaches high levels. The phase lasts 2-3 days and death may occur in about 1/3 of the patients.
- *The oliguria phase* is observed in patients who have survived previous phases. Blood pressure returns to normal, whereas oliguria-anuria with increased proteinuria and hematuria are detected. Petechiae disappear, but severe gastrointestinal or nervous system hemorrhages and pulmonary edema, may cause death in about 50% of the patients.
- The *diuretic phase* is characterized by the normalization of the kidneys' function, with intense diuresis of 3-6 liters/day. The regulation of fluid and electrolyte balance prevents shock.
- The convalescence is long-lasting, while anemia and fatigue persist for months.

The moderate form of haemorrhagic fever is determined by Seoul and Puumala viruses. The symptoms are mild; some phases may miss; the lethality is 0.1-15%.

Mild forms are caused by Seoul virus, although severe forms are not totally excluded.

Epidemic nephropathy is caused by the Puumala virus agent. The infection can exhibit same phases as a hemorrhagic fever, bleeding is minimal and cases of death do not exceed 10%.

Laboratory diagnostic

The methodology is similar to other viruses of the *Bunyaviridae* family:

- *hantavirus isolation* in cell culture is difficult, and experimental host spectrum is limited to wild or laboratory mice and rats.
- *The serological diagnosis* confirms the presence of specific antibodies (ELISA, IF). IgM is detected by ELISA and suggest recent infection, if antibodies are detected in the early onset of the disease.
- *The differential diagnosis* is made with rickettsiosis leptospirosis.

Control Methods

Preventive measures:

- Rodent control, prevention of rodent access into homes, buildings, etc.
- Preventing rodent access to food sources (humans, animals).
- Chlorine disinfection of contaminated areas.
- Specific vaccines (inactivated, recombinant, etc.) are still in the experimental stage.

Anti-epidemic measures:

- epidemiological investigation in order to find the source and detect the contact.
- reporting to the Local Authority Public Health.
- isolation, disinfection.
- quarantine is not imposed.
- house rodent control.

The treatment is symptomatic, directed to maintain the balance of fluids and electrolytes, as well as the maintainance of constant circulating blood volume, control of renal function and prevention of renal infections. The administration of ribavirin IV is recommended in the early days of the disease.

1.2.15. Lassa hemorrhagic fever

The pathogen agent

The Lassa virus was first described in 1969, in the town of Lassa, Nigeria. It belongs to the *Arenaviridae family*, *Arenavirus* genus. It is an RNA virus. It is a small, sand-like (lat. Arenaceus - sand) granule (ribosomes), identified inside the virus, hence the family name of these viruses. There are determined 4 subtypes of the Lassa virus, which circulate in different regions.

The virus is resistant to the external environment. It maintains its long-term viability in blood or secretions. It is inactivated under the action of ether and chloroform.

The reservoir and sources of pathogen

The natural reservoir of the Lassa virus is considered to be the synastrophic rodents of the *Mastomys* genus - the *Mastomys natalensis* rats, a sub-Saharan African indigenous animal. About 15-20% of the rodent population from the endemic regions are infected with the Lassa virus (Lassa virus carriers). Animals develop asymptomatic infection, hence they are lifelong sources of infection, whereas the virus is present in saliva and urine.

A sick person is also a source of pathogen throughout the disease. The virus is excreted with the urine within 3-9 weeks and with sperm - 3 months. The virus is found in blood, urine, nasal mucus and breast milk.

The incubation period lasts 3-21 days, more frequently 7-10 days. The patient is not contagious during this period.

The transmission mode

The Lassa virus is transmitted airborne to humans by exposure to infected rodent excrements (most important ways of contamination), by food and contact with contaminated objects.

Human to human virus transmission is mainly airborne, as well as by habitual contact and transplacentally. A parenteral contamination of mucosal or skin lesions via a direct contact with contaminated biological materials is possible. This mode of transmission poses a risk to medical workers.

The transmission of Lassa virus through breast milk was also observed.

Transmission through sexual contact has not been established.

Manifestations of epidemic process

Lassa fever is endemic in West Africa (Sierra Leone, Guinea, Senegal,

Nigeria, Mali, Liberia) and Central (Democratic Republic of Congo, Central African Republic).

The prevalence of antibodies in the population is 4 to 55% in endemic areas. Annually, the death rate is 500 cases per 100 thousand population, with the record of 300000-500000 cases of Lassa hemorrhagic fever diseases. The rates of deaths have been decreasing recently because of the ribovirin treatment.

A high density of *Mastomys* rodent population, the main reservoir of the virus, leads to diseases in endemic areas, which are common for both rural and urban regions throughout the year, with a higher incidence in January -February, because of the rats' migration to people's houses.

The disease can be imported to non-endemic countries. Cases associated with healthcare commonly occur.

The manifestations of infectious process

The disease onset is acute with general symptoms: fever, facial edema, fatigue, conjunctivitis and mucosal bleeding. The symptoms characteristic of affected organs also appear: gastrointestinal tract (nausea, often bloody vomiting, diarrhea that also can be bloody, abdominal pain, dysphagia, hepatitis), cardiovascular system (pericarditis, hypertension or hypotension, tachycardia), respiratory system (chest pain, dyspnea, pharyngitis, pleuritis), nervous system (encephalitis, meningitis, convulsions). A differential diagnosis is required for both Ebola and Marburg haemorrhagic fevers, as well as other febrile conditions such as malaria.

Laboratory diagnostic

The detection of the virus from the pharyngeal lavage, blood and urine is performed by the immunofluorescent method of diagnosis and the detection of specific antibodies - by IHA and CFR.

According to the WHO recommendations, the primary diagnosis of Lassa fever is made in cases of the presence of IgG antibodies in titers 1: 512 and higher, along with the parallel detection of IgM in patients with fever in endemic areas.

Control measures (see Ebola). The monitoring and deratization of *Mastomys rodents* is essential.

1.2.16. Marburg Viral Haemorrhagic Fever

Short history

Marburg disease was first reported in 1967, when it occurred

simultaneously in Marburg and Francfurt, Germany and in Belgrade, Yugoslavia (now Serbia) respectively. The first diseased persons were the workers from of the research laboratories, exposed to African green monkeys imported from Uganda.

The pathogen – *Marburgvirus,* with a constant diameter of 80 nm, is an RNA type virus. It belongs to the genus *filovirus, Filoviridae family*. There are 4 known serologcal variants of the Marburg virus. It is genetically similar to the Ebola virus. Multiplication occurs in *Aedes aegypti* mosquito. It can be grown in vitro, in cell cultures of monkeys and it is not cytopathogenic, and in vivo - in mice. It is not pathogenic for mice. Monkeys present clinical manifestations similar to those of Marburg disease in humans. It has a medium resistance in the external environment. It is thermally stable, but sensitive to the action of alcohol, chloroform, common chemical desinfectants and UV radiation.

The reservoir and pathogen source are not definitely established. It is assumed that the virus is circulating in the population of African green monkeys *(Cercopithecus aethiops)* and fruit bats from Africa, *Rousettus aegyptiacus*. Bats do not manifest clinical forms of the infection.

The Marburg virus has been found to reproduce easily in *Aedes aegypti* mosquito; it retains its viability in the *Anopheles maculipeunis* mosquito for 8 days; and in *Ixodes ricinus* ticks - 15 days.

The sick man is contagious during the incubation period, when the virus is already circulating in the blood, and throughout the disease. There has been described a case of contracting the infection from a convalescent on the 80th day of the didease onset.

Mode of transmission. The Marburg virus is transmitted directly through blood contact, body fluids, tissues of infected persons or animals, septic drops, but also by contact with items, contaminated with blood or secretions, tissues or organs of the animal or dead patient. The risk of contamination is higher for health workers that take care of patients, workers of research laboratories that have contacts with green monkeys, imported from endemic areas.

Manifestations of epidemic process

Marburg haemorrhagic fever has endemic features. The Marburg virus is spread in the same area as the Ebola virus: Central Africa, East and West and the south of the continent. Laboratory confirmed cases have been reported in Uganda, Zimbabwe, the Democratic Republic of Congo, Kenya,

Angola, South Africa. So far, there are 468 cases of Marburg haemorrhagic infection reported worldwide, of which 80% are fatal. The largest outbreak occurred in the Democratic Republic of Congo (in 1998-2001), when 154 cases occured, of which 128 (83%) had a lethal outcome, and in Angola (in 2004-2005) - 252 cases of disease and 227 (90%) cases of deaths. Most patients were young men, who worked in the gold mines inhabited by bats.

Cases of Marburg haemorrhagic fever were reported outside the African continent, such as during the outbreak in 1967 (Germany, Yugoslavia), Russia (in 1990), US (in 2008) and the Netherlands (in 2008). All of them had an African origin of contamnation (travelling or activities in African countries, contact with animals, especially monkeys imported from Africa).

The manifestations of infectious process

The disease is manifested by an acute onset, severe headache, high fever, abdominal pain and cramping. Severe diarrhea, nausea and vomiting appear on the third day. Many patients develop dry throat and cough, sore throat with open sores on the tongue and lips. Maculopapular rash similar to that of measles occurs on the 5th - 7th days, primarily on the face and torso, then all over the body. Patients develop severe bleeding, which particularly affects the gastrointestinal tract on the same 5th - 7th days. Subconjunctival, gingival, and vaginal bleeding are present. CNS disorders are manifested by paraesthesia, confusion, irritability and meningitis in severe cases. Death occurs after 7-16 days of illness due to haemorhages. Fever persists 14 - 16 days of the convalescence period.

Laboratory diagnosis

Electronmicroscopyofbloodandorgansamplesandimmunofluorescence or PCR for viral RNA detection are used as rapid diagnostic methods. Serological diagnosis by identifying IgM and subsequently IgG antibodies is possible on the 7th day of illness. Isolation of the virus from the blood, nasal mucus or urine by the cell culture inoculation lasts for 5-7 days. The high risk of transmitting the virus through blood requires a good handling of the specimens and diagnosing only in laboratories with high level microbiological safety.

Control measures

Marburg haemorrhagic fever refers to infections that can lead to an emergency, therefore Marburg disease is included in the International Health Regulations (2005), which specifies the epidemiological surveillance and

control measures. The undertaken actions are similar to those of Lassa hemorrhagic fever and Ebola.

All the three above mentioned infections are defined by the term „contagious viral haemorrhagic fevers", which emphasizes the role of humans as a source of pathogen and increased infectiousness. Specific and epidemic preventive measures are described in the section „Ebola Hemorrhagic Fever".

1.2.17. Ebola Haemorrhagic Fever

Short history

The disease was first described in 1976, when two simultaneous outbreaks occurred in Zaire (now Congo) and Sudan, Nzara and Maride regions, near the borders of Zaire. The first case of disease was recorded in Yambuku (Zaire), near the Ebola River, where the name of the disease comes from. The Zaire epidemic killed 284 people out of a total 318 patients, the lethality rate was 89.3%; and the Sudan epidemic reported 151 cases of deths out of a total of 284, the lethality rate being of 53.2%, therefore the WHO has determined the infection as a particularly dangerous one.

Pathogen agent

The causative agent of Ebola haemorrhagic fever is a virus of RNA type genome called *Ebolavirus*, which belongs to the *Filovirus* family, the *Filoviridae* family because of the filamentous form of these viruses. So far, five Ebola virus serotypes have been confirmed, distinguished by the antigenic structure, virulence and spread area: Ebolavirus Zair (EBOZ), Ebolavirus Sudan (EBOS), Ebolavirus Reston (EBOR), Ebolavirus Tai Forest (EBOTFV) The latest new virus Ebolavirus Bundibugio (EBOBDB). Four serotypes (EBOZ, EBOS, EBOTFV, EBOBDB) cause disease in humans. The most virulent is the EBOZ serotype, the less virulent are the EBOS and EBOBDB serotypes and the least virulent – the EBOTFV serotype. The EBOR serotype does not cause diseases in humans but is pathogenic to monkeys. There are new data regarding the spread of Ebolavirus Reston infection in pigs in the Philippines, with possible transmission to humans without apparent signs of the disease manifestation. Ebola virus is genetically very close to the Marburg virus and is characterized by high variability. It is grown on guinea-pig and African green monkey renal cultures. It has a moderate resistance in the external environment. It can be inactivated by heat (when heating up to

60°C for 30 minutes, and boiling - 5 minutes). It is destroyed under the action of alcohol and calcium hypochlorite.

The reservoir and source of pathogen

The natural hosts of the Ebola virus are the bats in the endemic areas, which maintain the reproduction and circulation of the virus without being affected by the virus. They infect monkeys (chimpanzees and gorillas), which unlike bats, develop lethal forms of the infection. The virus enters the human from natural nidi. There are data about viral contamination from bats, monkeys and antelopes through the contact with disased animals and their bodies.

The human becomes a source of pathogen only during the illness; the carriage is missing. The incubation period lasts 2-21 days, on average 7 days. During this period the patient is not contagious. The contagious period starts with the first symptoms and lasts untill the recovery (2-3 months). The contagiousness is higher in the first 2-3 weeks of the disease onset. The virus is found in blood (7-10 days), different organs, tissues, secretions (nasal mucus, urine and semen). The patient is dangerous to others persons, because of possible viral transmission from human to human.

The transmission mode. The Ebola virus is introduced into the human population from natural foci, through a direct contact with blood, secretions, organs or biological fluids of infected animals (chimpanzees, gorillas, bats, forest antelopes, etc.),while nursing, collecting material for laboratory research, working with infected material, skin, fur, meat processing, body autopsy; or indirectly, by contract with objects contaminated with blood or secretions of diseased animals. Food transmission has been is possible through unprocessed animal products.

The transmission to people mainly occurs parenterally or through a direct contact with blood, secretions, organs or body fluids of infected persons, manipulations on patients, autopsy of persons who died of Ebola; or indirectly, via a contact with contaminated objects, fluids of the patient, particularly needles and syringes. A human to human transmission of the virus occurs most commonlyin health care institutions and within families. An important epidemiogenic element is the traditional funeral rituals.

The airborne and sexual transmissions are not confirmed, but they are possible.

Manifestations of epidemic process

Ebola haemorrhagic fever is a predominantly endemic disease. The spreading area largely corresponds to that of Marburg hemorrhagic fever. Ebola fever foci are located in the forests of the tropical regions of Central and Western Africa (the Democratic Republic of Congo, Sudan, Gabon, Ugarda, Guinea, Nigeria, Liberia, Sierra Leone) *(Figure 55)*.

Figure 55. *Main foci of Ebola hemorragic fever in 1976-2014 (from Wikipedia).*

Serological investigations of human population and animals, allowed to detect antibodies against the Ebola virus in Madagascar and the Philippines. The most severe and frequent outbreaks of Ebola hemorrhagic fever were recorded in 1976-2013 in the Republic of Congo (in 1976, 1995, 2001-2003 2007, 2009, 2012), in Sudan (in 1976, 1979, 1996, 2004), in Gabon (in 1994, 1996, 1997, 2001-2002) and in Uganda (in 2001-2002, 2007-2008, 2011, 2012, 2013).

Cases of Ebola fever have been recorded outside the endemic area as well (in Great Britain-1976; in the USA-1990; in Russia-1996, 2004). During the period of 1976-2013, there were recorded 2355 cases of Ebola haemorrhagic fever, of which 1548 cases were with lethal outcome, the lethality rate - 65.7%.

However, the largest epidemic of Ebola haemorrhagic fever broke out in February 2014 in Guinea and northern Liberia and expanded into Sierra Leone and Nigeria. Cases of Ebola were recorded until the 08th September 2014. There were 4269 cases of Ebola with 2298 cases of deaths, the lethality rate being of 54%; and on the 30th December 2014, 7842 cases of death aut of a total of 20081 cases of the disease were reported, that is lethality rate was 40%. The epidemic led to serious economic disorders

(border closures, cancellation of air flights, decrease of the border trade etc.). Besides the human losses

Although in most epidemics the virus is introduced into the human population from natural foci, the epidemic process itself is maintained by forming family foci (during care, housing, ritual ceremonies, etc.) and nosocomial (direct contact with patients with severe forms of bleeding, parenteral manipulations, etc.).

Only in 2014, epidemic recorded over 140 cases of deaths among health care workers.

Outbreaks of Ebola hemorrhagic fever in endemic territories occur more frequently in spring and summer, during the active period of the natural sources of infection.

The manifestations of infectious process

The onset of the disease is acute, with a fever of 38°-39°C, fatigue, headache, myalgia, arthralgia, sore throat, nausea, shortness of breath, and pain when swallowing. From the 2nd to 3rd day, the disease is manifested by vomiting, chest pain, stomach pain, bloody diarrhea, bleeding on the injection site and mucous membranes (nose, vagina, gums). On the 5th - 7th days, the general hemorrhagic syndrome develops, which is manifested by skin hemorrahage similar to the maculopapular rash, ecchymosis and internal organs hematomas, and bloody vomiting. General hemorrhagic syndrome is an indicator of poor prognosis, which often leads to death, usually on the 2nd week of illness, most commonly between the 8th and 9th days of the first symptoms. The acute phase lasts 2-3 weeks and the convalescence period is 2-3 months in case of favorable outcome.

Laboratory diagnosis

The detection of viral RNA by polymerase chain reaction (PCR) and the detection of proteins by ELISA are used for rapid diagnosis of the disease in acute phase. On the 5th - 7th day, the detection of specific antibodies by CFT and IHAR is performed. The diagnosis can be confirmed by virus isolation in cell culture.

In order to make the diagnosis, it is important to take into account the epidemiological circumstances like: visiting Central and West African countries, care of infected persons or contacting patients with signs of haemorrhagic fever or of that of unknown aetiology, attending burial rituals of those who died of hemorrhagic fever, working in laboratories with Ebola virus or infected material, contacting with bats or monkeys.

Epidemiological surveillance

Ebola haemorrhagic fever is included into the International Health Regulations (2005) and the Moldovan Health Protection Regulations (2008), which regulate the epidemiological surveillance and the measures of control. The action plan aims at reducing the import of hemorrhagic fevers and avoiding the epidemic spread within the country and includes organizational measures, staff training, diagnosis, treatment, and eradication mesures in epidemic hemorrhagic fevers. According to WHO, all countries are oblidged to report immediately the cases (eruptions) of serious illnesses, acute hemorrhagic fever syndrome, especially if they are observed in persons coming from endemic territories, taking into account the incubation period.

Control measures

Preventive measures are related to individual security compliance while taking care of the patient, performing the parenteral maneuvers, management of the medical waste, clinical laboratory investigations, as well as the research on primates. The measures undertaken wihin medical institutions are particularly important in prevention of haemorrhagic infections as, following the rules of aseptic and antiseptic techniques, using protective equipment, good personal hygiene, information of the population about the risk factors for infections and protective measures. Specific prophylaxis vaccine is still under development.

Anti-epidemic measures

During the epidemic period within the endemic territories, early detection and isolation (hospitalization) of patients are important. The measures imply the active detection of diseased persons at border crossing points by identifying persons, suspected of hemorrhagic fever and their subsequent isolation. After the patient is diagnosed, the doctor is called for.

The patient is mandatorily consulted by a specialist immediately after the detection or hospitalization. The collection of pathological material for laboratory investigations is carried out by a health worker, trained in biological safety and under the epidemiologist guidance. If suspected cases of contagious haemorrhagic fever appear, blood is collected from the vein (10 ml in two 5 ml tubes), mucus - from the pharynx by a sterile swab, sputum and 100 ml of urine via a sterile catheter in a sterile vial, pathological samples of the skin by a sterile scarifier. The collected material is immediately analyzed in specialized laboratory or stored

usually in a sealed termocontainer according to the security requirements until the arrival of the specialist. The autopsy and collection of material for the laboratory investigation is not carried out in case of death of the patient, with Ebola hemorrhagic fever because of an increased risk of contracting the infection. The epidemiological data of anamnesis are taken into account: the arrival of the patient from countries with a high risk of contagious haemorrhagic infections (with the maximum incubation period of 21 days); the patient's possible contacts during the journey, at place of residence or at work; the transport means, used by the patient to arrive from the endemic territories (countries); finding residence in endemic areas, visiting natural foci or contracting wild animals (especially monkeys) in natural outbreaks or in laboratory conditions.

The cases are reported to both the Ministry of Health and Public Health Surveillance Service, within 2 hours from the moment the patient has been diagnosed.

The patient who is diagnosed or suspected of contagious haemorrhagic fever is temporary isolated and evacuated to the inpatient infectious disease department in strict accordance with the rules of working with particularly dangerous infections. Patients or persons suspected of Ebola hemorrhagic fever are isolated in an isolation ward with low-pressure air and internal ventilation, otherwise the ventilation is disconnected. The discharge is performed no sooner than 21 days after the onset of the disease and after 3 negative virological investigations.

It is recommended to apply 3% chloramine B - 120 min. exposition, 1.5% calcium hypochlorite - 120 min., 3% hydrogen peroxide - 180 min., or 6% - 60 min.; 8% Lysol - 90 min for current and terminal disinfection of the contagious hemorrhagic infections.

People who had contact with patients or are suspected of Ebola hemorrhagic fever, including the medical personnel, are isolated and supervised for 21 days. Laboratory investigations of close contacts are performed to detect non-symptomatic forms of the disease.

The medical personnel who care for patients with contagious haemorrhagic fever must wear protective equipment (anti-plague type 1).

In case of contacting an infected person, the emergency prophylaxis, includes processing of mucous membranes of the mouth and nose with 0.05% potassium permanganate solution, eye washing with 1% boric acid solution. The mouth and throat are to be further processed with 70% ethyl

alcohol or 0.05% potassium permanganate solution,and 1% boric acid solution.

In case of suspected presence of Ebola haemorrhagic fever, it is recommended to administer antiviral preparation virazole (ribamidine) 0.2 g - 4 times a day for 10 days.

1.3. Sapronosis

1.3.1. Tetanus

The disease has been known since antiquity. Its occurrence is associated with trauma or injuries. The first description was made by Hippocrates.

In 1854, Simpson estabilished the infectious nature of the disease. In 1884 Carle and Raccoons, succeeded in transmitting the disease to rabbits, by injecting a drop, taken from a tetanus wound into the sciatic nerve.

In 1884 The pathogen was described for the first time by Nicolaier (bacillus Nicolaier) and it was isolated in pure culture by Kitasato (1887). Later (1890) Kitasato got the tetanus toxin and antitoxin serum and,in collaboration with Behring, proposed to treat patients of tetanus. In 1923, Ramon got the tetanus toxoid,which was further used in specific prophylaxis.

Since 1954, anti-tetanus heterologous serum was replaced with human tetanus immunoglobulin without incidental risks to foreign protein.

The pathogen agent

Clostridium Tetani (the Clostridium genus, family Bacillaceae) is a gram-positive, spor-forming and anaerobic bacillus, which is 0.3-0.8 / 2.1 - 8.1 mm in size, and mobile due to peritrichi flagella.

Nontoxigenic strains, identical to those of toxins, were isolated and confirmed by comparative analysis of DNA. Clostridium tetani has a ubiquitous spread and it was isolated in 30-40% of the tested samples of soil, taken in Japan, Canada, Brazil (currently in Africa, Europe, Antarctica, Asia, Australia), and in human and animal feces, it makes 25-35% (intestinal carriage).

Clostridium tetani produces two biologically active toxins: neurotoxin, called tetanospasmin and oxygen-labile hemolysin, named tetanolysin.

Tetanospasmin is encoded by a gene, conveyed by a plasmid, in the absence of which the strain becomes nontoxic.

Therefore, the toxigenic testing of samples is mandatory.

The exact role of tetanus hemolysin in the pathogenesis is not well known.

The spores are extremely resistant to the external environment, maintaining their viability in the soil during many years. It is resistant to liquid media, tightly capped tubes, and cultures during 21 years. The culture can survive for 730 days in dry silk threads impregnated with exudates from a tetanus wound at room temperature. The spores resist to the most common antiseptics like alcohol. Boiling destroys the spores by autoclaving in 15-20 minutes, and by oxidizing disinfectants (hydrogen peroxide, potassium permanganate) in a few minutes.

The vegetative forms are very sensitive to the external environment.

The **pathogen reservoir** includes herbivorous animals, rodents, birds, and humans,where it normally lives in the intestine and is eliminated into the external environment by feces and soil and can survive for years, whereas under favorable conditions it multiplies and accumulates. The bacillus is very widespread in the external environment. It has been isolated from the soil, street dust, farms, manure, fish products (vacuum-preserved), catgut, commercial textiles, etc.

Mode, factors and transmission ways

The contracting of the infection typically occurs through wounds contaminated with spores of *Clostridium tetani.* It develops in deep wounds,particularly those of knives, tools, pins, wire, wood chips and animal bites. Women are at risk of contamination during the delivery of newborns or abortion, when the used tools are contaminated. The newborn can contract the infection through contaminated instruments during the removal of the umbilical cord, especially if the birth occurs outside the maternity department.

Tetanus-diseased humans are not contagious.

Receptivity. Pathogenesis. Immunity

Human receptivity to tetanus toxin is very high. The sporadic pathogen penetrates into the human body, predominantly through the damaged skin or mucous membranes to produce the disease. The spore multiplication at the wound level is followed by the release of exotoxin, which is favored by anaerobic conditions (lack of blood irrigation, necrosis, marked by a possible presence of calcium in the soil, etc.). The toxin diffuses into the muscle, whereby the blood circulation reaches the central nervous system, then it reaches the spinal cord and the nuclei of the cranial nerves through the neuron pathway.

There is practically no immunity, after a natural illness occurs, since the antitoxin levels are low (due to very low levels of toxin capable of

producing the disease) and unable to protect. Repeated cases of tetanus during the patient's life are known.

Manifestations of epidemic process

Tetanus spreads worldwide with an increased incidence in the past years. Only in 1973 there were about one million cases of death of tetanus worldwide, of which approximately 60% were due to neonatal tetanus. A higher incidence was recorded in South East Asia, Africa and the eastern Mediterranean.

Currently, the incidence of tetanus has a decreasing tendency as a result of vaccination coverage of the population that is growing each year.

The annual average of the incidence of tetanus disease in pre-vaccination period (in 1951-1960) constituted 5.26 cases per 100 thousand population in Moldova. The incidence of tetanus is continuously decreasing because of the implementation of planned vaccination of children in 1960, high vaccination coverage, and by providing an adequate caree during labor. In the last two decades, the incidence being of 0.01 - 0.02 cases per 100 000 population sporadic cases have been reported *(Figure 56).*

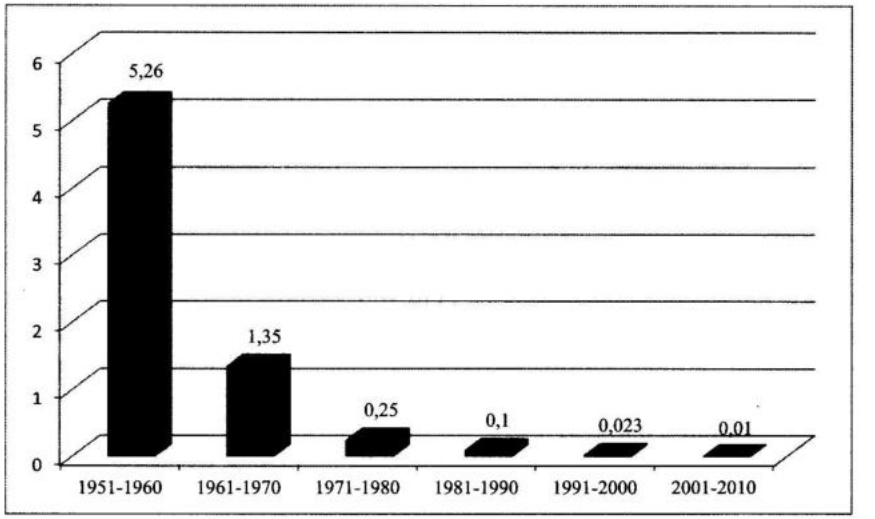

Figure 56. *The tetanus morbidity rate in the Republic of Moldova, in 1951-2010.*

Tetanus develops only by contaminating the wound with spores. It is not a contagious disease. The distribution of tetanus cases is universal, with a

greater frequency in warm and fertile climate of equatorial areas, which are rich in moisture and organic substances. In temperate areas, tetanus occurs mainly during hot and humid periods, when the pathogen multplies in the soil and people walk barefoot. According to the age, tetanus predominates among newborns, followed by children and young adults. The incidence is higher in men, due to a more frequent occupational exposure.

Farmers, gardeners, cattle caretakers, etc. are professionals that are at risk of contamination with spores. Some categories of patients who undergo surgical interventions, especially those with purulent sepsis or post-abortion are also at a great risk of contamination. Sometimes, even those who undergo invasive dental procedures with contaminated instruments are at risk of catching tetanus. Neonatal tetanus is one of the worst forms of tetanus (fatality is 50-90%).

Neonatal and obstetrical tetanus continues to be a problem in countries with an unsatisfactory level of vaccination and antiseptic regime at labor.

In recent years, tetanus is also associated with subcutaneous administration of injectable drugs.

Clinical manifestations

The period of incubation is about one week and varies from 3 days to 3 weeks. Cases with fulminant evolution have a short incubation period, being associated with the severity and the distance that the toxin travels from the wound to the central nervous system.

Localized tetanus is characterized by local painful spasms of the muscles near the tetanus wound, which last weeks or even months.

Generalized cases make about 80% of the reported tetanus cases, with trismus (lockjaw) and painful contraction of the facial muscles, which reveals clenched teeth similar to "Risus sardonicus". Hyperreflexia and hypertonicity occur at multiple muscular groups, causing a generalized tetanospasm, which determines the opistothonus. The spasm of the glottis causes asphyxia and immediate death. The reported lethal cases vary between 10 and 75%.

Cephalic tetanus occurs as a result of the injuries or lesions of the head and face with short incubation period of 1-2 days, characterized by the presence of trismus and cranial nerve palsies, involving particulary the III, IV, VII, IX, X and XII nerves. In newborns tetanus occurs after the infection of the umbilical cord in 4-14 days after birth and is manifested by trismus, difficulty of swallowing, opistotonus, continuous crying, refusal of breastfeeding. In most cases, the disease is fatal.

Laboratory diagnosis

The bacteriological diagnosis requires inactivation of the pathological product (30 minutes at 80°C) in order to destroy all vegetative forms associated with preservation of spores. Insemination is carried out in anaerobic broth and solid media. Pure culture injected intramuscularly to mice leads to a fatal outcome, with the phenomena of tetanus in 4 days.

Tetanus epidemiological surveillance includes the following main objectives:

- monitoring of vaccination coverage of the population;
- serological control of the tetanus immune status of the population;
- case-based surveillance and national reporting of each suspected case of tetanus and neonatal tetanus;
- providing medical assistance according to the national protocols in case of traumas, births outside the maternity department, and use of specific prevention measures;
- monitoring the tetanus vaccination status among fertile women.

Control measures

Preventive measures

The tetanus control strategy is based on providing a vaccine coverage of about 100% of population. In children and adults specific prophylaxis is carried out with tetanus toxoid vaccine in combination with diphtheria and cough vaccines (DTP, DT, Td). Recently, the tetanus vaccine has been included in the combined vaccine, which includes diphtheria, hepatitis B and Haemophilus influenzae type b vaccines. The pentavalent vaccine is given to children according to the vaccination schedule of newborns at 2, 4 and 6 months,with a subsequent revaccination at the age of 22-24 months – DTP revaccination, at the age of 6-7 years – DT, and later at the ages 15-16, 20, 25, 30, 35, 40, 50 and 60 years – Td revaccination.

Neonatal tetanus can be prevented by vaccinating fertile women with tetanus toxoid before the pregnancy, or pregnant women in the last trimester of pregnancy.

Tetanus prophylaxis also involves washing the wound, removal of foreign substances and dead tissues, drainage, irrigation and (depending on previous immunization) Td and anti-tetanus immunoglobulin administration. Emergency prophylaxis is performed in all types of injuries that affect the skin or mucous membranes as in second to fourth degree burns or frostbites, animal bites, abdominal perforation, abortions or labors

occurring outside the maternity department, gangrene or tissue necrosis, chronic abscesses or trophic ulcers.

It is essential to follow the aseptic rules during labor care, especially when the pregnant woman has not been vaccinated.

1.3.2. Legionelosis

Short history

The disease was first reported in 1976. The phenomenon is related to the American Legionary Congress held in Philadelphia in July 1976. There were 4,400 participants and 182 of them suffered from acute respiratory disease, followed by a severe pneumonia which resulted in deths of 29 persons. In January 1977, McDade et al. detected a gram-negative microorganism in a deceased legionnaire during the outbreak in Philadelphia, which proved to be the pathogen of this epidemic.

In 1978, at the International Symposium on Legionnaires' Disease, the newly discovered bacterium was named Legionella pneumophila, and the disease was called

"Legionnaires' disease" or "legionellosis".

The pathogen agent

Legionellae are Gram-negative bacteria, belonging to the *Legionella* family of *Legionella pneumophila*, which currently comprises 50 species and 70 serogroups. Not all species are pathogenic to humans. More than 90% of the clinical forms of legionellosis (Legionnaires' disease), including the nosocomial ones are caused by *Legionella pneumophila*, which includes 16 serogroups.

Legionellae are non-sporulating anaerobic small bacilli, with a size of 0.3-0.9 / 2.0 μm. They are mobile due to polar, subterminal or lateral flagella and have no capsule or a cell wall. They are cultivated only in the media with L-cysteine and iron salts. They are catalase-positive and oxidase-negative. They do not reduce nitrates.

Legionella populations are heterogeneous, with different virulence, depending on the environment they come from. The protozoa are more virulent, causing severe pneumonia; those from water are less aggressive, being involved in mild pseudo flu forms.

Legionella is resistant to the external environment. It survives up to one year in distilled water. At the same time, it dies within one minute under the action of 1% formalin solution, 70% ethanol and 0.002% phenol; and within 10 minutes under the action of 3% chloramine solution.

The reservoir and sources of pathogen

Legionella natural habitat is natural or artificial water reservoirs. Legionella are isolated from various reservoirs with huge spectrum of physical, chemical and biological features. Legionella multiplication is enhanced in the warm waters, but can be isolated in cold water as well.

High adaptive capacities allow their colonization in artificial pools, ventilation systems, shower facilities, jacuzzi, respiratory therapy equipment, etc. The survival conditions are more favorable in artificial sources than in natural ones, which leads to their accumulation in high concentrations. Legionella produces large amounts of glycocallyx, which favors their persistence and accumulation in rubber and silicon tubes and pipes, whereas iron, zinc and aluminum enhance their growth, forming the so-called "biofilms" on the surfaces of medical facilities and water pipes, where Legionella become more resistant to the action of disinfectants. The intensity depends on the temperature (25° -42°C) of water stagnation, formation of sludge, scale, biofilm and the presence of amoebas. The places where legionella can multiply are the water-cooling towers, evaporators, condensers, etc.

Way and factors of transmission

The mode of transmission is respiratory, transmission pathway - airborne, transmission factors are liquid aerosols. Almost all major epidemic outbreaks of legionellosis, as well as many sporadic cases, were caused by the spread of liquid aerosols containing legionella, generated by air conditioning systems. Another way of transmission is by drinking contaminated water (bathrooms, showers, taps, contamination of medical devices used to assist in breathing, etc). Sporadic cases of Legionella infection can occur where portable humidifiers and aerosols are used.

Pontiac fever (Acute Respiratory Disease of Legionellosis nature) was associated with the same sources and mechanisms. The risk of contamination is high in the hotels, but it is even higher in hospitals (baths, showers, hydrotherapy devices, sprays, air conditioning systems, etc.).

The high concentration of the pathogen in aerosols enables the direct entering of legionella into the lower respiratory tract, where the contact with alveolar macrophages occurs and virulent strains actively multiply.

Manifestations of epidemic process

The disease is prevalent everywhere. A larger number of illnesses have been recorded in the US and Europe. Legionellosis cases (Legionnaires'

disease, legionellosis pneumonia) were recorded in almost all EU countries *(Figure 57)*.

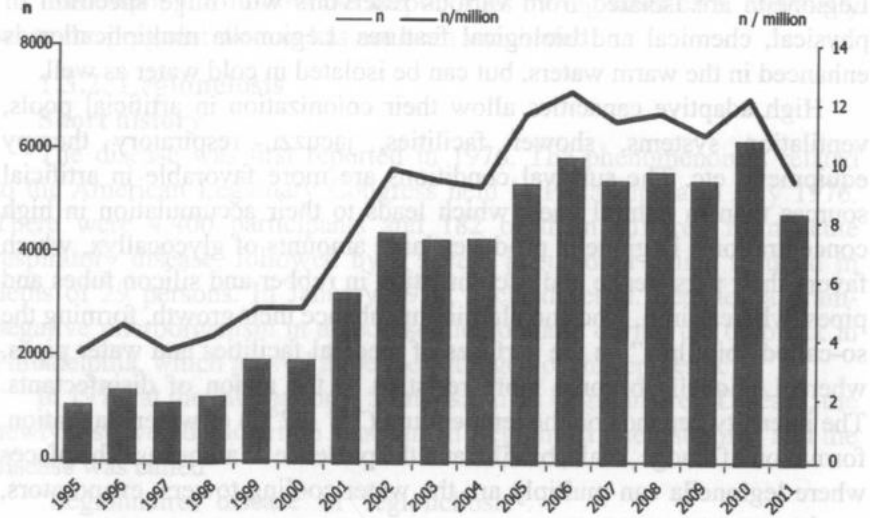

Figure 57. *The legionelosis morbidity rate in UE countries per 1 mln population (ECDC, 2011), in 1995-2011.*

The epidemiological survey of legionella outbreaks found that pneumonia occurred in 5-10% of people, who were in the areas with aerosols contaminated with Legionella, and Pontiac fever – in 80-100% of people. The risk for pneumonia is increased in the elderly *(Figure 58)*, smokers with concomitant pathologies and a higher degree of immunodeficiency, or receiving immunodepressant therapy.

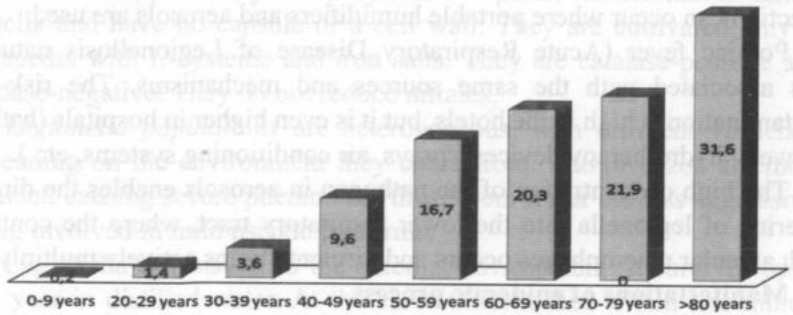

Figure 58. *The legionelosis morbidity rate per 1 mln population (ECDC, 2011), in UE countries, according to the age in 2011.*

Legionella causes 2-6% of total pneumonia and 10-15% of atypical pneumonia cases (pneumonia caused by mycoplasma, chlamydia, legionella and coxiella). Legionellosis is rarely diagnosed in children, usually it is based on associated pathologies.

Legionellosis can occur throughout the year, however, the incidence rate is higher in summer *(Figure 59)*.

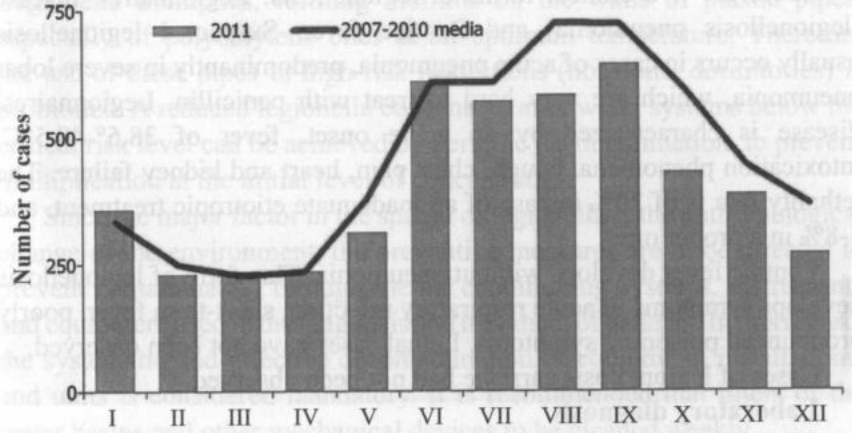

Figure 59. *Seasonal distribution of Legionelosis in UE countries in 2011 (N=4891) (ECDC, 2011).*

Both epidemic outbreaks and sporadic cases of legionellosis are predominantly recorded among hotel clients and staff, patients and hospital staff, institutional and industrial employees.

Recently, a particular attention has been paid to legionellosis associated with traveling (travel-associated legionellosa); which occurs during tourist or business trips and is usually diagnosed on returing home. Multiple and often fatal cases of legionellosis associated with hotels, have served as a basis for creating a unique international system for surveillance and control of travel-related legionellosis.

The risk of nosocomial legionellosis is caused not only by the possibilities of legionella contamination via water supply systems, air conditioning systems, medical equipment, but also by the presence of the immunodeficient individuals.

In the oncological or transplantation departments the legionellosis incidence rate can reach 15-20% in nosocomial pneumonia, due to

contamination of the water systems,, whereas the lethality rate is of 30-40% (Tartakovskii I.S., 2013).

The manifestations of infectious process

In Legionnaires' disease the incubation period is 2-10 days, in case of immunosuppression it may last up to 3 weeks; in Pontiac fever 4-60 hours (an avarage 36-48 hours).

There are two clinical forms of legionellosis: Legionnaires' disease (legionellosis pneumonia) and Pontiac fever. Suspected legionellosis usually occurs in cases of acute pneumonia, predominantly in severe lobar pneumonia, which are very hard to treat with penicillin. Legionnaires' disease is characterized by an acute onset, fever of 38.5°-40.5°C, intoxication phenomena, cough, chest pain, heart and kidney failure. The lethality rate is of 20% in case of an inadequate etiotropic treatment, and 5-8% in a proper one.

Pontiac fever develops without pneumonia. This form of legionellosis develops symptoms of acute respiratory infection, short-term fever, poorly pronounced poisoning symptoms. Lethal cases have not been observed.

Cases of legionellosis carriage had not been observed.

Laboratory diagnosis

The bacteriological method consists of isolation of *L. pneumophila* culture from the material obtained from biopsy, authopsy, bronchoscopy, pleural exudates, sputum or blood is the most accurate confirmation of legionellosis infection. Isolation and identification of culture from clinical samples takes 5-7 days.

The serological method is based on the indirect immunofluorescence reaction in serum, which results in a 4-fold increase of titer. Since the antibody titer increases no sooner than 2-3 weeks after the onset, the method is more retrospective in character. When investigating a single sample of serum, the presumptive diagnosis of legionellosis can be assumed if the antibody titer is> 1: 128.

In the acute period of the disease, the direct immunofluorescence method (bronchoscopic material, pleural effusion, sputum) and the Enzyme Immuno Assay method for antigen detection in urine (applied only for *L. pneumophila* serogroup 1) may be used.

Legionellosis epidemiological surveillance is based on investigations carried out to identify legionella in water and air conditioning systems in hospitals, hotels and other insitutions.

Preventive measures

Important measures are periodic mechanical cleaning of water systems presenting an epidemiogenic risk; water heating in devices used at temperatures above 60°C; minimizing the temperature or pressure variations in water systems; water decontamination.

It is reasonable that such constructions should be made of copper. Legionella multiplies, forming biofilms on the walls of plastic pipes, especially of polyethylene ones at an optimum temperature. Therefore the use of these pipes in high-risk institutions (hospitals, dormitories) is prohibited. A reduced legionella concentration in water systems below the critical risk level can be achieved by periodic decontamination, to prevent multiplication at the initial level of concentration.

Since the major factor in the spread of legionella is the anthropological change of the environment, the preventive measures are to be directed to prevent contamination through the air conditioning systems, instruments and equipment used in the diagnosis and treatment of patients. In this regard, the systematic and effective decontamination of equipment, installations, and units is considered mandatory. It is recommended that filters of the water basins and other mechanical devices to be cleaned weekly.

Anti-epidemic measures

Information about Legionnaires' disease or suspected persons is transmitted to the Center for Public Health.

Epidemiological investigation of the outbreak is aimed to detect objects that served as sources of pathogen, as well as persons who were at risk of contamination under the same conditions. The taken data should include the following: the presence of contact with conditioning, cooling or compressed air systems, wet aerosol, travelling, hotel stay (not earlier than 2 weeks before the onset); balneological procedures; smoking, presence of associated pathologies, treatment with immunosuppressants etc.

The bacteriological investigations of water samples and lavages of items that serve for the multiplication of legionella are done in order to determine which objects were the source and factor for pathogen transmission.

Hospitalization is performed in accordance with clinical indications.

Measures to clean and decontaminate air conditioning and water supply systems are undertaken. The exploitation of these objects is suspended until they are bacteriologically confirmed to be germ-free.

The isolation of contacts, emergency prevention and disinfection in the outbreak are not performed.

2. Epidemiology of invasive diseases

2.1. Protozoosis

2.1.1. Malaria

Short history

The disease has been known since ancient times. It is mentioned in the writings of Hippocrates and Celsius, being considered a miasmatic disease, caused by fumes emitted from the swamps, hence the name „malaria" (male illness, air = air). The French also call it „paludism" from the Latin word *palus that means pond.*

The English physician Thomas Sydenham (1624-1689) described malaria attacks in details, and their severity in particular. In 1880, the French physician Alphonse Laveran, discovered the pathogen of malaria in Algeria. The parasite was isolated from the blood during the examination of a malaria patient. Studying the morphology of these inclusions more thoroughly, he noted their animal nature. He named them *Oscillaria malarriae,* and later they were found to be *P. falciparum* (the Nobel Prize, in 1907). In 1886, the Italian physician Camillo Golgi described for the first time two forms of malaria – T*ertian and Quarta* (the Nobel Prize in 1906). Later, Giovanni Grassi and Raimondo Filetti described two parasitic species in humans (in 1890): *Plasmodium vivax* – the pathogen of malaria with a fever lasting 3 days and *Plasmodium malariae* – the pathogen of fever that lasts 4 days. In 1897, the American scientist William H. Walic identified the third species of Plasmodium – *Plasmodium falciparum*, and in 1922 I. Stephens discovered the fourth malarial parasite - *Plasmodium ovalae*. In 1897, in India the English physician Ronald Ross discovered (Calcutta) the vector of malaria transmission in humans – *the Anopheles mosquito* (the Nobel Prize, in 1902), which was confirmed by D. Grassi in 1898.

The pathogen agent

The pathogen belongs to the *Sporozoa Class*, *Plasmodidae family*, *genus Plasmodium*, and has 4 pathogenic species in humans: *P. falciparum, P. vivax, P. malariae and P. ovalae.*

P. vivax is the most common species in both tropical and temperate areas. P. *falciparum* is widespread in tropical and subtropical areas, causing severe forms of malaria.

Plasmodium life cycle may develop in two different hosts: a human is the intermediate host and the female Anopheles mosquito – the final host of plasmodia.

The asexual schizogony cycle takes place in the human body and develops in two stages: a) *the exo-erythrocytic tissue stage* develops in the liver; b) the *erythrocytic stage.*

A sexual cycle develops in mosquito's body (*sporogony*).

Exo-erythrocytic schizogony stage starts with the introduction of *Sporozoites* into the human body via infected mosquitoes. In a short time (30-60 minutes after the invasion) it attaches to the liver parenchymal cells. At this level, it continues to develop and multiply in hepatocytes, forming plasmoid multinuclei masses „primary tissue schizonts", of 40 μm in diameter, which are divided into 10 000-40 000 merozoites. Entering into the blood stream, they attach to the red blood cells. The red cells initiate the exo-erythrocytic cycle. The time is variable, depending on the parasite species: P. falciparum – 6 days, P. vivax – 8 days, P. ovale – 9 days, P. malariae – 15 days, according to the incubation period of the disease. This period is clinically latent. The parasites cannot be detected in the peripheral blood. The person is not infectious for mosquitoes during this period.

The tertiary and *ovalae* types of malaria occur in combination of two types of sporozoites. Some of them (*tachysporozoites*) start tissue schizogony immediately after their penetration into hepatocytes and after leaving the liver. The other spororozoites (*bradysporozoites*) remain inactive in the liver („latent") within 8-9 months or even 2 years. The latent period ends up with the secondary exoerythrocytic schizogony, malarial parasite exit out of the blood stream and relapses of the disease. P. malariae can persist in the blood in low doses for over 30 years, producing the carriage status without relapses.

Erythrocytic schizogony

The tissue merozoites penetrate the red blood cells and there they are transformed into the trophozoites (young forms) and then into mature schizonts with a potential multiplication. These red blood cells lyse, releasing a new generation of merozoites that invade new erythrocytes. The duration of erythrocytic cycle varies by species: 48 hours in *P. falciparum*, *P. vivax, P. ovalae* and 72 hours in *P. malariae*, hence the rhythm of fever attacks: the *tertian* fever for *P. falciparum, P. vivax and P. ovalae* and

quarta fever of 4 days for *P. malariae*. Some *merozoites* embeded into the red blood cells give rise to sexual forms of parasites – haematocytes males and females.

Sexual cycle (sporogony)

Sexual cycle (sporogonic)

The female Anopheles mosquito infects itself by aspirating human blood, infected with plasmodium gametocytes. The further development of the parasite occurs in the stomach of mosquito, where gametocytes turns into the male and female gametes, which after the fertilization turn into diploid zygotes, later on turning into ookinetes (egg cell). The ookinetes are implanted into the stomach wall, becoming oocysts. In the oocyst, sporozoites, which migrate into the salivary glands and are the infectious elements, are formed by meiotic reductive division. The average sporogon cycle lasts 15 days, varying from 10 to 40 days, depending on temperature, humidity and species of plasmodium.

Reservoir and the source of pathogen are the patients with acute or chronic form of malaria and gametocytes carriers.

Since natural and laboratory infections with simian malaria agents (P. knowlesi, P. cynomolgi, P. brasiliensis) have been identified, it is assumed that monkeys may be a reservoir of malaria parasites to humans, thus making it difficult to eradicate malaria in tropical areas.

The female Anopheles mosquito is considered to be a reservoir of malaria. It requires a „feeding" of blood for reproduction and a body, where the malaric Plasmodium will mandatorily pass all stages of metamorphosis. About 100 species of *Anopheles* are identified. The main mosquito vector is considered *Anopheles maculipenis.* The Anopheles lays its eggs on the surface of the water,thus aquatic larvae are also essential to the implementation of preventive measures. The resistance to Anopheles is directly related to variations in temperature, altitude, etc., which explains their different distribution across the globe.

Ways and means of transmission

Malaria is transmitted usually through mosquito bites parenterally, injected with saliva. The sporozoytes invade the human body through the bloodstream during the „feeding".

Malaria can also be transmitted during blood transfusion from donors with latent malaria, (post-transfusional malaria), by contaminated needles and syringes, or other medical devices contaminated with blood containing

merozoites. Congenital transmission is possible, but only through the placenta with lesions, the normal placenta constituting a barrier to the passage of the malaria parasite.

Manifestations of epidemic process

Malaria continues to be one of the most widespread infectious diseases. Fighting is considered a problem because of the enormous health and socio-economic impact.

According to the WHO data, around 350-500 million people worldwide catch malaria each year, of which 1.3-3 mln. die from this disease, most of which are children under the age of 5. About 2.5 billion people, or 40% of the world population, live in areas with increased risk of contracting malaria. About 90% of global malaria morbidity is recorded in the African population. Endemic malaria are found in India, Brazil, Sri Lanka, Vietnam, Colombia.

Currently, the risk of infection with malaria has increased due to the migration of European population to endemic areas. Over 30-35 mln. tourists visit these countries annually. EU countries record 10-12 thousand cases of imported malaria annually.

The most common is P. vivax. This species is widespread in countries with tropical, subtropical and temperate climates. Currently, local transmission of the malaria mosquito of this type occurs in more than 100 countries, especially in Central and South American countries, North and Equatorial Africa, the Middle East, Transcaucasia, Central and South Asia.

Tropical malaria pathogen (*P. falciparum*) is found mostly in Equatorial Africa, Latin America and Southeast Asia.

P. ovale covers the least area of spreading: Equatorial African countries, Guinea, Vietnam, Cambodia and Laos.

The areas of P. malariae spread have not been studied so far, the number of cases caused by quarta malaria being insignificant.

Outside the endemic areas, cases of malaria can occur due to the import of infection via a diseased person or contaminated mosquitoes from endemic areas, or by means of transport.

Malaria was one of the most widespread infections in Moldova. According to the statistics, there were 89 450 cases of malaria in Bessarabia in 1910, and in 1938 -96,000 cases. The malaria situation was further aggravated during the Second World War (1941-1945) and the early postwar period. In 1945, there were 126,733 cases of malaria, in

1947 – 130,886, and in 1948 – 155,929 cases. The incidence of malaria has decreased significantly after implementing a comprehensive plan for prevention and monitoring of malaria disease, which included: 1) active detection of patients and carriers of malaria parasites; 2) treatment of all detected patients and carriers of parasites; 3) individual chemoprophylaxis; 4) providing measures against vectors (indoor mosquitoes and destruction of mosquito larvae in pools of water, draining mosquito breeding sites for the Anopheles genus by hydraulic works, etc.), opening of antimalarial stations within the country. In 1950, the number of patients was reduced to 31 271 cases, in 1953 - up to 890 cases, and the last case of indigenous malaria was recorded in 1960, considered as the year of malaria eradication in the territory.

However, in the coming years the phenomenon of malaria import in the republic intensifies due to intense migration of the population, as well as to the endemic territories. Over the last 30 years, 12-57 cases of imported malaria have been recorded annualy, most of which were diagnosed in native population arrived from endemic countries: Sudan (90%), Cameroon, Kongo, Angola, Nigeria, Equatorial Guinea, Kenya, Iraq, Afghanistan, Russian Federation.

Most cases are with P. falciparum – 93.9%, whereas infestations with P. ovale and P. vivax constitute 4.0% – 3.2%.

Most cases of imported malaria are in males (95%) aged 21-50: professionals and workers temporarily employed in endemic countries – 84%, air or navy personnel – 10%, military staff – 5%, students – 1%.

The seasonality is determined by climatic conditions and biological features of both vector and parasite. The period of infectiousness of mosquito coincides with the period of the year, during which the climatic conditions, suitable for the development of malaria plasmodium in the mosquito body up to the sporozoite stage, are maintained. This period starts when the average temperature reaches 16°C for P.vivax and 17°-18°C for P. falciparum and P. malariae. Contagiousness of mosquitoes persist until the weather conditions ensure the possibility of a sporogony cycle development. This period ceases, when the average air temperature is lower 16°C.

Nowdays, the seasonality of malaria in non-endemic territories does not correspond to that of endemic territories. In Moldova most cases of malaria illness are currently recorded in the cold season. More than 60%

of annual morbidity are recorded from November to March, which is determined by people returning from endemic countries.

In Moldova local outbreaks of malaria can occur due to favorable climatic and natural conditions for intensive reproduction of Anopheles mosquitoes, systematic import and lack of immunity against malaria in the human population.

Clinical manifestations

Tropical malaria caused by P. falciparum is characterized by polymorphism of clinical simptoms. Following the incubation period of 6-16 days, the prodromal stage is manifested by depressed mood, headache, muscle aches, back pain, and persistent fever. The disease is manifested by irregular fever with long paroxysms (up to 24-36 hours and more), vomiting, sometimes diarrhea, hypotension, bradycardia, oliguria, albuminuria, uremia. Seizures are accompanied by strong headaches and back pain. Often there is pain in the abdomen, splenohepatomegaly (whereas the spleen is not enlarged) accompanied by jaundice and increased transaminases level. Tropical malaria evolution lasts about a year, sometimes more. When treated properly, it has a positive development and improves within a few days. Tropical malaria may result in serious fatal outcomes without a proper treatment.

Malaria caused by *P. vivax* evolves with two types of incubation period: short incubation – 8-21 days and prolonged incubation – 6-8 months, sometimes up to 1 year. The prodromal period is manifested by fever 38°-39°C, anorexia, nausea, vomiting, sometimes diarrhea, muscle aches and intense headache. Typical fever attack occurs during this period. Seizures usually appear in the first half of the day at the same hour, preceded by prodromal signs and are characterized by the triad as: chills, fever, sweating. The onset is acute, violent, lasting about an hour; fever lasts 3-4 hours, reaching 40°-41°C with dry skin; profuse sweating, which lasts 3-4 hours, then the temperature drops abruptly to the normal body temperature. After a fever attack, the patient feels relieved until the next access, which occurs after 40 hours. The duration of untreated malaria can last 2-2.5 years.

Malaria caused by *P. ovalae* has the incubation period of 10-16 days. The fever attack have an intermittent character. One of the features of this form is that fever attack occur in the evening or at night. Malaria ovalae is characterized by a milder development, few paroxysms, mild fever without

manifested chills. The duration of clinical evolution is approximately 2 years.

Quarta malaria caused by *P. malariae* is characterized by a long-term clinical evolution. The prodromal period is usually asymptomatic. The disease begins with intermittent fever with afebrile intervals lasting 2 days. Sometimes double triggers occur – two days consecutively, after a day interval (double quartan). Sometimes, the character of the temperature curve can change, which makes it difficult to make the diagnosis. Splenomegaly develops slowly, but it may become pronounced. Parasitemia gradually increases without reaching a high level. *Malaria quarta* lasts 4-5 years in most cases, but the development can last longer (10-45 years) – that is characteristic of carriage state.

Laboratory diagnosis

Parasitological diagnosis is make by the detection of the parasite in the capillary blood taken from the finger. Since the development of P. vivax, P. ovalae, and P. malariae occurs in the blood stream the parasites can be detected at all stages of the development both in smear and thick drop. In tropical malaria, only young trophozoite forms or mature gametocytes of P. Falciparum can be found in the peripheral blood, as the development of schizons occurs in the capillary masses of internal organs. P. falciparum is detected in severe forms, in the peripheral blood (malignant), as a result of microcirculation disorder.

In case of pathogen detection, not only the species but also the number of parasites are to be recorded, since the severity of the disease is directly proportional to the level of parasitemia. The WHO recommends the approximate assessment of the droplet parasitemia level. The intensity of parasitemia is indicated by pluses ranging 1-4.

Serological diagnosis

Serological methods are used in malaria, especially in epidemiological investigations, retrospective diagnosis, examination of donors for the prophylaxis of post-trausfusion malaria and as an auxiliary method used for diagnostic purposes. The tests used are: indirect hemagglutination, indirect immunofluorescence, ELISA and PCR (determine the species of parasite and mixed infections). In 1992, the WHO adopted to implement two new tests to diagnose malaria: Para SightF (Becton Dickson, USA) and ICT (ICT Diagnostics, Australia). These tests are used in quality diagnosis of malaria caused by P. falciparum, based on the immunochromatographic reaction of HRP-2 antigen plasmodia.

In malaria **epidemiological surveillance** provides investigation of the people arriving from endemic countries, collecting and analyzing data on imported cases or local entomological surveillance of the situation, predicting the epidemic situation, and assessing the measures used in the focus.

Control measures

Preventive measures

It is important to prevent re-emerging of the disease and possible conditions of its imports, prevention of the spread of the Anopheles mosquito in the countries, where malaria has been eradicated.

In the early detection of malaria cases the important measures are: the haematological examination of the persons with clinical suspicion of malaria, cases of fever of unclear etiology in the first 2-3 days after arrival, prolonged fever, people who returned from endemic areas and present fever or low-grade fever for 6 months on arrival, persons with febrile injuries within 3 months after a blood transfusion, and donors implicated in the case of malaria transfusion, students arriving from endemic countries. The World Health Organization regularly provides data on risk countries for malaria.

Travelers, seasonal workers, military persons travelling to malaria endemic countries are recommended to take chemoprophylaxis with antimalarials. The dosage differs depending on the itinerary, drug tolerance, physiological status (age, pregnancy), design and medicines available in different countries. Chemoprophylaxis starts 1-2 weeks before entering the endemic area and continues for 4 weeks after the leaving, in order to prevent possible and acquired infection on or before departure.

A continuous updating data on the resistance to chemoprophylaxis is required for an effective treatment. The bulletins issued by WHO on the species of P. falciparum resistance to various chemotherapeutic remedies and appropriate chemoprophylaxis schemes can serve as an annual guidance. In areas,where resistance to antimalarials has not been established, the prophylaxis of choice is made with chlorine (delagil) as it is effective against all 4 Plasmodium species, and it can be given to both pregnant women and young children. The occurrence of side effects to antimalarials (nausea, vomiting, accelerated transit) are not the reasons for discontinuation of prophylaxis.

An effective method of prevention is the control of malariogenic

mosquitoes, which is carried out by drainage works, irrigation, spraying with insecticide (K-Othrine, Solfac, Cislin etc.), insecticides, larvicides (Baycidal) or biological methods (fish larva-phage).

An effective and low-cost method of prevention is to avoid mosquito bites by application of repellents to the skin (diethyl methylbenzamide, ethylhexanediol, permethrin, dimethylphthalate), wearing long-sleeved clothing and long trousers, the use of anti-mosquito nets, application of an insecticide (permethrin) or repellents on clothing, pyrethrin spray or electric insecticide diffusers for the night.

It is also important to raise awareness amongst the population about the risks and preventive measures of malaria, especially among people travelling to endemic areas.

The WHO recommends that every person travelling to the Malaria regions should follow the principles of ABCD:

A. Being aware of the risk, incubation period and main symptoms;

B. Avoid mosquito bites especially between dusk and dawn;

C. Take medications against malaria (chemoprophylaxis);

D. Immediately request diagnosis and treatment for a fever which lasts more than a week after entering a malaria risk area and up to 3 months after the departure.

There is no effective vaccine. However, efforts to find an effective vaccine are encouraged by the possibiity to cultivate the parasite in vitro and obtaining specific pure antigens.

Perspectives on the control or eradication of malaria are reduced due to the following factors: malar resistance, which is increasing for P. falciparum and appearing resistance to P. Vivax; the mechanism that provides protection against infection is still unknown; an increasing insecticidal resistance of anopheles mosquito populations.

Anti-epidemic measures

Malaria is one of the diseases that is reported to Center for Public Health by telephone or electronic system within 24 hours. In the medical record (form Nr. 025/e) about the patient who was detected malaria and came from endemic country the inscription „He/She was in the tropics" is required.

Hospitalization is mandatory. It is necessary to avoid the contact of the sick with mosquitoes. The therapy is administrated according to the

sensitivity of patient to the treatment. The discharge is done only after the disappearance of parasitemia proven by two successive negative hematological examinations with the intervals of 48 hours.

The follow up of the patient lasts: 2 years in case of P. falciparum and 3 years in case of P. vivax and P.ovalae, in case of P. malariae – 5 years. During this period the carrier is periodically examined clinically and hematologically.

Each confirmed case requires epidemiological investigation within 24 hours and taking effective anti-epidemic measures. The origin of the case and the factors that led to the spread are to be determined during the epidemiological investigation. Malaria suspects are subjected to blood testing. The measures against vectors include spraying insecticides in the epidemic territory and other 5-7 neighboring households. Within a 3-5 km area, water basins and marshes are examined and a decision regarding the need for anopheloid surfaces to be processed is taken. The surveillance of the outbreak is complex.

Following a post-transfusion case, all donors are to be serologically investigated. Patients with chills or fever lasting 3 months after the transfusion are to be subjected to haematological control for malaria. People who have contracted malaria or whose clinical condition or laboratory examinations cause suspicion are excluded from blood donation programs.

2.1.2. Leishmaniasis

Short history

The disease has been known since ancient times. However, the first descriptions of cutaneous leishmaniasis pathogen were presented by L. Cunningham (1884) and Firth (1891). In 1898, P. Borovsky detected the causative agent in cutaneous ulcer of small grains and related it to the protozoa. Later W. Leishman and S. Donovan (1900-1903) the agent of visceral leishmaniasis – protozoa detected in the spleen of kala-azar patients. It was morphologically identical to the agent detected by Borovsky.

In 1903, R. Ross assigned the described microorganisms to a new genus Leishmania. Serjana E., A. Donation and Parro (1921) established the mode of transmission of the parasite - by phlebotomy.

The pathogen agent

Leishmaniosis is caused by protozoa belonging to the genus Leishmania, the family Tripanosomidae, class flagella. There are several

species that cause human disease: *L. tropica, L. major, L. mexicana* – the causative agents of cutaneous leishmaniasis; *L. donovani, L. infantum, L. Chagas* – the causative agents of visceral leishmaniasis; *L. braziliensis, L. panamensis* – the causative agents of leishmaniasis mucocutaneous (American).

The parasite is present in two forms: amastigotes and promastigotes. The first form is found in humans and the other in vertebrates. It is small (2-3 μm), round or oval in shape, intracellular, with localization in reticuloendothelial cells.

The second form, promastigote, is mobile, larger in size (15-25 μm / 1.5-3.5 μm), consists of the central nucleus, kinetoplast at the anterior pole and it is found in the digestive tract of sand flies.

Reservoir and sources of pathogen

Leishmania affects a great variety of mammals, including humans. Dogs, foxes and jackals may serve as natural reservoir of visceral leishmaniasis („kala-azar"). The human reservoir of visceral leishmaniasis is found in India and Sudan. The parasite is transmitted from human to human (visceral leishmaniasis).

Natural reservoir of cutaneous leishmaniasis is found in different rodent species in desert habitat and rarely in humans or dogs.

Natural reservoir of mucocutaneous leishmaniasis is represented by mammals of the tropical forest.

Factors and transmission routes

Parenteral transmission is realized by female sand flies bite, infested with promastigoti. Sand flies are small insects, measuring 3 mm in size. Only the female is haematophagous.

Leishmaniae multiply in the gut of sand flies as flagella (promastigot) and get into the pharynx, proboscis, which blocks them. Before the bite the female evacuates the content of the digestive tract along with the parasites by vomiting or feaces.

Human-to-human transmission is provided by sand flies anthropophilic species. In some parts of the world leishmaniasis is a zooanthroponosis. Animal-to-human transmission occurs through a bite of a zoophilus sand flies.

Manifestations of epidemic process

The geographical distribution of leishmanisis is limited to tropical and subtropical regions associated with the sandfly habitat. The disease

is endemic in 88 countries. According to some assessments, the number of patients with leishmaniasis exceeds 12 million worldwide. Annually around 2 million of new cases are recorded. 90% of the total cases of visceral leishmaniasis occur in Bangladesh, India, Brazil and Sudan; 90% of cases of mucocutaneous leishmaniasis are found in Bolivia, Brazil, Peru, and 90% of all cutaneous leishmaniasis infections are recorded in Afghanistan, Brazil, Iran, Saudi Arabia and Syria.

The number of illnesses by leishmaniasis, within the endemic areas, is constantly increasing due to human activities and climatic changes (intense deforestation, construction of dams, irrigation systems, increased migration of population).

Periodically, epidemics with high lethality rate occur especially in non-immune population of endemic areas,. In 1990, for example, in Sudan, there were 100,000 of deaths, as a result of the outbreak of visceral leishmaniasis and, in 1999, the epidemic of over 200,000 cases were recorded in Afghanistan. Children were main affected especially in endemic areas, since adults have a natural immunity. The outbreaks of leishmaniasis are missing in European countries, only imported cases were registered.

The transmission of the parasite through blood transfusions, injections with promastigoti culture during laboratory investigations, both congenitally and sexually is possible.

Clinical manifestations

After a long incubation period (months or years) visceral leishmaniasis is manifested by irregular fever, adeno-hepato-splenomegaly and pancytopenia, being the most severe form of leishmaniasis, which, if untreated may lead to death. Chronic skin lesions, which require long-term and costly treatment, may occur.

Visceral leishmaniasis has a fulminant evolution in people with HIV. Parasitosis instigates the onset of AIDS by double action: immunosuppression and stimulating the virus replication.

In cutaneous leishmaniasis the diagnosis is made based on epidemiological history, ulcerous skin diseases and laboratory investigations. It has a benign evolution.

Laboratory diagnosis of cutaneous and visceral forms is based on revealing the parasite: 1) direct examination by taking puncture of the skin or bone marrow (sternal in adults and tibial in children); 2) promastigote forms develops in a week, if it is grown on an agar medium with blood at

27°C; 3) inoculation of leishmania on hamsters, lead to deth in 3-6 months.

Preventive measures include avoiding sand flies bites in the endemic areas. Personal protection is ensured with nets impregnated with insecticide and repellents. The leishmaniasis morbidity was eliminated in some countries (China, Azerbaijan, Kazakhstan, Turkmenistan, Israel), in urban areas, by taking individual protection measures, applying insecticides, as well as monitoring and treatment of the diseased persons.

2.1.3. Amoebiasis

Short history

The pathogen was discovered by F. Lesh (1875) in Russia. In 1883 R. Koch isolated amoebae from the feces of the sick. It was described as an independent nosological form, called „amoeba dysentery", by William F. Counsilman and Leffler in 1891.

In 1903 F. Shaudinn described in detail the pathogen of *amoeba dysentery* and named it *Entamoeba histolytica*. In 1925 E. Brumpt assumed the existence of two species of Entamoeba: a pathogenic invasive *virulent species*, and the other non-pathogenic *non-virulent* one, which he called *Entamoeba dispar*. This idea was confirmed in 1993.

The pathogen agent

Amoebiasis is caused by Entamoeba histolytica, a protozoan belonging to the Entamoeba genus, the Sarcodina class. E. histolytica occurs in two forms: cystic and vegetative (trophozoites).

Trophozoites are irregular in shape, 8-30 μm in size and consis of the spherical core (3.5 μm), central kariozom and chromatin arranged at the periphery. The cyst of spherical shape represents the invasive stage. It is 10 -15 mm in size, with 4 mature nuclei, morphologically similar to trophozoites. It penetrates the bowel tissue thus causing the disease by forming amoebiene wounds, which can penetrate other organs through the blood stream, that can lead to the formation of amoebae abscesses.

The cysts are removed from the human body into the environment with feces. The pathogen enters into the body with water or food and reaches the stomach; the cyst gives birth to other 4 small amoebas, further dividing up and giving rise to new vegetative forms. This is the life cycle of the parasite.

Outside the body, the vegetative form of the pathogen dies very quickly, whereas its cystic forms are resistant to the external environment: they

persist up to 4 weeks in the feces, and 8 months in water. However, dry conditions destroy them.

The reservoir and source of the pathogen

Humans with acute or chronic forms of amoebiasis are the only source of parasite.

Mode and transmission routes

The mode of transmission is of the fecal-oral type, which is carried out predominantly via water or food infected with feces, or habitual – by contaminated hands. Houseflies and cockroaches can contribute to food contamination. The direct mode of transmission can be found in homosexuals.

Predisposing factors: the use of water for habitual purposes, including drinking, from open sources, as well as unprocessed food; summer season with plenty of herbs, vegetables and fruits; poor personal hygiene.

Manifestations of epidemic process

Amoebiasis is spread everywhere, but it manifests more intensely in endemic countries with warm, tropical and subtropical climates. Africa, Southeast Asia, Latin America are regions where amebiasis represents a serious public health problem. It is estimated that more than 10% of the world population is affected by this protozoa, which is responsible for about 100,000 deaths per year, being second after malaria.

Natural receptivity is high and general. In endemic countries, the risk is increased among children aged 2-10 years. There is a higher incidence among girls.

Amebiasis accounts for 2-4% of the population of the developed countries, being spread among immigrants, students and tourists arriving or returning from endemic areas, as well as in immunocompromised (HIV) homosexuals. The risk of invasion is increased during the summer period.

Clinical manifestations

After a period of incubation lasting from one week to 3 months (sometimes longer), amoebiasis is manifested by ulceration in the intestine and occasionally the formation of abscesses in the liver (tropical or amoebaic abscesses), lungs, spleen, testicles or brain.

The main symptoms include diarrhea, indigestion, abdominal pain, rectal tenesmus, weight loss, and anemia. The invasive forms include fever, signs of organ damage depending on the location of the abscess.

Laboratory diagnosis is made based on microscopic examination,

which helps to detect the parasite in the feces or rectal exudates in the intestinal forms, or by biopuncture in case of extraintestinal forms.

Serological investigations are used in extra-intestinal amoebiasis.

Preventive measures

Amoebiasis preventive measures are aimed at an early detection of *E. histolytica* through regular parasitological check-ups and everyday cleaning, as well as leading a healthy lifestyle in patients or carriers, especially among persons who arrived or returned from endemic areas, persons from risk groups (children's institutions, food sector, drinking water supply facilities). Vegetables are to be consumed after treating them with vinegar for 15 minutes. Decontamination with chlorine is also effective.

Anti-epidemic measures

The patients are subjected to the treatment with metronidazol or other preparations (minidazol, clorchin, ornidazole), according to the schemes (amoebian dysentery), depending on the type of invasion.

Hospitalization is performed based on clinical indications, possible complications or side effects and the need for parasitological control. Discharge is performed after receiving a course of full treatment and when a complete recovery occurs, as well as after the three negative results of the fecal mass analysis to identify the parasite presence (vegetative or cystic forms) are performed at intervals of 1-2 days.

The follow up is carried out for 12 months, with parasitological investigations of fecal masses in 1, 3, 6 months after the discharge. The parasite carriers are not allowed to work in the risk sectors.

Feces can be decontaminated with 5% Lysol and laundry-by soaking in 3% lysol solution.

People who were in contact with the patient are to be subjected to the parasitological examination, followed by the detection of chronic and carriage forms or ulcerations, with their subsequent treatment. Isolation and contact chemoprophylaxis are not performed.

2.1.4. Giardiasis (Lambliasis)

Short history

The parasite was observed by Antonie van Leeuwenhoek in 1681, while he examined his own feces. In 1859, Vilem described lamblia in

detail, isolating it from stools of children with diarrhea. Later, some authors called it *Lamblia,* while the others named the species found in humans, *Giardia,* in honor of the French professor Alfred Giard.

The pathogen agent

The etiological agent *Giardia lamblia* belongs to the genus Giardia (Lamblia) of the Flagella class. The genus currently comprises 6 species.

Giardia lamblia (also called intestinalis or duodenalis) is found in the species that affects humans, as well as other mammals, including pets and farm animals.

There are two forms of parasites, which show two stages of development: trophozoite and cyst. The cycle starts with the ingestion of the cyst that releases troodosites in the duodenum. They attach to the surface of the intestinal endothelium and multiply by a binary division. Seclusion formations occur in the colon. The cyst, which is inactive, oval-shaped, 11-14 μm in length and 7-9 μm in width, is excreted with feces in the external environment. Elimination of the cysts from an infected body occurs intermittently, known as „negative periods" which takes 7-10 days, sometimes 2-3 weeks.

The vegetative form of Giardia is destroyed in 5-10 minutes in the external environment. The cyst form, vice versa, is resistant to the environmental conditions. It persist at room temperature from 2-3 days to 2 weeks; in moist soil – up to 60 days, and in water - 35-80 days. At the same time, the cysts are destroyed in dry conditions within 1-2 minutes, at + 70°C – in 30-40 seconds, and instantly at boiling.

Normal concentrations of chloramine and other disinfectants at do not have a destructive action on lamb cysts.

The reservoir and the source of pathogen

The sick and healthy carriers are the main sources of the pathogen. There is evidence that animals can be infected (dogs, cats, pigs, cattle) as well and the human can be a source of pathogen for animals.

Mode and transmission routes

Pathogen transmission from the source to a healthy person occurs through fecal-oral mechanism. The infective dose is 1-10 cysts. Ingestion of cysts occurs mainly through contaminated water used for drinking (by water, since chlorination does not destroy the cysts).

There are descriptions of several outbreaks of giardiasis, especially among travelers returning from countries with endemic giardiasis, therefore the disease is also called „tourist diarrhea".

Giardia cysts can be transmitted through contaminated food, particularly raw vegetables. The transmission can result from a casual contact (underwear, dishes, and toys, as well as through hands). This mode of transmission occurs more frequently in children's communities (nurseries, kindergartens, schools), prisons, refugee camps, asylums for mentally disabled persons. According to the bibliographical sources, the infestation of children in nurseries, kindergartens, orphanages varies between 18-20% and 60-75%.

Manifestations of epidemic process

Giardiasis is spread worldwide, in hot, temperate or cold climates, especially among children under 14 years old. Annually, the estimated number of cases of giardiasis is about 200 million in the world. In the US, the incidence of giardiasis is 15 cases per 100 000 population and in the Russian Federation – 90.9 cases per 100 thousand population, whereas the general giardiasis morbidity rate is estimated at 70% of the detected cases.

The incidence of giardiasis is higher in developing countries (Africa, Asia, South and Central America), where the access to water is limited. Under these circumstances almost all children are contaminated with giardiasis, and the parasite prevalence in young children may reach 10-30%.

In European countries and the US, the invasion is associated with contaminated water consumption, travelling abroad and swimming in the pools, whereas the incidence of diarrhea constitutes 2%-5%.

In the Republic of Moldova, the incidence of giardiasis is 9.0-9.5 cases per 100 thousand population, while the percentage of children, under 17 years of age, constitute 81% of the overall morbidity rate. The incidence of giardiasis is the highest in the age group of 3-6 years – 77.0 cases per 100 thousand population, compared to 41 cases in the age group 0-2 years, 24 cases in the age group 7-17 and 9.0 cases in adults *(Figure 60)*.

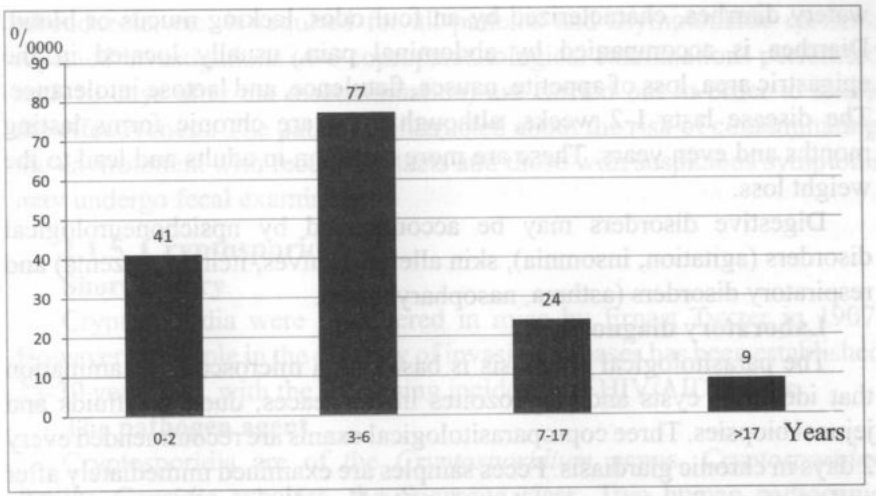

Figure 60. *The incidence by giardiosis according to the age group, in the Republic of Moldova.*

Giardiasis is more prevalent among the rural population with an overall morbidity rate of 77.61% compared to 23.39% in the urban population *(Figure 61)*.

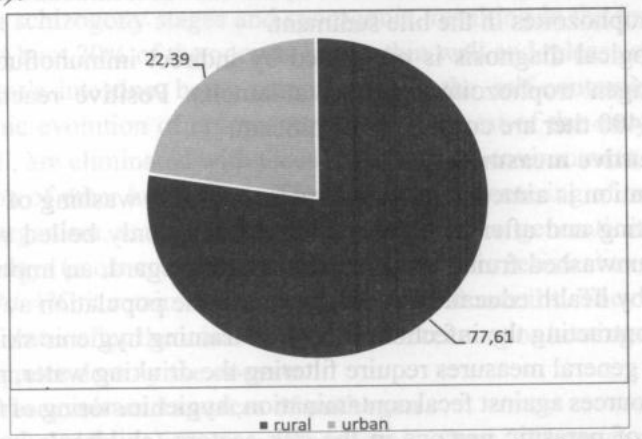

Figure 61. *Distribution of giardiosis cases according to the place of residence in the Republic of Moldova.*

Clinical manifestations

After the incubation period of 2-3 weeks, the disease begins with a

watery diarrhea, characterized by an foul odor, lacking mucus or blood. Diarrhea is accompanied by abdominal pain, usually located in the epigastric area, loss of appetite, nausea, flatulence, and lactose intolerance. The disease lasts 1-2 weeks, although there are chronic forms lasting months and even years. These are more common in adults and lead to the weight loss.

Digestive disorders may be accompanied by npsichoneurological disorders (agitation, insomnia), skin allergies (hives, itching, eczema) and respiratory disorders (asthma, nasopharyngitis).

Laboratory diagnosis

The parasitological diagnosis is based on a microscopic examination that identifies cysts and trophozoites in the feaces, duodenal fluids and jejunal biopsies. Three coproparasitological exams are recommended every 2 days in chronic giardiasis. Feces samples are examined immediately after collection or after preserving them in 19% formalin or polyvinyl alcohol.

The cysts in feces are examined microscopically, for at least three times within 10-20 days, because of the intermittent excretion of cysts.

Microscopic examination identifies trophozoites in patients with profuse diarrhea, which are easily recognized by shape and mobility. If the parasite is not detected in the stools, the duodenal intubation is used to identify trophozoites in the bile sediment.

Serological diagnosis is performed by indirect immunofluorescence, using antigen trophozoite fixed on a lamella. Positive reactions with 1 / 50-1 / 400 titer are considered significant.

Preventive measures

Prevention is aimed at personal hygiene: proper washing of the hands before eating and after using the toilet, drinking only boiled water, and avoiding unwashed fruit and vegetables. In this regard, an important role is played by health education in order to make the population aware of the risks of contracting the infection, as well as training hygienic skills.

Other general measures require filtering the drinking water, protection of water sources against fecal contamination, hygienic storing of feces, and detection of parasitic persons in the risk sectors (children's institutions, food sector, drinking water supply facilities) via periodical parasitological check-ups.

Anti-epidemic measures

The appropriate therapy with metronidazol, tinidazol, furazolidone,

albendazole, etc. is required for all patients and asymptomatic carriers; repeated investigations (4-6 coproparasitological examinations performed in 7-10 days after the discontinuation) are carried out in order to check the effectiveness. The patient is instructed about the risk of contaminating the environment with feces. Contacts and those with suspicious symptoms may undergo fecal examination.

2.1.5. Cryptosporidiosis

Short history

Cryptosporidia were discovered in mice by Ernest Tyzzer in 1907. However, their role in the etiology of invasive diseases has been established 25-30 years ago, with the increasing incidence of HIV/AIDS cases.

The pathogen agent

Cryptosporidia are of the *Cryptosporidium* genus, *Cryptosporidae* family, *Coccidia* subclass, the *Sporozoa* class. Two human pathogenic species are recognized: *Cryptosporidium muris* and *Cryptosporidium parvum*. In human pathology the main role belongs to the species *Cryptosporidium parvum*, which is pathogenic for cattle. Cryptosporidia are intracellular parasites with the habitat in the epithelial cells of the digestive tract and lung. The complete development cycle takes place in a single host, including schizogony stages and sporogonia, resulting in the formation of oocysts. About 20% of the oocysts have a thin wall and release sporozoites in the host's intestine, being responsible for the self-contamination and the chronic evolution of cryptosporidiosis. The rest of the oocysts with a thick wall, are eliminated with feces in the outside environment, where the infestation of other hosts occur. The process from entering of oocysts into the host organism, as well as the elimination of a new generation of oocysts lasts 3-7 days (most commonly 5 days). Oocysts are resistant to exogenous factors. At 4°C it retains its viability for several months. They survive in drinking water after the usual chlorination. Freezing or heating up to 65°C for 30 minutes destroy the oocysts.

The reservoir and sources of pathogen

Cryptosporidiosis is a zooanthroponosis. Over 30 known species are hosts of cryptosporidiosis. Some hosts (mice, rats, guinea pigs, rabbits, cats, dogs, chickens, geese) do not show clinical signs after infestation. Other hosts (calves, lambs, piglets, goats, deer, monkeys, humans) can manifest pronounced clinical symptoms.

The source of cryptosporidios for humans is both healthy patients and carriers, as well as diseased or cryptosporidic animals. It is considered that the calves up to one month represent the main reservoir of cryptosporids. It has been found that 1 g of feces in infected calves contain from 1 mln. up to 74 mln. of oocysts. The diseased man eliminates oocysts throughout the disease and other 2-3 weeks after the disappearance of the clinical symptoms.

Carriage among humans is a rare phenomenon, but it is common in immunocompromised individuals (13-17%).

Ways and means of transmission

In cryptosporidiosis The main mode of transmission is fecal-oral and the respiratory one (by solid aerosols containing oocysts) is not very common. There are four ways of transmitting oocysts: by water, food, airborne and a habitual contact. Human-to-human transmission occurs under unsanitary conditions, during consumption of the contaminated products, inhaled oocysts, or through sexual intercourse with homosexual men. However, the waterway is the most common one. Contaminated water epidemics have been reported everywhere.

The pathogen is mainly transmitted by ingestion of food, contaminated with oocysts from diseased animals or carriers of cryptosporidiosis, as well as other by elements of external environment contaminated with their feces.

Manifestations of epidemic process

Cryptosporidiosis is spread everywhere; the epidemic shows a greater incidence in tropical and subtropical countries, whereas it is less spread in the Central and Northern Europe.

The responsiveness is higher in children and in immunocompromised persons. Cryptosporidiosis is more common among children up to 6 years of age. In the Republic of Moldova, the cryptosporidiosis incidence rate among children up to 2 years of age is about 60% and among those of up to 6 years of age – 86%. The incidence is higher among children in the urban areas compared to those in the rural ones.

The incidence is 4 times higher in immunocompromised individuals compared to the immunocompetent ones, being of 47-50% in HIV patients. Also, it affects the infants and children who are artificially fed three times more often compared to those that are breastfed. The incidence was found to be higher in children in overcrowded communities. In adults

cryptosporidiosis commonly occurs among farm workers, and those who keep dogs, cats and other animals or birds inside their flats.

Cryptosporidiosis infestations are favored by visits to endemic areas, accompanied by disturbances in food regimen and poor personal hygiene.

Cryptosporidiosis is predominantly sporadic, but outbreaks and epidemics, especially those related to water contamination are reported within developed countries as well. In the UK, for example, in 1991, there was cryptosporidiosis epidemic involving 516 people, whereas the transmission factor was the consumption of drinking water. Another epidemic transmitted by drinking water was recorded in the US (1999), affecting thousands of people with serious symptoms in the elderly and immunocompromised persons.

The period of maximum seasonal incidence is from May to August and it is favored by swimming in extensively polluted water, as well as abundant human consumption of water (especially from unknown sources), unwashed vegetables and intense migration of population and activation of sources.

Clinical manifestations

Cryptosporidiosis develops in two clinical forms: in immunocompetents persons it develops as an autoimmune diarrheal disease, and in immunocompromised persons - as a persistent diarrhea associated with malnutrition.

In 30-40% cases of immunocompetent persons, cryptosporidiosis evolves in subclinical or carriage forms, sometimes with poorly pronounced clinical manifestations (aqueous fluid stool, slight fever, weakness, nausea and vomiting (1-2 times a day). The disease progresses with the following pronounced clinical manifestations: chills, fever 37°-38°C, malaise, loss of appetite, nausea, vomiting (1-2 times a day) and aqueous fluid stool (up to 10 times per day). Diarrhea can last 3-48 days, while the disease lasts 2 weeks and ends up with spontaneous healing.

The disease progresses more severely in immunocompromised individuals:high fever (38-39°C), repeated vomiting, progressive diarrhea leading to the loss of a large amount of fluid (1-12 l in 24 hours), dehydration of the body, and essential weight loss. The disease can last for 6-8 weeks. In these patients, cryptosporidiosis is often associated with toxoplasmosis, candidosis, lambliosis, pneumocystis or cytomegalovirus infection, which lead to a malignant development of pronounced cachexia and, if not treated properly, to patient's death.

The bronchopulmonary form, of cryptosporidiosis, is manifested by fever, lymphadenopathy, chronic cough with poor mucosal sputum (rarely mucopurulent), dyspnea and cyanosis. The radiological examination reveals diffuse damage to the trachea, bronchi, bronchiole and even alveoli.

Laboratory diagnosis

Nonspecific clinical manifestations of cryptosporidiosis require, necessarily, confirming the diagnosis by laboratory investigations.

The following methods of cryptosporidiosis diagnosis are applied: microscopic, serological and biological.

The microscopic method has gained a wider practical application, which consists in detecting cryptosporidic oocysts in smears prepared from feces and other materials (bronchial, sputum, tracheal aspirate, biopsy of lung and duodenum) and stained by one of the accepted methods (Ziehl -Neelsen, Romanovski-Giemsa, and others). In order to enhance the effectiveness of the method, preventive material can be concentrated by floatation, sedimentation or sedimentation + floatation methods.

Oocysts can be identified in feces by direct immunofluorescence using monoclonal antibodies specific to the determinant antigen of oocyst wall.

ELISA and IFIR used in the detection of circulating antibodies are more sensitive and specific methods which are commonly used in the diagnosis of cryptosporidiosis.

Preventive measures

The prevention of cryptosporidiosis can be ensured by general prophylaxis measures: neutralization of oocysts on various objects in animal farms, medical stations, children's institutions, pest control measures, actions aimed at strengthening the infant immunity through by promoting natural nutrition, measures to prevent HIV infection and other immunodeficiency conditions, increasing the level of hygienic culture of the population, protecting the environment from pollution with human and animal wastes, ensuring the human population with qualitative drinking water and food, supervision of the sanitary-hygienic regime in children's institutions, hospitals, asylums, etc.

Anti-epidemic measures

People diagnosed with cryptosporidiosis are subjected to the treatment according to the approved protocol and medical surveillance. There is no etiotrope therapeutic means in cryptosporidiosis. Symptomatic therapy is used. People infected with cryptosporidiosis undergo the same measures as in giardiasis.

2.2. Helminthiases

2.2.1. Geohelminthiasis

2.2.1.1. Ascariasis

The disease has been known since ancient times. The terme was introduced by Hippocrates. The life cycle of the parasite was described in 1916.

The pathogen agent

Ascaridiasis is a parasitosis caused by *Ascaris lumbricoides worm*, a long intestinal human nematode. In the adult female measures about 30 cm and the male – 20 cm. Adult worms live in the intestinal lumen, from 6 months to 2 years. The female deposits up to 200,000 eggs daily, which are eliminated by feces in the external environment. Unembryonated eggs have thick coatings and are highly resistant to temperature up to 45°C and to dry conditions. It can resist in the external environment for many years.

Embryonated eggs (geohelmintiasis), under favorable conditions of temperature and humidity, resist in the soil during 10 days at 30°C and 50 days at 17°C. Further biological cycle occurs only in a human host, by entering into the human body through contaminated food or water.

The eggs hatch in the duodenum and the released larvae migrate via the hepatic portal vein, liver (3-4 days), heart, lungs (10 days), larynx and pharynx, then they return to the intestine where grow into an adult form.

The reservoir and the source of pathogen

The soil is considered to be the reservoir of ascariasis, where the ascaridae eggs are eliminated by feces and develop invasive stage (contagious), mandatory in the development cycle of the parasite.

The source of invasion or the host is the diseased man. This eliminates a large number of unembryonated eggs by feces that further mature in the external environment (in the soil).

Therefore, the ascariasis-diseased person is not contagious, since the eggs mature in the soil.

The factors and transmission routes

The mode of transmission is fecal-oral. Contamination occurs orally, through drinking water or food, especially vegetables, fruits, vegetables, eggs contaminated with ascarides; by ingestion of soil (common in children), contaminated hands or toys.

Predisposing factors: the warm seasons, increased soil humidity,

contaminated soil, frequent contact with soil, poor personal hygiene of the population.

Manifestations of epidemic process

Ascariasis is widely spread all over the world, with greater frequency in tropical countries, where it is hyperendemic. According to the WHO, annually, more than 1 million people are infected with ascariasis worldwide, and 60,000 die from this invasion. Ascariasis is recorded at 105-200 cases per 100 thousand population, affecting about 0.15-0.20% of the population in Moldova.

The incidence is higher among the rural population compared to the urban areas. The increase morbidity (incidence) among rural population is corelated to the increasing number of people who are engaged in gardening. The risk of contamination is increased in areas where human excretions are used for soil fertilization.

Children get sick more often then adults *(Figure 62)*. This is due to poor hygiene among children and low immune system. Children are infected more frequently when playing around the house, children's playgrounds, where the soil is contaminated with ascaride eggs.

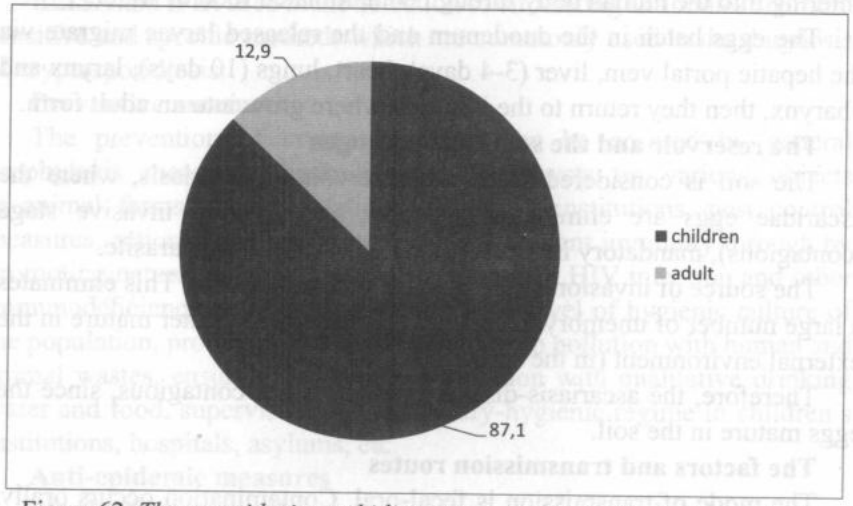

Figure 62. *The ascaridosis morbidity rate according to the age group in the Republic of Moldova, in 2000-2013.*

Ascariasis is characterized by seasonality and focality. Ascariasis infestation occurs mainly during the summer-autumn period in temperate

territories. The focality is determined by the level of ascariasis- diseased population and is estimated by both the number of microfoci recorded in the village and the number of affected people *(Table 6)*.

Table 6

Types of foci in ascariasis

Type of focus	Affected population, %
High intensity foci	≥10 and more
Medium intensity foci	3-10
Low intensity foci	<3

Clinical manifestations

The clinical symptoms vary in both stages of the evolutionary cycle the localization of the parasite in the host organism, as well as the intensity of infestation of the body.

During the invasion of the lungs, which lasts about eight days, the disease is manifested by fever, cough, lung opacities, hypereosinophilic blood. The adult worms cause gastrointestinal symptoms during the intestinal invasion: vague abdominal pain, transit disorders, especially diarrhea, nausea or even vomiting. It is characterized by nutritional disorders, especially in children.

Nervous phenomena like irritability, accompanied by sleep disorders, are common. Ascaridosis inhibits the development of other infectious diseases. Ascarids have an immunodepresive action and complicates the development of pregnancy and births. Complications can occur in the form of intestinal occlusions due to mechanical obstruction of the lumen by ascarids, spasm reflux, intussusception of intestinal loops, or peritonitis, especially after a digestive tract surgery.

Laboratory diagnosis

Parasitological diagnosis includes the detection of adult parasites and ascaride eggs via fecal examination.

It is important to consider the leukocyte formula, that shows hypereosinophilia (40%) associated with hyperleukocytosis in the primary lung stage, with maximum values on the 20th day, which then decreases, stabilizing at 5-20%.

Preventive measures

The main measures for the prevention of ascariasis include the prevention of contamination of the environment, particularly that of soil, with helminth eggs and hygienic education of the population.

Individual measures are important: proper hygiene, washing the hands and vegetable food. Prevention of soil infestation with helminth eggs can be achieved by providing the population with sanitary facilities, waste water treatment, prohibition of using feces to fertilize the soil.

Anti-epidemic measures

Ascariasis patients are subjected to an outpatient treatment, whereas the excrements must be decontaminated with lime chloride (200-300 g per 1 kg of weight) or with boiling water in a closed bowl during 40 minutes.

In intensive outbreaks, where the incidence exceeds 10% of the population, the deworming is carried out in mass among infant population (up to the age of 18 years) and adults in microfoci. Mass deworming should be carried out twice a year for the maximum effect: the first deworming - in early season of mass infestation (March-April), aimed at treating people infected the year before; the second deworming – at the end of the mass population infestation season (November-December), aimed at deworming the infected persons during the summer-autumn period. The elective remedies for mass treatment are: piperazone-Adipine, decaris, vermox (mebendazole), and albendazole according to the approved schemes.

In medium intensity outbreaks, all members of microfoci (families, private households where at least one ascaridosis patient has been detected during the last 2-3 years and the current year) are subjected to deworming. The treatment is carried out twice per year at the same time, using the same means as in case of intensive outbreaks.

In low intensity outbreaks, with the incidence of less than 3%, the epidemiological and laboratory examinations of all microfoci and infected persons are carried out, followed by a further treatment of the identified patients.

2.2.1.2. Trichuriasis (Trichuroza)

The pathogen agent

Trichuriasis is caused by the nematode *Trichuris trichiura* (*Trichocephalus trichiura*). The female parasite measures 35-55 mm, and the male one – 30-45 mm. Its body is divided into two parts: the anterior, longer and as thin as a hair, and the posterior one which is shorter, but much thicker. *T. trichiura* lives in the anterior part of the colon mucosa. Its lifespain is 4-6 years. The female lays from 1000 to 3500 eggs daily in the lumen of the intestine. The eggs are eliminated with feces in the external environment.

Maturation of the eggs occurs in the soil at temperature of 15°- 40°C, in the presence of oxygen and a relative humidity of about 100%. The maturization (embryo) of the eggs lasts 17 days at 30°C and 4 months at 15°C, and they remain invasive for several years.

The larvae are released from the egg into the small intestinal mucosa of the human body, where they develop within 10-12 days, then move into the lumen of the intestine and migrate to the colon. They become adults after a month. The release of the eggs into the external environment starts in approximately 6 weeks after the infestation.

The reservoir and source of the pathogen are the same like in ascariasis.

The reservoir is considered to be the soil, where the parasite eggs develop up to the invasive stage, because only embryonated egg on the soil (geohelmint) is the important element in human infestation.

The parasite source is a man sick with Trichuriasis, rarely monkeys.

Risk factors and transmission routes

Human infection occurs by ingestion of embryonated eggs with contaminated water or food, soil dust and contaminated hands.

Contributing factors are analogous to those of ascariasis.

Manifestations of epidemic process

Trichuriasis is more common in hot and humid climates and poor sanitary conditions. Responsiveness is general, but the infestation is higher in children aged 5-15 years, as a result of not following the rules of daily hygiene and increased receptivity.

In adults the incidence is however higher compared to that in ascariasis, representing almost a third of overall Trichuriasis morbidity *(Figure 63)*.

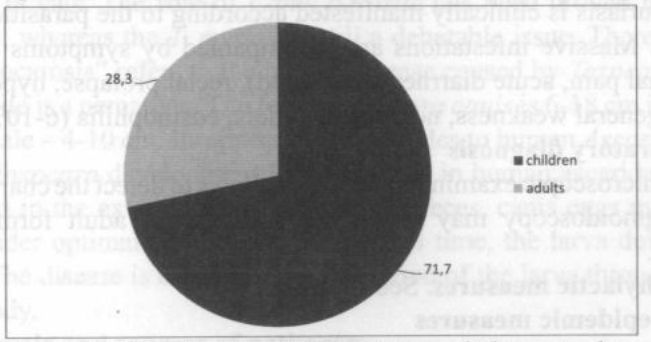

Figure 63. *Distribution of trichuriasis morbidity according to the age group in the Republic of Moldova.*

The incidence is higher in the warm season of the year, especially among rural population, where the contact with soil more commonly occurs, whereas the sanitary facilities are poor.

About 82% of Trichuriasis cases are recorded among the rural population and 18% among the urban population in Moldova.

The multiannual dynamics of Trichuriasis morbidity is decreasing *(Figure 64)*.

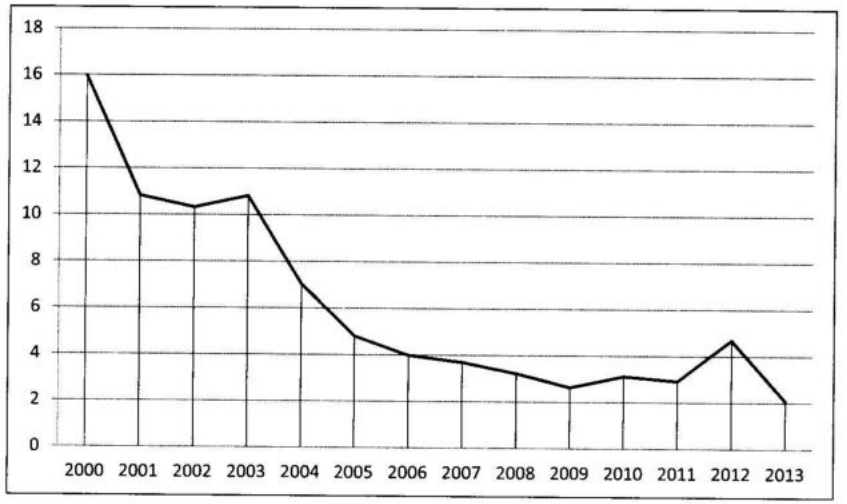

Figure 64. *Dynamics of Trichuriasis morbidity in the Republic of Moldova, in 2000-2013.*

Clinical manifestations

Trichuriasis is clinically manifested according to the parasitic level of infection. Massive infestations are accompanied by symptoms of colitis (abdominal pain, acute diarrhea with blood), rectal prolapse, hypochromic anemia, general weakness, nervous disorders, eosinophilia (6-10%).

Laboratory diagnosis

The microscopic examination of feces allows to detect the characteristic eggs. Sigmoidoscopy may reveal the presence of adult forms of the pathogen.

Prophylactic measures. See ascariasis.

Anti-epidemic measures

Patients undergo an out-patient treatment with mebendazole or albendazole. The excrements are mandatorily decontaminated to prevent

soil contamination with trichiura eggs. Decontamination is the same as with ascariasis.

The foci of Trichuriasis are clinically supervised during a period of 2 years with parasitological investigation of all family members twice per year, in spring and autumn. The measures of sanitation and elimination of the conditions that contribute to repeated infestation of family members are performed in the outbreak.

The focus is considered eliminated if during the medical supervision of the family, no Trichuriasis patients have been detected and the sanitay conditions meet the requirements.

The supervision of the false foci is discontinued after three negative feces examinations of all family members.

2.2.1.3. Toxocariasis

Short history

The first studies were conducted by Fulleborn in 1911, who assumed the possibility of invasion with ascaridea, uncharacteristic of *Ascaris* genus.

In 1952, Beaver described the migrating larvae in humans and animals and called it „larva migrans" (migrant larva), proposing to be approved as a diagnostic form.

In 1969, Beaver suggested the induced invasion of dog ascaridae (Toxocara canis) to be called, Toxocariasis.

The Toxocariasis pathogen belongs to the family of *Anisakidae* (*Ascaridae*), the *Toxocara* genus.

Two *Toxocara* species are known: Toxocara canis- in dogs and *Toxocara mystax* – in cats. The role of *Canis parasite* has been proved in human pathology, whereas the *T. mystax* is still a debatable issue. Therefore, the term „toxocarosis" refers to the human disease caused by *Toxocara canis*. The parasite is a nematode. The female *toxocara canis* is 6-18 cm in length, and the male – 4-10 cm. Its appearance resembles to human *Ascaridia*. The cycle of *Toxocara* development is the same as in human ascaridae. Being eliminated in the external environment with feces, canis eggs mature for 5 days under optimal conditions. During this time, the larva develops in the egg. The disease is caused by the migration of the larva throughout the human body.

Reservoir and sources of pathogen

Toxocara canis parasitizes in the intestine of dogs, wolves, foxes and

other canines. Mature parasites are localizated in the small intestine and the hosts' stomach. The life cycle lasts 4-6 months. The female lays more than 200000 eggs per day. The environmental pollution with *Toxocara* eggs is enormous since the animal is invaded by parasites, usually, by hundreds of specimens. Toxocariasis is widely spread among animals. It is favored by multiple modes of transmission: fecal-oral (infestation with eggs from the environment), transplacentar (infestation of the fetus with larvae through placental blood), by breastfeeding (transmission of larvae through milk).

Eggs mature in soil at 24°-30°C, in the presence of humidity and light and keep its viability for a long time. Therefore, the soil is considered a necessary condition for its development in nature.

The human is believed to be an occasional host (intermediate host) of *Toxocara* and does not present potential epidemiological risk, since the larvae do not develop into adult forms in human body.

Risk factors and transmission routes

Toxocariasis is transmitted by a fecal-oral mode. Human invasion is possible by eating food (especially vegetables, fruits, berries), drinking water, as well as through hands contaminated with *Toxocara canis* eggs.

Predisposing factors: the increased dog population, especially that of stray dogs, lack of decontamination of animal excrements, intense contamination of children's playgrounds with helminths eggs, occupation of the adults (dog breeders, farm workers, gardeners, etc.).

Manifestations of epidemic process

Toxocariasis is ubiquitous, being more frequent in countries with hot and humid climates, ecxept for the Nordic countries. The incidence of *Toxocariasis* is increasing worldwide. It is considered that about 20% of the population of dogs and almost 80% of puppies, that contaminate intensively the environment with eggs, are infected with *Toxocara*. The soil contamination reaches 10% in the examined samples, collected from many places. Toxocariasis is a particular problem for urban areas. *T. canis* eggs were found in 18-42% of soil samples, collected from children playgrounds in public areas of Italy, UK, USA, Czech Republic, Russian Federation etc.

Children up to the age of 14 years are more commonly affected compared to the adults.

The invasion is more common in high-risk adult groups (dog breeders, vegetable growers, housing and communal services workers etc.).

Seasonality refers to summer-autumn period, when the number of invasive eggs in the soil and contact with the soil are at maximum.

Clinical manifestations

The incubation period is very short and lasts from a few hours to a few days, that coresponds to the release of the larva from the egg into the small intestine and its further penetration into the bloodstream. Clinical manifestations depend on the massive invasion, frequency of reinfection, method of larva migration through the body, its spread to certain organs and tissues, and the degree of host immune response.Toxocarosis is characterized by a long-lasting development and recurrent evolution (from a few months to several years).

Toxocariasis is manifested mainly by two clinical forms - visceral and ophthalmic. Approximately 30% of cases are found to be asymptomatic.

Visceral toxocarosis is the result of massive infestation with larvae. The symptoms include general asthenia, recurrent fever, arthralgia and specific organ damage: lung disorders, hepatosplenomegaly, lymphadenopathy, neurological signs. The body temperature is usually subfebrile, rarely febrile, and is commonly found during pulmonary manifestations.

Pulmonary disorders are recorded in about 65% of patients with visceral Toxocariasis. The clinical manifestations vary from catarrhal symptoms to severe asthmatic forms with catarrhal inflammation, bronchitis and recurrent bronchopneumonia. Patients suffer from frequent nocturnal attacks of dry cough and in some cases severe wheezing,dyspnea and cyanosis. Toxocariasis was detected in 19% of patients, diseased with asthma and bronchitis.

Recurrent eruptions (erythema, urticaria, Quincke edema) are observed in 30% of cases. Hepatomegaly is recorded in 80% of cases, and splenomegaly – in 20%. The migration of the larvae to the brain is characterized by seizures, epileptic seizures, paresis and paralysis. In some cases, visceral toxocarosis evolves with myocarditis, eosinophilic pancreatitis, kidney disease, muscle and joint pain.

Eosinophilia is constant and long-lasting.

Ophthalmic Toxocariasis is associated with a low level of larvae infestation. There are two predominant forms of manifestation: solitary granuloma and chronic exudation endophthalmitis. As a rule, Toxocariasis affects one eye, where a single larva is found. In cases of the intraretinal migration, the larvae can be seen at ophthalmoscopy, whereas the scotoma migrant appears in the patient's field of view.

Ophthalmic nerve damage by larvae can lead to unilateral blindness.

Laboratory diagnosis

In Toxocariasis parasitological diagnosis is difficult due to the difficulties to identify the migratory larvae. However, parasitological diagnosis is based on the identification of larvae in tissue biopsy.

Immunological diagnosis plays a primary role in the immunological testing of Toxocarasis. Currently there are immunoenzymatic diagnostic systems for identification of the parasite. The specific antibody titer of 1: 800 and higher indicates visceral manifestations and titers 1: 200-1: 400 - in carriage of visceral toxocarosis or in the ophthalmic form of Toxocariasis.

Preventive measures

Complex preventive measures include: planned deworming of dogs, arranging special areas for dog walking, protection of children's playgrounds, public gardens, places to walk the animals, good personal hygiene (washing hands after the contact with animals and before meals), thorough washing of vegetables, fruit, berries, vegetables before eating, knowledge of epidemiology, symptoms, risk factors, danger and prevention measures in Toxocariasis.

2.2.1.4. Strongiloidosis

Short history

In 1876, Joseph Normand detected the pathogen in fecal samples of French soldiers affected by diarrhea. R. Leuckart (1882) confirmed that fact and named the parasite *Strongyloides stercoralis*. In 1928, Nishigori demonstrated the parasite invasiveness by endogenous autoinfestation. A special role in studying the Strongiloidosis belongs to Acad. K.I.Skreabin.

Strongyloides stercoralis pathogen belongs to the Nemathoda class, *Rhabditida* order, *Strongyloidae* family, *Strongyloides* genus. The parasite lives in the intestine of a human and in the soil. S. stercoralis is a small, thin and thread-like worm (it is the smallest pathogen for humans). It has a small size - the female - of 2.2 / 0.77 mm, and the male - of 0.7 / 0.05 mm. The female parasitizes in the submucosa of the duodenum or jejunum, affecting the villous stroma and Liberquin glands. In cases of massive invasion it can affect the whole intestine. It can also penetrate the bile and the pancreatic ducts.The males are eliminated with the excrement after fertilization, whereas the females live for about a year and lay daily about 50 eggs in the Liberquin glands, from which the rabditoid larvae are born. These are active (but not invasive), they reach the intestine lumen, where

they are eliminated with feces in the external environment to continue their biological cycle in soil, water, feces, at a temperature of > 10°C and sufficient humidity. Depending on the conditions, rabditoide larvae can evolve in two ways: a) direct – in 1-4 days, it turns into invasive filariform larvae (Strongyle); b) indirect – in 4-6 days, it turns into a free generation of females and males that produce a new generation of rabiditoid larvae in the external environment, which in turn evolve either directly or indirectly. The *strongylide larvae* survive in the soil for 12 days and can penetrate the intact skin of a host. Strongyle larvae reach the lung, then enter the respiratory tract, and on swallowing they reach the duodenum (on the 17th day), where they turn into adults (on the 27th day), giving birth to a new biological cycle. In individuals with slow intestinal transit (constipation, intestinal diverticula), rabditoide larvae may develop into filariformed forms directly in the intestine, causing self-infestation and chronic disease.

The reservoir and source of pathogen

The source of parasite is the sick man,who may eliminate larvae for years, especially in cases of self-infestation. In rare cases, dogs and pigs infected with S. stercoralis may serve as a source of parasite. The parasite reservoir includes both human population, where it is transformed into an adult and deposits eggs, and the environment (the soil), where the rabditoide larvae turn into the invasive ones.

Mode and transmission routes

Filariform larvae penetrate the body through the intact skin, by contact with polluted soil, oral mucosa and esophagus after consuming water, herbs, vegetables, fruits and berries contaminated with filariform larvae. The contamination of infants via breastfeeding or organ transplants is less common..

Manifestations of epidemic process

Strongiloidosis is universally widespread, with an increasing incidence in tropical and subtropical regions (Africa, South America, Asia), where the moist soil and warm climate favor the rapid multiplication of generations of the parasite. Annually, about 35 million cases of Strongiloidosis are recorded all over the world, of which 21 mln cases occur on the African continent. The invasion is more often detected in moderate climates, including Europe. Strongiloidosis is reported to be spread in sub-Carpathian rural areas of Romania. In Western Ukraine, its incidence is 1.5-4.1%. Strongiloidosis is ranked the 6th among all helmintiasis types in

Moldova, with the record from 1 to 46 cases every year. There is a constant increase in morbidity.

Responsiveness is general, but the infestation more commonly occurs among the adults from rural areas. People with immunodeficiencies are at a higher risk of being infected.

Persons with an increased risk of infestation are: rural workers engaged in agricultural work, greenhouse workers, biological wastewater treatment plants, sewerage networks, pig farmers, people who work or travel in endemic areas. Foci have been described in psychiatric institutions and retirement homes.

The seasonality refers to the summer time. The season epidemic lasts 4-4.5 months (May-September) in moderate climates. In tropical areas, infestations occur throughout the year.

Clinical manifestations

The strongiloidosis symptoms correspond to the three stages of the disease: skin, lung and the intestinal one.

The skin stage is characterized by the appearance of pruritic dermatitis, small papules and macules within 24 hours at the site of invasion of filariform larvae. In case of exogenous self-infection, the syndrome of „currens larvae"occurs, which is manifested by the appearance of linear lesions, located at the site of invasion (the anus, buttocks) and extending along the larval migration pathway.

The *Lung stage* or the *early stage* corresponds to the stage of larval migration and is manifested by fever, chills, diffuse pain in the abdomen, dry and painful cough, predominantly nocturnal, pruritus, urticaria, edema, allergic skin, especially on the face, pronounced eosinophilia in the blood, increased size of the liver, enlarged lymph nodes, mild catarrhal symptoms, eosinophilic infiltration in the lungs detected by radiologic examination.

Intestinal or chronic stage is mainly manifested by disorders of the digestive system, characterized by abdominal pains, which can simulate ulcerative disease, cholecystitis or gastroduodenitis, presence of diarrhea with mucus, sometimes with streaks of blood, alternating with the periods of constipation, abdominal bloating, nausea, vomiting, anemia, dehydration. Allergic manifestations may occur: recurrent chronic urticaria, Quincke edema, skin itching and edema of the skin after scratching, perianal pruritus, conjunctivitis, high blood eosinophilia.

Secondary anemia occurs after a long-term development. Radiological examination reveals signs of duodenal dyskinesia.

The latent form of invasion is observed in 7-10% cases. Blood eosinophilia and detection of rabditoide larvae in feces can serve as diagnostic indicators. This form is more commonly observed in patients with rheumatism, tonsilitis, liver cirrhosis and renal cancer.

Laboratory diagnosis

In silyloidosis, laboratory diagnosis is based on parasitological diagnosis and pathogen detection in feces or duodenal content, rarely sputum, urine, blood, and cephalosporidic fluid.

The contents of the feces and duodenal ulcer, as well as other fresh substrates are to be examined by special laboratory methods, such as Baermann method with 92-95% efficacy.

In 82-84% of cases, specific antibodies can be detected in the blood serum of the patients via serological tests (IFIR, IHAR, ELISA). Serological tests are used in the diagnosis of chronic invasive forms and difficulties associated with the coproparasitic examination.

The epidemiological surveillance aims at the detection and supervision of Strongiloidosis cases in the territory by identifying the time and space of spreading, determining risk factors, assessing the efficacy of the measures performed, prognosis of the epidemiological situation, proposing prophylaxis programs. An important element is to train health workers and those working in analytical laboratories about the diagnosis of Strongiloidosis.

Preventive measures are aimed at protecting the environment against pollution with larvae, prohibiting the soil fertilization with human and animal waste; promoting education of the population regarding the epidemiology, risk factors, clinical signs and preventive measures; prophylactic examination of workers from stone mines, brick factories, sewage systems, waste water treatment plants; examining the greenhouse workers, cynologists; examination of persons directed for hospitalization; examination of persons with allergic symptoms, especially with repeated rash, uncertain eosinophilia, chronic diseases of the gastrointestinal tract, liver and gallbladder, people with HIV; examining persons (based on clinical indications) who returned from endemic areas (tropical and subtropical), foreigners arriving from endemic countries for the early detection of strongilloidosis.

Anti-epidemic measures

The case of strongilidosis is reported to the CPH.

Patients with strongilloidosis, regardless of the presence or absence of clinical manifestations, are to be treated within in-patient departments (hospitals or infectious disease units).

In case if a large number of patients are detected within a collective, the treatment is to be organized on the spot, under the supervision of an attending physician and parasitologist. It is mandatory to treat and examine all the patients. The feces eliminated by the patients during the treatment are to be decontaminated with boiling water with a 3% carbation solution (1 : 1 ratio) or lime chloride (200 g at 1 hour exposure).

The epidemiological investigation is to be carried out in the strongylidosis focus. In the outbreak all family members, as well as the neighbouring persons (especially in rural areas) who have contacted with those in the focus, are to undergo three parasitological examinations with 1-2 day intervals.

Planned examination is to be organized in communities of Strongiloidosis higher than 1% incidence. Wet cleaning of the rooms with Lugol solution is to be performed. The solution can be also used for hand decontamination.

It is necessary to comply with the security measures during the examination of the pathological samples in laboratory (using the rubber gloves). Laboratory utensils are to be decontaminated by boiling or dipping them in 5% phenol or lysol solution during 5 hours.

The polluted places are to be urgently treated with Lugol solution, in case of contamination of uncovered parts of the body, clothes or furniture with infested material.

2.2.2. Biohelminthiasis

2.2.2.1. Echinococcosis (Hydatidosis)

Short history

The disease has been known since ancient times. It was first described by Pallas (1760). The name of the parasite was proposed by Karl Rudolph (1801). The evolutionary cycle of the parasite was established by C. von Siebold (1853), Küchenmeister (1861) and R. Leuckart (1862).

The pathogen agent

The pathogen is presented by the larval form of *Echinococcus granulosus* parasite of the class *Cestoda, Taeniidae family, genus Echinococcus*. It is the smallest cestod that measures between 2 and 6 cm. It consists of the head (scolex), neck and strobila, which consists of 4 proglottids. The first and the second proglottids are asexual, the third is a hermaphrodite and the fourth – a distal one; the longest one contains a uterus in which there are from 400-800 to 2000 eggs. The adult form of *E. granulosus* parasites in large numbers (hundreds and thousands of copies) in the front third of the small intestine of dogs, wolf, jackal, fox and coyote. The parasite develops and reaches sexual maturity within these hosts. The development of the parasite in the host ceases in 2-3 months and the life span is 5-10 months. The terminal proglottids, filled with eggs, detach from the strobila into the intestine and, along with excrement, spread to the external environment. As they are able to move, they can get to the perianal folds, causing a severe itching, which makes the animal to scratch, rubbing the perianal area against the earth, grass, and thus polluting the environment including children's playgrounds with eggs.

Being ingested by intermediary hosts (sheep, cattle, pigs, camels, some rodents and even humans), hexicant embryosare, under the action of digestive juices and especially of the bile, are released from the eggs into the gastrointestinal tract, which further actively pass through the intestinal wall, reach the bloodstream and then the liver via the portal vein. Most of them remain in the liver, and those that cross the hepatic lining via the bloodstream, reach the lungs. Some excess embryos pass through the lungs and through the systemic circulation, and may locate in any organ or tissue. The embryo (oncospheres) forms characteristic mature larval hydatid cysts. The cyst may be localized in any organ of the body.

The hydatid cyst is initially univesicular (unilocular), and within 6-8 months, the vesicle becomes fertile, forming the inner membrane, where protoscolles are formed. When hydatid is ingested, protoscoleces attach to the intestinal wall and turn into the adult tenia. Their number may be quite large, as each hidatida contains a huge number of protoscoleces *(Figure 65)*.

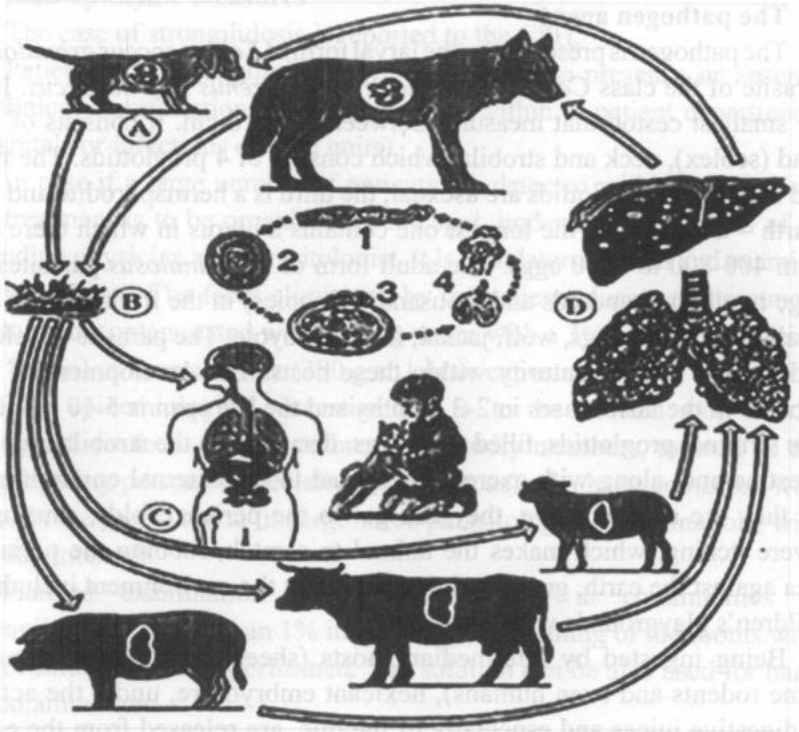

Figure 65. *The biologic cycle of E. granulosus: A –adult stage in dog's body (final host), B –polluted environment with dog's excretions, C – the larval stage in the human body and farm animals (intermediate host), D –hydatid cyst in liver and lung.*

The reservoir and the source of pathogen

The reservoir of *E. granulosus* invasion is a diseased animal of the canine family. Dogs are the main source of the disease especially in human foci and xenanthrope wild canines-in natural foci.

The transmission of the pathogen from the intermediate host to the final one occurs in various ways: the dogs are infested by food wastes from slaughterhouses, after being fed with organs of diseased animals that are slaughtered at home; or with corpses of animals.

Intermediate host infestation pathways vary as well: farm animals are infested after ingesting parasite's eggs and oncospheres by water or food contaminated with infected dog's feces. The pastoral dogs play a big role

in polluting. Shepherd dogs show the highest index of infestation, which explains the increased incidence of helminthiasis among sheep.

Humans are infested by water, fruits, vegetables, and berries contaminated with proglottids or *E. granulosus* eggs. A direct contact with the dog still remains the commonest way of human infestation, along with poor personal hygiene. Consumption of raw milk and fresh sheep or goat's cheese represents a major danger for the infestation. An experimentally proven infestation pathway is the aerogenic one.

Predisposing factors: a large number of homeless dogs; high population of cattle, especially sheep; insufficient deworming of dogs; poor personal hygiene.

Manifestations of epidemic process

Echinococcus granulosus is a parasite present on all populated continents. It is considered that trading of livestock, mainly sheep, from Europe to other countries caused the intercontinental spread of the invasion. The tapeworms are found in all climatic zones. Human hydatidosis is recognized as a major public health problem, especially for countries with intensive farm development. The most active outbreaks of echinococcosis are recorded in South American countries: Argentina, Brazil, Chile, Peru, and Uruguay. The highest rate of morbidity was reported by Uruguay - 20.7 cases per 100 thousand population. The incidence rate of dogs infected with E. granulosus is from 11.36 to 27.69% in Brazil.

Hydatidosis is also very widespread in the countries of Central and South Asia, where the incidence of morbidity ranges between 5.0 and 25.0 cases per 100 thousand population.

Hydatidosis has an uneven spreading across Europe, showing a less morbidity rate in the Northern countries. Thanks to the National Program for combating hydatidosis in Iceland, once a hyperendemic area where every 5th person developed hydatidosis, the disease has been eradicated. The last case of hydatidosis was recorded in 1960. In the UK, cases of human hydatidosis are determined by outbreaks recorded among sheep. Sporadically, echinococcosis is recorded in Poland, Switzerland, Austria and Germany. The situation is different in Southern Europe, where countries such as Italy, Greece, Bulgaria, Romania and countries of the former Yugoslavia are considered endemic and hyperendemic for echinococcosis.

For example, the incidence of human echinococcosis in Italy, especially in mountain regions, is 5.6 to 9.4 cases per 100 thousand

population, due to the intensive development of sheep breeding, large number of illegal slaughtering and homeless dogs.

Hydatidosis is considered endemic in Romania, where in 1979-1988, there were hospitalized 8557 patients with hydatid cyst,whereas the average incidence of morbidity was 5.6 cases per 100 thousand population. Echinococcosis is ranked the first among all parasitic zooanthroponoses recorded in Romania in terms of prevalence in humans and animals.

In 1980-2010 in the Republic of Moldova, 4703 persons with echinococcosis were recorded; the average incidence of the morbidity rate was 3.63 cases per 100,000 population.

Over the years, the number of echinococcosis cases is increasing *(Figure 66)*, the phenomenon determined not only by the increase of actual incidence, but also by the advance of imaging diagnostic methods and laboratory tests.

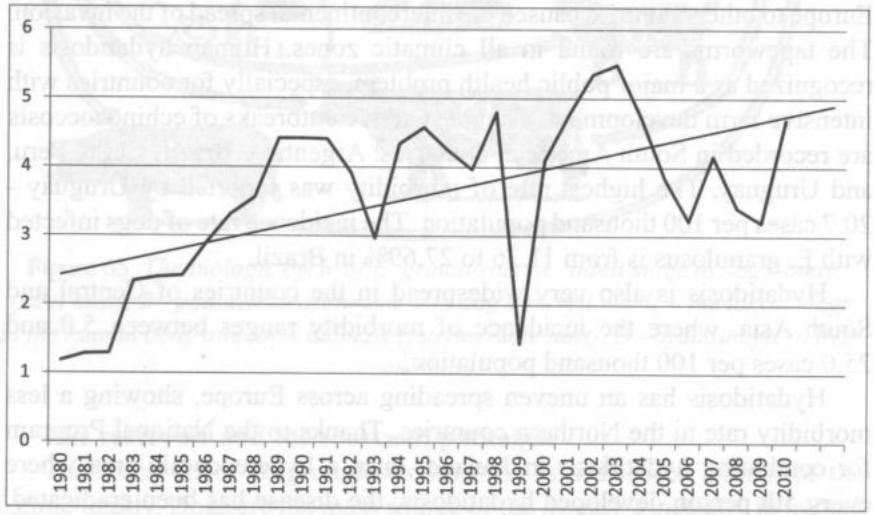

Figure 66. *Dynamics and tendency of echinococosis morbidity in the Republic of Moldova ($^0/_{0000}$), in 1980-2010.*

Although, the invasion occurs most commonly in childhood, due to the long - term evolution of the disease, the majority of cases of hydatidosis are recorded in young adults (18-30 years old) and constitute 23.9%. However, children (0-17 years) constitute in 20.1% of cases, and 24.5% – people aged ≥50 years.

Most cases of the illness, (89.7%) occur in the rural areas *(Figure 67)*, where the risk of contracting is higher.

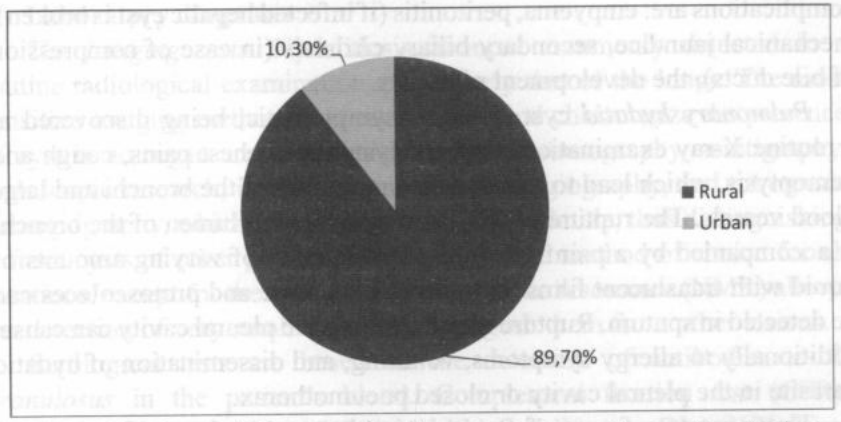

Figure 67. *The morbidity rate of echinococosis among the rural and urban population in the Republic of Moldova (in 1980-2010).*

The distribution of cases by gender shows that both men and women have the same rate, being of 50.7% and 49.3% respectively.

The frequency of outbreaks with a single case of echinococcosis is 97.7%, whereas with two or more cases – 2.3%.

A direct correlation between the number of livestock (including sheep) per capita and the morbidity index in these areas, with a coefficient of + 0.72 was found.

E. granulosus infestation can occur throughout the year and is determined by the resistance of oncospheres to environmental factors. However, there are periods with an increased risk of infestation, such as the warm season of the year, which practically coincides with the gardening, harvesting, vegetables and fruits season.

Clinical manifestations

Clinical manifestations are determined by the location and number of hydatid cysts.

Cystic liver echinococcosis is manifested by hepatomegaly - abdominal tumor formation, which is localizated in the right upper quadrant or epigastric area, causing pain associated with feeling of nausea and vomiting. The pain radiates to the right shoulder and scapula, simulating cholecystitis, in case when the cyst is located in the right lobe. Echinococci death may lead to

the development of septic or aseptic necrotic focus (abscess), accompanied by fever, increased pain, enlarged liver, leukocytosis, increased ESR. Other complications are: empyema, peritonitis (if infected hepatic cyst is broken), mechanical jaundice, secondary biliary cirrhosis (in case of compression of bile ducts), the development of ascites.

Pulmonary hydatid cyst is often asymptomatic, being discovered at a routine X-ray examination. Hydatid cysts cause chest pains, cough and hemoptysis, which lead to a massive compression of the bronchi and large blood vessels. The rupture of a hydatid cyst into the lumen of the bronchi is accompanied by a painful cough, expectoration of varying amounts of liquid with translucent films. Fragments of capsule and protoscoleces can be detected in sputum. Rupture of the cyst into the pleural cavity can cause, additionally to allergy symptoms, vomiting, and dissemination of hydatid parasite in the pleural cavity or closed pneumothorax.

There are two forms of *Brain hydatidosis* : 1. primary form occurs when the embryo is implanted in the brain by the bloodstream (it is usually solitary) and 2. secondary or metastatic form is determined by protoscoleces derived from intracardiac rupture of a hydatid cyst, rarely liver or lung (usually multiple). In primary cerebral hydatidosis the clinical manifestations vary depending on the patient's age and location of the parasite. The asymptomatic period lasts for 6-8 years in children, after which disproportionate shape of the cranium, intracranial hypertension, hydrocephalus, unilateral headache, deacreasing in the morning after vomiting occur. Gradually, new symptoms like sensory, vision, speech, balance disturbances, seizures, behavioral problems and sometimes mental changes develop. In adults brain hydatidosis, starts by sensomotory or hemiplegic seizures. The disease may evolve into a brain tumor, stroke or other clinical disorders based on the location.

Cardiac hydatidosis can cause ventricular rupture, pericarditis, cardiac tamponade with the dissemination of the parasite in other organs and tissues.

Bone hydatidosis invade the marrow cavity and gradually erode the bone, causing pathological fractures.

Spleen hydatidosis starts with a moderate pain in the left upper quadrant, which radiates into the low back or left shoulder. Lower localizations cause gastrointestinal disorders, and those located in the upper level are associated with pulmonary symptoms.

Renal echinococcosis has a slow evolution. The patients complain of a local but persistent pain.

Laboratory diagnosis

The imaging tests. Hydatidosis is more commonly detected in a routine radiological examination, particularly that of the lungs. The most sensitive imaging methods that can identify and characterize the presence of cyst in an organ are: ultrasound, computed tomography, scintigraphy, endoscopic research, intravenous cholecysto-angiography, and retrograde colangiography, which reveal more details of hydatide, thus distinguishing it from cysts of other genesis. Ultrasound is the method of choice for all locations, except for the chest. Nuclear magnetic resonance (NMR) allows the detection of very small cysts and distingush them from other lesions.

Serological tests are based on the detection of antibodies to *E. granulosus* in the patient's blood. Complement fixation test (CFR), electrophoretic method and IHAR, ELISA and Western Blot (WB) have been used in recent years. Numerous studies have been conducted and they proved that the maximum efficacy of ELISA and specificity of WB test.

Correct diagnosis can be made by detecting the parasite before the surgery.

Epidemiological surveillance includes:

- Determining the structure and density of final and intermediate hosts in the territory;
- Systematic study of echinococcosis morbidity in the human population to determine the epidemiological features;
- serological screening of high-risk groups;
- Detection of echinococcosis outbreaks and microfoci;
- Developing programs for the prevention and control of echinococcosis at different levels of epidemiological surveillance.
- **Preventive measures**

Preventive measures are aimed at interrupting the life cycle of the pathogen, therefore medical and veterinary measures are necessary to be carried out. Preventive measures include:

1. Sanitary and veterinary activities related to the final host:

- Dogs (for guarding, herding, service, entertainment) are to be registered and given a pet passport, which includes the information on investigations and treatment performed;
- Mandatory helminthological examination of all dogs with the

prophylactic deworming of animals affected by E. Granulosus should be carried out once every 45 days during the period of December to April and once every 30 days from May to November, whereas the hunting dogs are subjected to compulsary deworming at the biginning of the hunting season and monthly throughout the hunting season;

- Reduce the number of homeless dogs;
- Prevention of dogs' presence in the territories of slaughterhouses or cemetries;
- Prevention of feeding the dogs with slaughter waste, including hunting remnants, without being investigated to larval tapeworms or being thermally processed.

2. Veterinary activities related to intermediate host:

- slaughtering only in special areas;
- Ensure the control of the meat and the destruction of the intestines of the slaughtered animals in biothermal or burning pits;
- Ensuring the sanitary-hygienic conditions control within livestock units.

Medical activities:

- Health education of the population and among risk-related groups, especially in endemic areas. It is necessary to inform of the risk factors of dog cohabitation, consumption of unprocessed vegetables, strict personal hygiene.
- Periodically screening (annually) of high-risk groups through the use of imaging and serological tests for early detection of the disease;
- Initiating and implementing campaigns to combat the pathogens in dogs and humans.

Anti-epidemic measures:

- Notification of echinococcosis cases (performed by a family doctor or surgeon to the place of patient's residence) through the Automated Information System and filling in the urgent notification of cases of infectious disease (form 058/e), case registration in the Register of Infectious Diseases Records (form 060/e);
- Follow-up of a patient for a period of eight years with clinical and laboratory examinations every two years.
- Epidemiological investigation of the outbreak along with the veterinary service, by means of identification, investigation and supervision of people at risk of contracting echinococcosis.

2.2.2.2. Teniarinchosis

Short history

In 1782, Goeze distinguished *Taenia saginata* from *Taenia solium*. In 1863, Leuckart proved cattle to be intermediate hosts for *Taenia saginata*.

Taenia saginata pathogen (Taeniarhynchus saginatus) belongs to the *Taeniidae family*, *Cestoda* class, such as *Taenia* tapeworms, which is of 4 to 12 m long.

Biological cycle

T. saginata parasitizes in the human body only in the adult stage, with localization in the intestine. The hydatid larva develops in the adult within 7 months of infection. Strobila consists of 500-2000 segments (proglottids). Each proglottis contains both female and male sexual organs, which are responsible for producing eggs. Elimination of final proglottids begins when strobila is 6-7 m long, about 3 months after infection. Proglottids are actively detached, one by one, and move to the anal sphincter, being discharged together with the feces. Under the action of physical and chemical factors, proglottids are destroyed in the environment, whereas the eggs together with water, grass or other forage enter the digestive tract of an intermediate host (cattle), where they migrate through the blood vessels and lymphatic tissue to the interfascicular muscle, being localized mainly in the most active muscle (masseter, tongue, infarction, etc.). Furthermore, they turn into cysticerci (Cysticercus bovis) within 10-70 days, which are vesicular larvae with a diameter of 2-15mm, containing invaginated scolex for the future tapeworms. Cysts remain viable for 1-2 years and more. Following human ingestion of the infected meat, the cysts get into the duodenum, where the invaginated scolex comes out and attaches to the mucosa of the intestine. It develops and grows into an adult tapeworm *(Figure 68)*.

In some cases, humans may serve as an intermediate host for T. saginata, the disease being called cysticercosis (see Teniasis).

The reservoir and source of pathogen

The source of pathogen is almost exclusively found in humans, who are the definite hosts and cattle - intermediate ones.

The source of the pathogen is a sick man, who is a carrier of the symptomatic and asymptomatic forms of T. saginata adult specimens. Persons who take care of animals present a specific risk in transmitting the invasion.

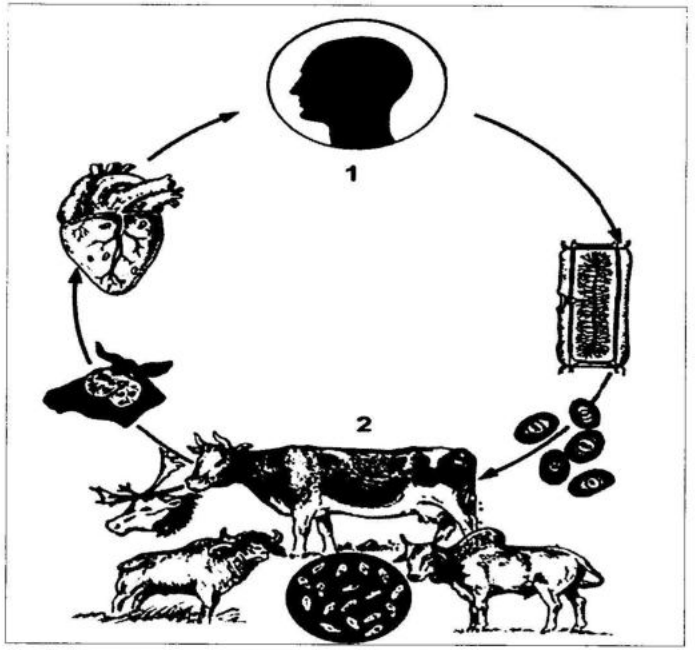

Figure 68. *The biologic cycle of Taenia saginata (N.I. Briko et al., 2013).*

Factors and transmission routes

Transmission from humans to cattle occurs via food or water, grass or animal food, contaminated with human excrement that is infested with proglottids of *T. saginata* or eggs. The transmission may also occur by direct human contact with the cattle-carrier of T. saginata, particularly in calves during the animal care.

Invasion transmission from cattle to humans occurs exclusively by insufficiently cooked or roasted meat, infected with cysticercus.

Predisposing factors: insufficiently cooked meat; a habit of tasting the meat before cooking; low maintenance and hygienic standards during the animal care; low quality of veterinary check-ups.

Manifestations of epidemic process

It is commonly found in the Russian Federation, the Caucasian countries, Mongolia, China, Europe (Bulgaria, Poland, Romania, etc.). In the Republic of Moldova sporadic cases of teniarinchosis have been recorded.

Teniarinchosis is more common among rural population and in districts with high intensity of cattle growing. Receptivity is general, but the adult population is commonly affected. The most-at-risk population includes workers of the slaughterhouses, the livestock farms, people who deal with cooking. Seasonality is determined by the periods of mass slaughtering of the animals.

Clinical manifestations

The disease is clinically manifested by dyspeptic disorders, abdominal pain, nausea, vomiting, intermittent diarrhea, weight loss despite an increased appetite, anemia, inconsistent eosinophilia, apathy, vertigo, depression and even epileptic seizures. Biliary and appendiceal colics may occur due to the parasitic toxicity.

Laboratory diagnosis is made based on the evidence of proglottids or eggs. The parasitological diagnosis is not useful in the first 3 months of infestation due to the absence of proglottids. Hypereosinophilia is characteristic of this phase, which drops to the normal level after the first proglottids elimination. The diagnosis is easir after the tapeworm matures. Tapeworm proglottids, which are daily eliminated can be easily seen on the patient's bedding. Samples for the laboratory examination are collected by scraping of the perianal creases, where a significant number of eggs - oncospheres remain stuck.

Preventive measures

A close collaboration between veterinary and medical services is required to increase the efficiency of the preventive measures.

Veterinary measures:

- mandatory veterinary expertise of bovine meat, mainly from individual households. The infestation is considered weak when there are not more than 3 cysticerci on an area of 40 cm2. Meat can be consumed only after sterilization by freezing or heating. Meat is considered unsuitable for use, if more than 3 cysticerci are found on the same surface;
- creating proper hygienic and sanitary conditions on livestock farms.

Medical measures:

- active diagnosis and treatment of patients;
- regular parasitological control of workers in livestock farms;
- education of the population, emphasizing the importance of avoiding the contamination of the environment with eggs, as well as

excluding the consumption of food of an unknown origin, which is either insufficiently cooked or infested.

Anti-epidemic measures

The complex of anti-epidemic measures in teniarinchosis includes:

- notification of the teniarinchosis case to the Center for Public Health;
- deworming of sick people within in-patient departments to produce a complete elimination of the parasite and avoid the contamination of the environment with proglottides. Feces are decontaminated with boiling water;
- follow-up supervision of persons during a year and regular parasitological control;
- carriers of T. saginata are not allowed to work at cattle farms.

2.2.2.3. Taenia solium infection (Teniasis)

Short history

In 1865, Leuckart described the life cycle of Taenia solium, which proved that the larval stage of the parasite present in the pig muscle is infectious to humans.

The etiologic agent of taenia solium belongs to the same class of Cestoda as T. saginata.The parasite is 3-6 m long. The evolutionary cycle is similar to that of T. saginata except for the intermediate hosts for T. - the pigs.

Humans are the final hosts. The parasite becomes an adult after two months from infestation and when the last proglottids (5-6) are detached and passively eliminated in the external environment with the feces. Each proglottid contains 30-50000 eggs, with oncospheres. These are infested forms that, in order to continue the life cycle of the parasite, need to be ingested by intermediate hosts such as pigs or wild boars.

The proglottis membrane is dissolved in the digestive tract under the action of digestive juices of intermediate host and then embryos penetrate the intestinal mucosa, blood vessels and lymphatic system, then get into the interfascicular muscle tissue of various organs (brain, eye, etc.), where within 3-4 months they grow in cysticerci (hydatidic larva), *Cysticercus celulosae.*

The infestation of pigs with Cysticerci is asymptomatic. The infected meat, consumed by humans, practically initiates a new evolutionary cycle of T. solium

Cysticercosis. Both T.*saginata* infection and T.*solium* infection (common) use humans as intermediate hosts. The phenomenon occurs more frequently in carriers of tapeworms, when the mature proglottids of strobila reach the duodenum by vomiting or duodenal peristaltic movements, where under the action of digestive juice, oncospheres are released from eggs and penetrate the intestinal mucosa and then through the bloodstream reach interfascicular muscle tissue in the eye and the brain, where they develop into cysticerci (self-infestation).

Infestation, however, can occur as a result of poor personal hygiene. The humans, who carry the adult forms of tapeworms, may become self-infested by contaminated hands with the eggs of tapeworms, directly from the perianal areas, or indirectly through the consumption of fruit and vegetables contaminated with the eggs of the parasite.

The reservoir and source of pathogen

Humans are the reservoir of the parasite in nature, as definitive host, and pigs - as intermediate hosts. The source of pathogen is considered to be both the diseased individuals, carrying the adult forms of T. solium and the infested pigs.

Factors and transmission routes

The pigs are infested by ingesting feces containing human T. solium eggs.

Humans contract the disease exclusively by consuming infected pork, which is insufficiently cooked or smoked. The chefs and housewives can infest themseves by tasting raw minced meat.

Manifestations of epidemic process

Tenia solium infection is widely spread in all the regions of the world, where people eat pork. There are three main foci of *T.solium* infection, the level of damage to pigs is 1.5-2.0% and higher:

1. Asian focus – the most immense one (China, India, Indonesia, Philippines, South Korea, Laos, Taiwan). In the provinces of China, for example, pork invasiveness reaches 10-20%

2. The countries of Latin America (Mexico, Guatemala, Nicaragua, Honduras, El Salvador, Colombia).

3. African focus (Cameroon, Madagascar, Kongo, Nigeria).

The spread of teniosis is sporadic in Europe, including Moldova.

The responsiveness is general, although, adults get infected more often as a result of consumption of insufficiently cooked meat products, snack chips and raw meat.

Clinical manifestations

Teniosis-diseased patients complain of abdominal pain, nausea, vomiting, diarrhea alternating with constipation, loss of appetite, anemia, weight loss and nervous system disorders (apathy, headache, dizziness, irritability, and insomnia). The alergic signs are pronounced. However, cysticercosis is the most serious complication of teniosis. *Muscle cysticercosis* is characterized by intensity of fluctuating muscle pain according to the number and degree of larvae spread, which often may be confused with rheumatic disease. Ophthalmic cysticercosis is found in serious sight disorders (the patient sees sparks and destorted outlines); the visual acuity gradually decreases intil its total loss.

Cerebral cysticercosis is manifested by fever, headache and swelling due to the absorption of toxins released by the parasite, which may result in autolysis of oncospheres, as well as the development of encephalitis or meningitis.

Subcutaneous cysticercosis is recognized by the presence of cysts of ellipsoidal shape with the size up to 10 mm, which are palpable and slide under the pressing finger.

Laboratory diagnosis

The diagnosis is made based on the detection of T. solium proglottids in feces, which need to be differentiated from those of T. saginata, being assessed by the number and type of uterine branches. T. solium eggs are similar to those of T. saginata, but they are rarely detected in the feces.

The primary diagnosis of cysticercosis can be confirmed by biopsy. Configurations of the larvae can be easily diagnosed by radiologic and imaging tests. Ophthalmic cysticercosis diagnosis is based on the fact that the larvae attach to the posterior chamber of the eye. When administering adrenaline in the eye, in addition to pupil dilation, there may be observed how the scolex leaves the vesicle by means of ophthalmoscope.

Preventive and anti-epidemic measures: see Teniarinchosis. Pigs are the specific intermediate hosts in teniosis.

2.2.2.4. Trichinosis

Short history

Human trichinosis was first described by J. Tiderman (1821) in Germany and by A. Peacock and I. Owen (1835) in the UK, who revealed the larva secluded in the infested human muscles. The evolutionary cycle

was described by R. R. Leuckart and Virchow in 1855-1859, independently of each other.

The pathogen belongs to the trichinellidae class, Trichinella nematode (from the Greek *Thrix-trihnos* – hair). It includes five species: *Trichinella spiralis, Trichinella native, Trichinella britovi, Nelson Trichinella, Trichinella pseudospiralis.*

T. spiralis,which is the most widespread parasite, is a small, white parasite barely visible with the naked eye. The male species measures 1.5 × 0.04, and the female one 3-4 × 0.06 mm. Both stages of the parasite-adult and larva develop in the same host. The adult species is localized in the small intestine of the humans, pig or rodents, and survives for several weeks. The male species lives for about a week and dies after fertilization, whereas the viviparous female species produces about 500 larvae in 2 weeks, then it is eliminated with the feces. The larvae reach the skeletal, diaphragmatic, laryngeal, ocular, intercostal, masticatory muscles and even the adipose tissue, where they begin to develop and grow in length within 17-18 days; they twist to form spirals. Within 2-3 months, a capsule is formed around them, which is impregnated with calcium salts in 6 months.

The calcification process ends about two years after the infestation and does not affect the viability of the larvae. There have been cases where larvae retained their viability and pathogenicity for 20 and even 30 years. Being ingested by humans, when eating raw or undercooked pork, in case when pigs ingest infested rats, the larvae reach the stomach where, under the action of gastric juice, the larvae are released and reach the small intestine. Here, within 2-3 days, the larvae reach sexual maturity, resuming the biological cycle.

The reservoir and sources of pathogen

In home conditions, the pathogen reservoir is represented by pigs, rats, cats, dogs; boars, bears, wolves, minks, weasels, beetles and other carnivores - in natural conditions; the main source of human infestation being the slaughtered pigs which have not undergone the vet control, rarely wild boars or bears may be the source of infection for humans.

A man can serve as a source of invasion when a human corpse with T. spiralis is devoured by rats or other wild animals.

Ways and factors of transmission

The transmission of the parasite from animals to humans occurs via the ingestion of raw or insufficiently processed pork, wild boar or bear (bacon,

ham, and other roasted smoked or salted meat, etc.) containing live larvae T. spiralis (trichinated meat).

Predisposing factors: the widespread consumption of pork or boar; unsatisfactory hygienic standards of the pig farms; high population density of rats within the pig farms; food traditions of using raw or smoked meat; and unsatisfactory vet control measures for meat products.

Manifestations of epidemic process

Trichinellosis is a widely distributed helminthiasis around the world, forming human foci, maintained by domestic (pigs, dogs, cats), synanthropic (rats), and natural animals(xenanthropic carnivores).

It is commonly found in Eastern Europe, throughout the Russian Arctic regions in the Eskimos (the incidence constitutes 50%), Asia, South America and Eastern Africa.

The trichinosis outbreaks are recorded in Belarus, Lithuania, Ukraine, Romania and Moldova. Trichinellosis is recorded more frequently in pig farms. The epidemic process is manifested both sporadically and eruptively or even epidemically among the hunters and their families.

Responsiveness is general, being more frequent in adults.

Seasonality is characteristic of eruptive epidemic (group) outbreaks. The human outbreaks more commonly occur in the autumn-winter months (the period of mass slaughtering of pigs), and the natural foci are related to the hunting season.

Clinical manifestations

The severity of clinical manifestations depends on the number of larvae ingested and the immunological status of the host. There are several phases of the disease evolution. The intestinal phase is characterized by diarrhea, nausea, vomiting, abdominal pain. These symptoms occur within 1-2 days after eating meat. The dissemination phase of the larvae in the blood, which lasts 7-10 days, is manifested by fever, facial and eyelid edema, and eosinophilia up to 50-80%. The long-lasting tissue phase of larval seclusion into the muscles is accompanied by functional myalgia, adynamia, sometimes by such serious complications as encephalitis, myocarditis and pneumonia.

Laboratory diagnosis

In chronic forms, when the symptoms of the disease are not typical, a serological or parasitological laboratory diagnosis is required. Anti-Trichinella antibodies appear in the blood 2-3 weeks after the infestation

and persist for many years. The detection of antibodies, in the presence of clinical symptoms, enables the diagnosis to be made.

During the localization of larvae in the muscle an accurate diagnosis can be made by identifying them in muscle biopsies (deltoid or soleus).

Preventive measures

The preventive measures are general and consist of strict veterinary control of pork (domestic or wild), mainly by trichinoscopic examination (the detection of a single, free or encapsulated larvae leads to the withdrawal of the meat from consumption, regardless of its macroscopic appearance); mass deratization procedures carried out at pig farms (4 times per year) with the immediate afterward collection of rat corpses; installation of new intact storehouses for collection and storage of the dead rats, withdrawn products and wastes of slaughtered animals; public education (underlining the danger posed by the consumption of illegal meat) and training of the veterinary staff. The most effective measure of individual prophylaxis is to consume pork, wild boar or beef only after being thermally processed,cooked or roasted at the temperature over 77°C orit being kept in freezing conditions (minimum 8 days at -30°C).

2.2.2.5. Diphyllobothriasis

Short history

The parasite was described and classified for the first time by K. Lyunei in 1778. The epidemiology and the biological cycle were studied by M. Braun (1883) and I. Ianitschi (1917).

The pathogen *Diphyllobothrium latum* (synonymous of Bothriocephasus latus) is a cestode with a length of 2 - 9m (it can reach up to 20m long) in humans and about 6m in dogs, being the longest tapeworm. The final hosts are humans, dogs, cats, sometimes pigs; wild animals – fox, mink. The mature parasite lays eggs through the uterine orifice, located in the anterior part of the proglottids. The eggs, which are eliminated with feces, develop and ultimately give birth to adult specimen that further require an aquatic environment (running water, lakes) and release ciliary hexactic embryos (oncospheres), which afterwards must be swallowed in 1-12 days (during its lifespain) by an intermediate host crustacean, the intestine of which turns these into larvae in 2-3 weeks. They are called procercoids. Crustaceans are swallowed by various species of fish, such as pike, perch, trout, etc., which become additional intermediate hosts. In the stomach of the fish, the prokaryotes are released by the action of the gastric

juice and penetrate the wall of the intestine, invading the muscle tissue and the internal organs (especially the liver and ovaries), where within a few weeks they turn into plerocercoids - infested vermiform larvae.

Plerocercoids, being ingested by humans or animals, grow further into adult parasites within the small intestine *(Figure 69)*.

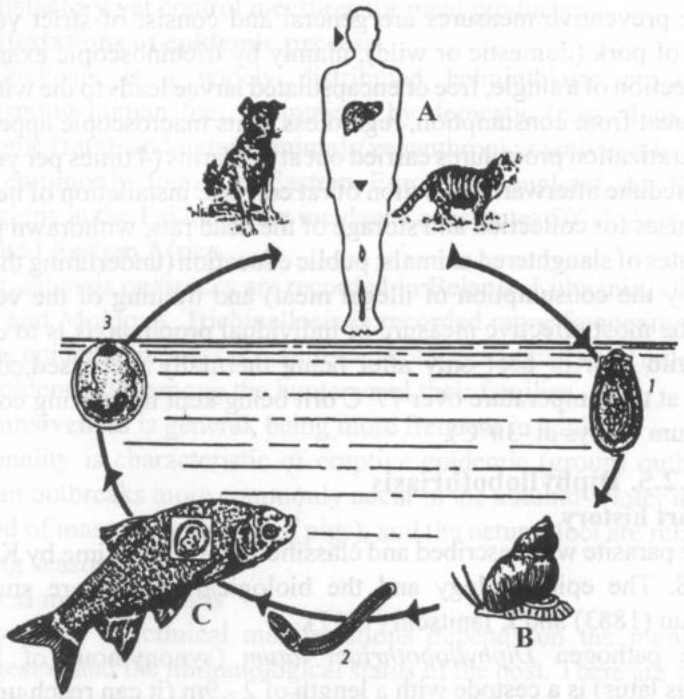

Figure 69. *The biologic cycle in Diphyllobothrium latum (N. I. Briko et al., 2013).*

The reservoir and sources of pathogen are represented by both the final hosts – humans, dogs, cats and other animals feeding on fish, and intermediate hosts - crustaceans and fish in which mandatory metamorphosis stages of the parasite occur.

Modes, routes and factors of transmission

The humans ingest invasive larvae, together with various fishery products (eggs, especially of pike, smoked or semi-processed fish). These are most commonly found in the genitals, liver, and muscle tissue of the intermediate host, which resist both freezing and salting.

Dogs, cats and other animals can be infected by consuming fish or wastes from canning factories and from the kitchen, with plerocercoids.

Manifestations of epidemic process

Diphyllobothriasis is common among the population who live on the banks of the large rivers, natural lakes and reservoirs. Endemic foci of Diphyllobothriasis have been reported in several European countries, especially in the areas of rivers and lakes on all the continents. In Romania, the Danube delta is considered endemic and in Moldova - the South (the basin of Lake Manta).

The receptivity is general, but it is more commonly found in adults. Infestations more often occur during the summer-autumn months, being related to the fishing season.

Clinical manifestations

Due to its mechanical, toxic-allergic and debilitating actions of the adult parasite, cause disorders, manifested by a pronounced anemia, loss of appetite, and dyspeptic phenomena, which are accompanied by diffuse abdominal pain, constipation or mild diarrhea, flatulence and salivation. Furthermore, glossitis appears which is manifested by red painful spots and small cracks. These are spread over the entire mouth, glottis and esophagus, and make the patient feel burns on the tongue, especially when the food is salty or sour, and pain while digesting the food. The temperature is usually subfebrile and,in multiple invasions, it may occasionally reach 38°-39°C. Diphyllobothriasis is also associated with apathy, edema, spleno- and hepatomegaly.

Laboratory diagnosis

The etiological diagnosis is made by the detection of the presence of strobil fragments in the feces and by identification of eggs of D. latum in the feces (and by direct smear as well). Proglottids are identified by their characteristic form and shape (large) and speckled appearance of the uterus and eggs. The haematological examination may reveal a significant decrease in the number of the red blood cells, which, unlike pernicious anemia, is accompanied by the presence of gastromucoprotein in the stomach juice.

Preventive measures

The complex of prophylactic measures in Diphyllobothriasis includes:

- the destruction of copepod crustaceans – intermediate hosts of the parasite (using molluscids);

- veterinary control of fish and fishery products; prohibiting the consumption of fish and fishery products (eggs and smoked pike fish);
- regular investigations of the workers from fishery households for the detection of D. latum carriers;
- Periodic anthelmintic treatment of the final hosts within the endemic areas;
- no feeding of dogs, cats and pigs with fish and fishery wastes;
- arrangement of the sanitary units and closing of those with a leakage to the running waters or lakes. The emergency room of boats or liners are provided with devices to ensure the sterilization of feces with antiseptics (chlorine lime, etc.);
- inactivation of sewage and wastes from industrial fishery enterprises;
- raising health education of the population, especially in endemic areas;
- sufficient heat processing of the fish products.

Anti-epidemic measures

The complex *Diphyllobothriasis* anti-epidemic measures includes:

- mandatory notification of a *Diphyllobothriasis* case to the public health system;
- epidemiological investigation of the outbreak;
- diphyllobothriasis-diseased patients undergo the in-patient treatment in hospitals, with mandatory decontamination of the feces;
- follow-up of the former carriers of D. latum during 6 months after the treatment, with periodic helmintocoprologic investigation;
- the non-admission of D. latum carriers to fishery activities.

2.2.3. Contagious helminthiases

2.2.3.1. Enterobiosis

Short history

The disease has been known since ancient times. The pathogen was described and classified for the first time by K. Linney in 1758. The life cycle of the parasite was described by R. Leuckart in 1865.

The pathogen Enterobius vermicularis (the old name – Oxyuris vermicularis) belongs to the Nematoda class, Oxyuridae family. It is a small cylindrical worm (female – 9-12 mm, male – 3-5 mm), very mobile, which habituates in the caecum. The oxyur is a monoxenous parasite, which develops only in one host - the humans. The mature female contains

10-12,000 eggs in the uterus, which are lodged in the perianal and perineal region in the night, where maturation takes place in 4-6 hours. The eggs are resistant to the external environment. The contaminated clothes and the bed linen remain infested for 3 weeks.

Being ingested by humans, including the same host, the infesting eggs hatch into the stomach and duodenum, giving rise to rabidito larvae that migrate to the ileocelia where they become adults (10-12 days). The total time is 15-43 days from the ingestion of the eggs until the sexual maturation of the pinworms. The life span is 3-4 weeks. After copulation, the males die in the ileocecal region and are eliminated with feces, while females detach from the colonic mucosa and actively migrate to to the anal region (perianal and perineal sometimes), then lay the eggs and later are eliminated.

The reservoir and source of pathogen

Enterobiasis is a typical anthroponosis. Humans are the only hosts of E. vermicularis, therefore the only source of pathogen is the diseased person.

Mode and routes of transmission

The mode or mechanism of transmission is typically fecal-oral. The main factors of transmission are the hands contaminated with pinworm eggs from the perianal area. The elimination of the female parasites through the anus produces nocturnal irritation and the inflammation of neighboring tissues (sometimes the pinworms also reach the vulva, causing pruritus and vaginal discharge), which make the patient scratch, thus leading to the presence of parasite eggs under the nails and on the fingers. Thus, the person infected with pinworm eggs may transport these from the perianal region into the oral cavity, either directly, from the fingers or indirectly, by contaminating different objects or food. Frequent self-infestation in children explains the massive and sometimes chronic infestations with pinworms (autoinfection). Hand contamination can also occur by sharing towels, pajamas or bed linen contaminated with pinworm eggs. Infestation occurs when eggs are ingested directly through dirty hands and indirectly through contaminated water and food.

Manifestations of the epidemic process

Enterobiosis is the most common helminthiasis and represents a global public health problem. It is found anywhere, but with a higher incidence in moderate climates, including highly developed countries. About 300 mln.

people are affected worldwide. In the Republic of Moldova the incidence of enterobiosis among general population varies between 400 and 500 cases per 100 thousand population, and is ranked first among other human helminthiases. The incidence rate of enterobiosis is 75-78%. Receptivity is general, but children are more affected. The incidence by enterobiosis reaches 20-21 cases per 1000 children among those aged 0-17 years, and 40-47 cases per 1000 children among those aged 3-6 years *(Figure 70)*.

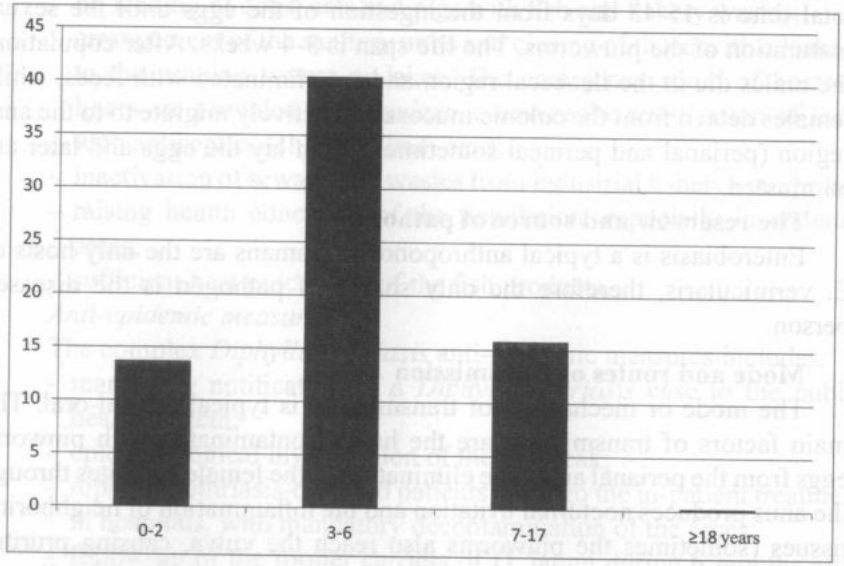

Figure 70. *The incidence rate by enterobiosis according to the age group (‰).*

In general morbidity by enterobiosis the rate of children aged of 0-17,, is 95.2% compared to 4.8% in the adult population.

The incidence of enterobiosis is higher in rural population, with an annual average of 598 cases per 100 thousand population, compared to 290 cases per 100 thousand population from the urban areas. The percentage share of morbidity by enterobiosis is 75% in rural population and 25% - in the urban one *(Figure 71)*.

This phenomenon can be explained by a low level of both hygienic conditions and habits of rural population, including that at school and pre-school institutions.

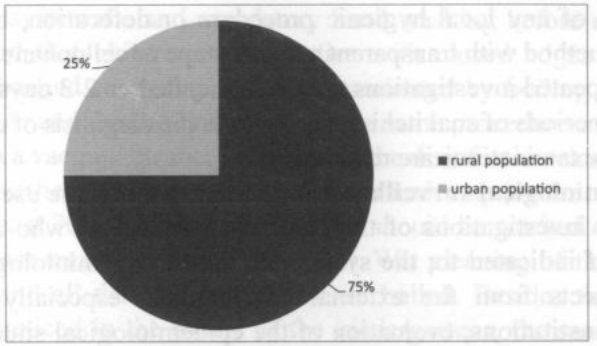

Figure 71. *The structure of the enterobiosis morbidity rate according to the residence of the Republic of Moldova.*

The incidence is higher among institutionalized children, especially in overcrowded collectives that are poorly sanitized, thus creating favorable conditions for the parasitic transmission via a casual contact. The level of involvement of children can reach 20-30% within these collectives. Pinworm eggs are found throughout the human body, especially in perianal zones, hands and various objects of environment (linen, floor, tables, toys etc.), which may lead to an intense repeated infestation among children, family and institutional outbreaks that may be long-lasting if no preventive measures are carried out.

Clinical manifestations

Symptomatic forms are manifested by intense, nocturnal, anal or perianal pruritus, associated with diarrhea, abdominal pain (in the caecum region), nerve disorders. Children suffering from severe infections may experience sleep disturbances, irritability, and nocturnal teeth grinding.

At the cutaneous level, many scratching lesions can be observed around the anal orifice, which, as a result of the microorganism's penetration, can spread through and produce linear dermatitis or even perianal abscesses requiring surgical treatment.

In girls, the parasite can cause vulvo-vaginitis, and in adults, appendicitis and prostatitis.

Laboratory diagnosis is not complicated to perform. Patients themselves can see and recognize the parasite. The parasitological diagnosis is based on the detection of eggs in perianal lavage or fingerprints. The eggs are collected at the anal margin, preferably in the morning, after waking

and before of any local hygienic procedure or defecation, by using the Grahman method with transparent adhesive tape or cellophane. In negative reasults, repeated investigations are recommended on 2-3 days, commonly during the periods of anal itching. To exclude the diagnosis of enterobiosis, 7 repeated examinations are required.

Epidemiological surveillance is directed towards the use of selective or planned investigations of the population and those who address to a physician if indicated to; the systematic sanitary-helmintological control of the objects from the external environment, especially within the children's institutions; evaluation of the epidemiological situation and of the undertaken measures, developing of the control programs.

Prophylactic measures

Prophylactic measures in contagious helminthiasis, transmitted from human to human, are directed to neutralizing the pathogen source and interrupting the transmission mechanism. These include:

- detection and treatment of the patients;
- preventing (not admitting) the environmental pollution with helminth eggs;
- respecting the strict sanitary and hygienic rules within the rooms;
- respecting the personal hygiene standards (get rid of the harmful habit of introducing fingers in the mouth; systematically hand washing with soap before and after the meals, cutting the fingernails, cleaning daily underwear and bed);
- respecting the hygienic regime in children's collectives;
- promoting hygienic education, especially among children. A systematical training of the hygiene skills among children is required.

Taking into account the increased incidence by enterobiosis among preschool children and primary school pupils, sanitary-hygienic measures should be a priority in children's institutions, boarding schools, orphanages, and general elementary schools.

Due to the intensive removal of parasites in 2-3 days after treatment, a cotton wool pad immersed in vaseline is recommended to be applied on the perianal region to children during the night, which prevents the elimination of the pinworms from the anal orifice, thus preventing the spread of the eggs. Hygienic measures are also applied by washing of the perianal and buttocks region with water and soap, in the morning and evening,

nail cutting, systematic and mandatory hand washing before meals. It is recommended that children wear panties with closed edges overnight, which are changed every day and decontaminated by boiling or ironing. The underwear and the bed linen undergo the same procedures. Carpets are cleaned with a vacuum cleaner or treated in the disinfection chamber. Toys that can be washed are treated with hot water and soap (water temperature must be at least 70°C) and the doll clothes should be washed and ironed. The potties are treated with boiling water. Wet cleaning of the rooms is carried out and all the cleaning cloths are boiled. Besides the hygienic measures practiced in the rooms of the kindergartens, the sand from the sandboxes is changed systematically or treated with boiling water.

2.2.3.2. Himenolepidosis

Short history

Dwarf tapeworm was first described by T. Bylharëh (1851) and V. Afanasiev (1890) and finally systematized by Blanschar P. (1891).

Pathogen agent

The pathogen of hymenolepisosis *Hymenolepis nana* is a small-sized tapeworm, whose length does not exceed 3-5 cm. Helminth H. nana represents a helminth with a simplified cycle of development, found in the human body only, which is both intermediate and definitive host. Parasite eggs are eliminated in the external environment with feces. Being ingested orally, the oncospheres hatch from the eggs into the small intestine, penetrate into the mucosa and develop up to the stage of cysticercosis. Within 5 to 7 days, cysticercids migrate to the intestine lumen, then fix to the mucosa, usually in the lower part of the small intestine, where it develops into an adult specimen. The development cycle lasts for a month.

The reservoir and the source of pathogen

The reservoir is represented by human population, whereas the source of the parasite being the diseased person.

Mode, factors and routes of transmission

The transmission of H. nana eggs from the diseased to the healthy man takes place via the fecal-oral mechanism. Ingestion takes place orally following consumption of fruit, vegetables, infusions, infested water, dirty hands, soil, infested toys, night pots, door handles and other household items infected with H. eggs nana.

Flies may also serve as mechanical transmitters. The eggs are stored from a few minutes to 3-4 weeks in the external environment. In

himenolepidosis, transmission is largely due to direct contact with the patient.

Manifestations of epidemic process

Himenolepidosis is widely spread, mostly in tropical and subtropical climatic countries and in populations living in overcrowded or non-hygiene conditions. Although receptivity is general, the invasion is more common among children *(Figure 72)*.

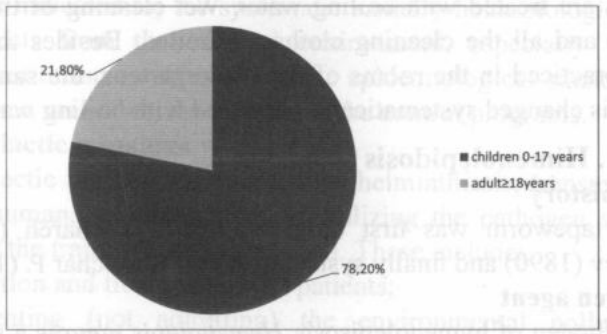

Figure 72. *The himenolepidosis morbidity rate in children aged of 0-17, in the Republic of Moldova, in 2000-2013.*

In the Republic of Moldova, the incidence of himenolepidosis varies between 1.07 and 5.81 cases per 100 thousand populations. The dynamics of morbidity is characterized by periodicity and a decreasing tendency *(Figure 73)*.

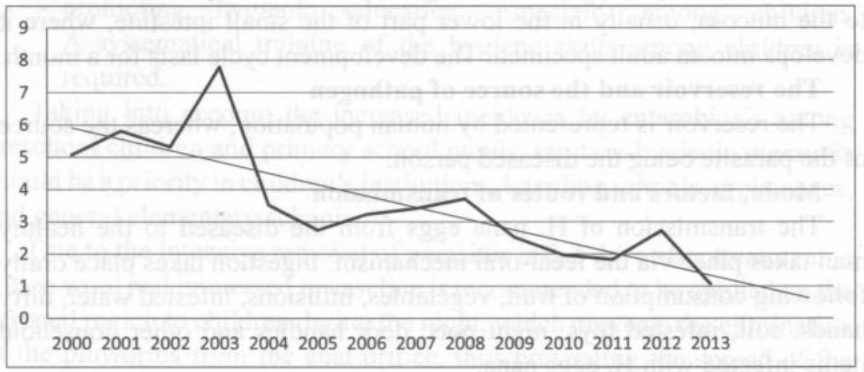

Figure 73. *The dynamics and tendency of morbidity by himenolepidosis in the Republic of Moldova, in 2000-2013.*

The incidence is higher among the rural population and children from institutions, particularly in overcrowded and poorly sanitized ones.

Clinical manifestations

The clinical manifestations of himenolepidosis are polymorphic, being prevailed by dyspeptic syndrome. In the pathogenesis of himenolepidosis, an important role is posed on the mechanical damage caused by both the larvae and the mature parasites upon the walls of the small intestine, which leads to the necrosis of the mucosa and the formation of the ulcers.

Patients complain of nausea, loss of appetite, vomiting, heartburn, dull pain in the abdomen, sometimes with seizures, diarrhea, often with mucus and rarely with streaks of blood, which requires differentiation from other forms of dysentery. Patients present general weakness, malaise, periodical anxiety, poor physical and mental development compared to their peers, epileptiform siezures, twitching, and loss of consciousness. Skin rash and itching may develop in some patients. One-third of those infected with himenolepidosis do not present clinical signs.

Laboratory diagnosis

H. nana eggs are easily destroyed in the external environment, thus the parasitic diagnostics require fresh faeces. Under these conditions, the diagnosis of himenolepidosis is not a problem. The diagnosis is based on the detection of H. nana eggs in the patient's faeces.

As regarding the cycle of parasite egg removal, 3 investigations within 2-3 days intervals, are recommended.

Prophylactic and antiepidemic- measures - see Enterobiasis.

B. EPIDEMIOLOGY OF NONCOMMUNICABLE DISEASES

1. Epidemiology of Cardiovascular Diseases

Introduction

Cardiovascular diseases (CVD) was the biggest „killer" of the XX century and remains a major public health problem. This issue is based on data recorded on mortality and morbidity level. Another argument is based on the consequences that CVD produce at the individual level (DALY: Disability-Adjusted Life Year) and at the community level (economic disruption and social) (1, 2).

CVD Group is characterized on various clinical forms, the most prominent is: high blood pressure (hypertension), coronary artery disease, including ischemic heart disease (ID), cerebrovascular accidents (CVA) and other cardiovascular diseases. The complexity of clinical manifestations and long survival of those suffering from these diseases oriented towards all research to improve the possibilities to detect, treatment and optimize the quality of life of those who suffer of this disease (3). Also, a priority in medicine was to identify all risk factors involved in the producing these diseases and find methods of detection and early neutralization. Observational studies of Framingham have brought numerous information on the association of multiple factors. Currently, more than 200 of such factors are known, based on individual or environmental conditions. According to the theory of epidemiologic transition, launched by Abdel Omran, this stage (fifth) is dominated by diseases that are called „of civilization". CVD followed by cancers, diseases of metabolism and psychic prevail (4). In many countries the prevention of these diseases is a priority of health policies, both being driven by professional, governmental, and patient organizations., a significant progress in CVD prevention programs was implemented by knowing the components of the epidemiological process. That were applyied to the risk population groups, very young ages. It leads to the significant results by the reducing the impact of these diseases (5).

Short history

In 1768 William Heberden first describes the clinical form called, angina', and in the XIX century was set aut the clinical anatomical concept.

Scarpa A. (1804) and Johann Lobstein (1833) define the concept of „coronary artery atherosclerosis" and Rudolf Virschow (1856) – on the „thrombosis". 1910 W. Obrastzow and N.D. Straschesko (Russia) and then in 1912 Herrick (USA) introduced the concept of clinical „myocardial infarction". The different clinical forms (angina and myocardial pectoris) correlate with changes in the 50 anatomic and clinical, atherosclerosis" and „thrombosis". In the same period there are conducted prospective epidemiological studies (studies of Framingham). 6,000 volunteers were enrolled in a population study that was consist of 10 thousand of men and women. There were supervised every 2 years to record new cases of disease caused by atherosclerosis (coronary artery disease, cerebral and peripheral arterial). It hypothesizes causality, which is considered the aetiology of diseases harmful association is dominated by simultaneous exposure to risk factors. K. Popper suppose the risk amplification due to increased cholesterol, blood pressure, smoking, obesity, diabetes, alteration hematocrit and hyperuricemia. In the 1957-1959 Dowber reported first results of the Framingham study and confirm the increased risk accoeding to gender, age, hypercholesterolemia, hypertension, smoking, and set out the foundation of Ancel Keys studies on metabolism (the influence of dietary fat). This research are to be essential to conduct prospective studies by Seven Countries Study (14 cohorts monitored simultaneously: Japan, USA, Yugoslavia, Greece, Italy, Netherlands, Finland), which had similar results to those of the Framingham study by highlighting the corelation between general population and saturated fat, and incidence of coronary heart disease. In Europe a number of studies were made after 1960: Prospective study Parisian, Withehall Study, study Goteborg Study of Banks in Belgium. First studies in migrant populations was initiated (Japanese living in Japan, Hawaii, USA or Yemenis immigrants to Israel). In 1962-1967 Cornfield and Truett realized the first analyzes of risk and predictive factors: hypertension, hypercholesterolemia, smoking, age and sex. First studies on primary prophylaxis was *Surgeron General* (1964) – the first monograph, which specifies the role of tobacco in cancer. The first studies of primary prevention (hypercholesterolemia, hypertension) were: study in psychiatric hospitals, American veterans, New York Anti Coronary

Study, Study of Veterans from Administration (the latter that is clinical trial demonstrated the hypotensive medication). Framingham study highlighted the role of serum lipid fractions with different protective effect (HDLc) Vs. the risk (LDLc). Study on London drivers (1966, Morris) emphasizes the protective effect of physical activity. In 1977, the Framingham Heart Study proved that the vascular cardiodiseases in diabetes patients are more common than in the general population. The Prospective Study made on Diabets in United Kindom (UKPDS) allow to publish the first results in 1998 and later in 2007. G. Rose (1985) publish in the article *Sick individual's sick Population* and estabilish that it is posible to prognose the coronary events at persons with risk factors.

This evolution of epidemiology on CVD reveals that we need a strategy prevention to reduce the consequences of this disease to be able to detect persons with high risk (clinical strategy) (6).

Risk factors

Risk factors (RF) are much discussed topic by epidemiologists and clinicians because of necessary of prevention program and monitoring of CVD, determination of the risk and improving the potential effects of identified factors. RF is the term used to define the elements that characterize a healthy person. This term combines the classical concept of causality (type „cause – effect") and the relationship with probability, prediction and prognose. As example: persons who has RF to ischemic heart disease (cholesterol, smoking) are associated with disease events, but also have a direct causal connection with aterogeneza- atherosclerosis. Risk factors are associated with risk of CVD or other atherosclerotic disease have not yet demonstrated a causal link with atherogenesis-atherosclerosis. These factors are called „predictive" factors of CID including: obesity, hyperuricemia, hypertriglyceridemia or hyperinsulinism. The difference between two concepts is relative, because what is today an „indicator" of risk over time can become a causal factor, as a result of future scientific progress in this area. It is accepted common classification of modified and unmodified Risk factors (RF) in adults. Modifiable RF that are most commonly mentioned are: high cholesterol, high blood pressure (hypertension), smoking, obesity, diabetes. Modifiable factors are age, personal or family history of CVD etc.

Other classifications of RF are divided into: traditional or conventional: hypertension, hypercholesterolemia, smoking, diabetes and non-

conventional: abdominal obesity, microalbuminuria, anemia, metabolic syndrome.

The INTERHEART study are listed as RF: ratio apoB / apoA1, smoking, diabetes, hypertension, abdominal obesity, psychosocial factors, physical activity, regular alcohol consumption and low consumption of fruit and vegetables.

Recent research has allowed to define the metabolic syndrome, which is a combination of metabolic abnormalities with increased risk of CVD. In this syndrome are identified RF as: abnormal fat distribution, insulin resistance with or without hyperglycemia, atherogenic dyslipidemia (high triglycerides, low HDLc, LDLc small dense particles), hypertension, prothrombotic status, proinflammatory status. RF includes the cardiometabolic risk that are associated with cardiovascular risk and risk of diabetes.

It is important to understand the significance of RF action. Thus, some factors act: a) asymmetric and asynchronous; some acting during childhood (familial hypercholesterolemia), others in adulthood (smoking, obesity, hypertension), their intensity is different from one individual to another. In this sense it uses the concept of global risk or risk status; b) an individual acting on several FR, and the result is not so much their sum as the exponential increase in risk. Most commonly are associated with moderate hyperglycemias, mild hypertension, obesity and smoking degree; c) the risk posed by a factor distribution is continuous, not a real dichotomy between increased risk and no risk. For this reason the limit values are set somewhat arbitrarily applied and are indicative only at the individual level. Currently, are used certain indicators to assess the risk at the individual level such as relative risk; the absolute risk of developing clinical event in a defined period. Long-term risk (risk liftime) identifies the risk of CVD and type 2 diabetes over a person's lifetime risk of mortality due to disease or current in the next 30 years. The residual risk for microangiopathy and macroangiopathy is the risk that remains after controlling mono- or multifactorial applied correctly.

The relative risk at the population level is based on value risk in population; the absolute risk is a clinical event occurred in a population in a certain period; the attributable risk is attributable absolute risk of exposure to RF (calculated as the difference between the rate of occurrence of disease in populations exposed subjects or situations to those not exposed to RF).

Status of risk or overall risk refers to the overall outcome of the action on the body FR. Evaluation is done by rapid methods or estimated by complex methods, based on quantification (7).

In clinico-epidemiological issue is important to know the incidence and prevalence of cardiovascular RFs. It was made four populations with profile:

1. Low risk: men <55 years and women <65 years with hypertension grade I; without factors of risk (the risk of major CV event in the next 10 years is 15%);

2. Medium risk: patients with hypertension of different levels; the presence of several risk factors (risk of major CV event in the next 10 years is 15-20%);

3. High risk: patients with hypertension grade I and II which have three or more factors risk associated, among them diabetes, plus target-organ damage; patients with severe hypertension without concomitant risk factors (risk of major CV event to 10 years is 20-30%);

4. Very high risk: patients with hypertension grade III and one or more factors associated risk; patients with clinically manifest CVD or renal disease (risk of major CV event to 10 years is ≥ 30%) (8).

Protective factors

Epidemiological studies have shown that CVD, in addition to RF, have protective factors, to which reference current prevention strategies. The most known are: increasing of HDLc, physical activity, and moderate alcohol consumption estrogens. The mechanisms by which HDLc provides cardiovascular protection act due to the role of this faction to carry cholesterol from tissues to the liver where it is metabolized. Physical activity influences the control of the RF: hypertension, obesity, diabetes. It is closely related to improving of lipid fractions as triglycerides and LDLc, but also by increasing of HDLc in cardiovascular protective element. Changing the physical and mintal tone (eustress) has a major influence in limiting the risk for CVD. Epidemiological studies have demonstrated that consumption of moderate amounts of alcohol (one glass of wine or 25 ml of brandy or 200 ml of beer per day) may help to reduce the cardiovascular risk. The phenomenon, called „French paradox", is justified both by vasodilation effect of moderate alcohol and the presence of antioxidant molecules, especially in Ceará wines. The increase in cardiovascular risk of postmenopausal emphasizes the role of both estrogens in cardiovascular protection by complex lipid fraction modulation (increase of HDLc and

LDLc decrease) and by stimulating the release of nitric oxide which decreases contractility of the injured muscle cells (9).

The manifestations of epidemiological process / extent of phenomina/ the incidence, prevalence and mortality

Cardiovascular diseases are the leading cause of death worldwide. It is estimated that over 17.5 million people died in 2012, which represent 31% of total mortality. Of all CVD, 7.4 million were due to ID and 6.7 million – AVC. As a general phenomenon, more than a quarter of these deaths were registered in countries with a developing intermediate or low economy, which underlines the trend of these diseases to spread even in the poorer areas of the world where in previous decades the death rate of CVD was much lower. By age category, 16 million of those deaths by non-communicable diseases were aged up to 70 years, of which 37% CVD and 82% coming from underdeveloped countries.

Data reported in Europe estimates that 4 million people have died because of this diseases, which is 47% of the total. In women it was recorded a mortality rate of 52%, which shows that compared to males, women are at higher risk of illness and death. On the European continent 1.5 million of deaths occur before the age of 75 years and 710,000 of these are considered premature deaths, were recorded in persons under the age of 65. In 2010 the morbidity recorded in Europe was of 2,500 cases per 100,000 population, which shows that, it had a stabilizing trend compared to 2000 *(Table 7)* (10).

Table 7

Mortality of cardiovascular diseases in Europe (10)

	CVD (total)	coronary artery disease	cerebrovascular disease	Other CVD
Male				
Total deths (all ages)	1 862 774 **42%**	876 017 **20%**	429 756 **10%**	557 001 **12%**
Deths >75 ani	939 698 **36%**	473 501 **18%**	201 780 **8%**	264 417 **10%**
Deths < 65 ani	508 132 **31%**	253 432 **16%**	95 249 **6%**	159 451 **10%**
Women				
Total Deths (all ages)	2 219 326 **51%**	903 330 **21%**	627 227 **14%**	688 769 **16%**

Continue

Deths >75 ani	536 712 **37%**	232 683 **16%**	155 702 **11%**	148 327 **10%**
Deths < 65 ani	201 492 **27%**	77 166 **10%**	54 470 **7%**	69 856 **9%**
Total				
Total Deths (all ages)	4 082 100 **46%**	1 779 347	1 056 983 **12%**	1 245 770 **14%**
Deths >75 ani	1 476 410 **37%**	706 184 **18%**	357 482 **9%**	412 744 **10%**
Deths < 65 ani	709 624 **30%**	330 598 **14%**	149 719 **6%**	229 307 **10%**

CVD cost 51% of total government spending for health care, which shows that they have a considerable economical burden. Of total spending to 54% are direct costs by 24% and 22% productivity loss due to informal care of patients. EU countries spend on average 60 billion / year for coronary artery disease and 38 billion / year for individuals with stroke (11).

Clinical manifestations

Cardiovascular diseases are characterized by a series of events that affects the heart and blood vessels. Among the most common clinical forms are observed, ischemic heart disease (IHD), due to the function to supply the heart muscle; cerebrovascular disease by localizing lesions in the cerebral blood vessels that supply the brain; ensuring peripheral arterial circulation of the upper and lower limbs; Rheumatic carditis affecting muscle and heart valves after repeated streptococcal infections; congenital heart malformations by changes in heart present at birth; deep vein thrombosis and pulmonary embolism resulting from venous obstruction of the lower limbs due to migration produced a blood clot that releases of plaque and progressing bloodstream to the heart and lungs.

Acute myocardial infarction (AMI) and stroke (stroke) are the main events of acute character, produced as a result of brutal arterial obstruction which triggering a devastating effect on the heart or brain. Most frequently these events occur abruptly with death or, in the case of survival, generates significant disabilities (3).

Paraclinically diagnosis (laboratory, imaging, pathology)

Paraclinically diagnosis in CVD is determined by a complex and varied explorations that associates biohumoral testing parameters, detecting specific tests of heart lesions, morphological exploration of abnormalities,

bioelectrical, biomechanics and exercise tests useful for assessing cardiovascular function.

The biohumoral parameters identified as nonspecific tests, but frequently used in clinical practice are those that measure: total cholesterol, HDLc (High Density Lipoproteins) and LDLc (Low Density Lipoproteins), triglycerides, apolipoproteins or low density lipoprotein. Other tests are: plasma glucose, uric acid, etc. Among the specific parameters are mentioned: Creatine phosphokinase (CPK), lactic acid dehydrogenase (LDH) especially indicated in the evaluation of myocardial fiber damage and the degree of necrosis of the IMA.

Morphological explorations are possible by performing classical radiological examination, plain radiographs, radiographs and serial echocardiography using various techniques (one-dimensional, two-dimensional). Scintigraphy offers the possibility of cardiac markers using a scan type: technetium - pyrophosphate (99Tc-PYP), monoclonal antibodies, Thalium-201. Cardiac catheterization and angiocardiogafia bring useful information for diagnosing a fault or conformations of the cardiac chambers, valves and vessels of the heart. Electrocardiography and vectorcardiografia, which can add standard ECG monitoring and Holter type are extremely useful for determining aspects of a possible cardiac dysfunction. The test measures the ability of physical exercise, being possible to detect coronary artery disease in subjects with latent risk (3).

Early detection (screening)

The active detection RF should be a continuous action of measures in general population or risk groups. The methods used are varied and may consist of simple techniques, depending on the detection. One example is the screening of dyslipidaemia, which is achieved by determination of total cholesterol, if the state of risk is absent, and can be extended to the investigation of the lipid profile (total cholesterol, HDLc and LDLc, apolipoproteins A and B, apolipoprotein A low density).

The principles underlying the achievement of screening are:

- detection of RF must be global in the sense that it will consider evaluation of all possible factors involved in increasing the probability of disease;
- occasional detection of RF requires research (eg smoker with a family history of CVD will be evaluated on lifestyle, the blood pressure, lipid profile and tolerance to carbohydrates);
- screening must be permanent;

- predictive action of risk factors will depend on the number of investigated persons.

The measurements are performed (eg in case of repeated dosing of total cholesterol to estimate risk real predictive power will increase by 50%).

Screening involves organization of detection of a good standard strategies, their development is likely to be at each preventive or curative medical consultation or in case of actions involving various specialists in multidisciplinary teams.

Various charts are applied to estimate the risk in terms of screening.

Thus, they have evolved Framingham score by estimating the overall risk for 10 years by using 6 RFs (sex, age, smoking status, total cholesterol, HDLc, systolic blood pressure), for risk stratification: low risk <10%, moderate risk 10-20% increased risk 20% to Framingham charts [Chart cardiovascular risk for men fractions using CT and LDLc. It takes into account age, CT (or LDLc), HDLc, blood pressure, diabetes and smoking. It is estimated cardiovascular risk over a period of 10 years based on the Framingham experience in men aged between 30 and 74 years. Environmental risk estimation is based on typical subjects Framingham and risk estimation is based on idealized tenisunea optimal blood pressure, CT = 160-199 mg / dl (or LDLc = 100-129 mg / dl), HDLc = 45 mg / dl in men, Smoking and non-diabetics. Using fractions LDLc is appropriate when rapid LDLc measurement is available. Chart cardiovascular risk for women is based on risk assessment and risk idealized environment, using as variables: optimal blood pressure, CT = 160-199 mg / dl (or LDLc = 100-129 mg / dl) = 55 mg HDLc / dl in women, diabetic and non-smoking. Using fractions LDLc is appropriate when rapid LDLc measurement is available]. The advantage of this chart is to be easily usable and disadvantages that are based on an American population (the study included Fremingham); underestimate the risk in people with more moderate FR expressed or expressed aggressive one factor; does not provide prediction for cerebrovascular territory. Other methods of risk assessment are: coronary risk prediction chart developed by the Joint British Societies; Euro'97 chart or diagram quantifying risk stratification and prognosis after the blood pressure according to European Society of Hypertension Guidelines 2003 SCORE diagram can be used to demonstrate to young people with low absolute risk that their relative risk compared with those of group their age, may sometimes be several times higher than that recommended. This may help motivate decisions such as

avoiding smoking, a healthy diet and physical activity, and to identify those individuals who may require medication. Since February 2009 SCORE chart was translated into Romanian Romanian Society of Cardiology and is applied to the entire population *(Figure 74)* (12).

Advantages of SCORE Diagram are multiple: based on European population, is easily usable, offers prediction for 10 years, is based on five risk factors (gender, age, smoking status, total cholesterol, systolic blood pressure) and provide stratification in classes risk: <1% (low risk); 1-5% moderate risk; 5% increased risk. The disadvantage of undervaluation of risk in certain categories (people with diabetes, metabolic risk, relatives of people with premature cardiovascular disease).

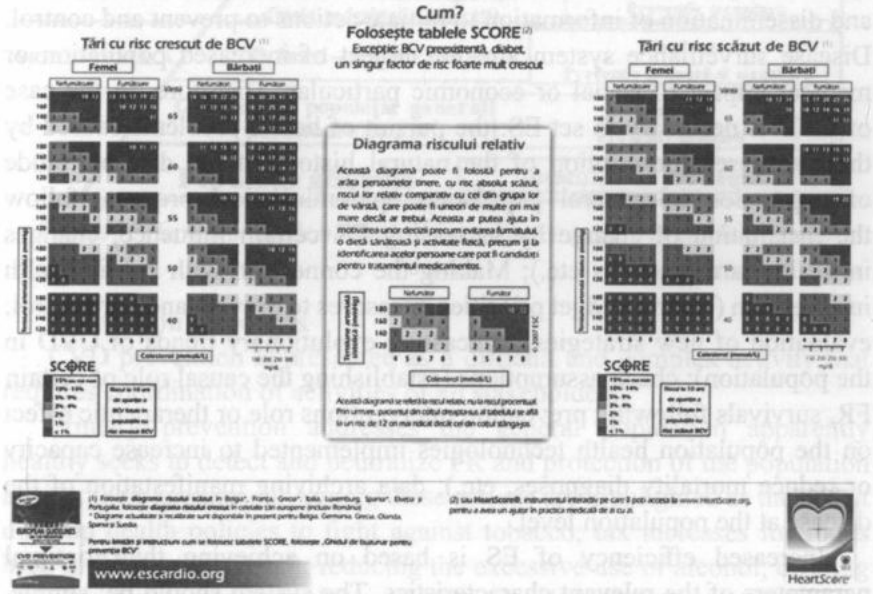

Figure 74. *SCORE chart recommended by Romanian Society of Cardiology (12).*

Currently used for risk estimation and various software such as UKPDS, launched in the UK. It applies specifically to people with type 2 diabetes and quantifies: coronary morbidity, cardiovascular morbidity, mortality coronary, cerebrovascular mortality. FR included in the evaluation are: age, duration of diabetes, gender, ethnicity, presence or absence of atrial fibrillation, smoking status, HbA1c, total cholesterol, HDLc, blood

pressure. The method has the advantage of being applicable to specific and cardiometabolic risk diabetic patients, and in turn, prevents the drawback is that it requires the use of a computer.

Archimedes model brings together recommendations resulting from the use of data from several randomized trials have quantified the risk of cardiovascular disease (MI, stroke), diabetes mellitus and microvascular complications of diabetes. Are measured over 20 variables (including medication) model is an educational and interactive.

Epidemiological surveillance

Epidemiological Surveillance (ES) is defined as a continuous and systematic process of collecting, processing, analysis, data interpretation and dissemination of information to initiate actions to prevent and control. Disease surveillance system are the subject of increased population or medical impact of social or economic particular. Therefore, in the case of CVD is necessary to set ES: the pursuit of health problems caused by these diseases (description of the natural history of the disease, mode of expression in general population epidemiological process, follow the installation of change by increasing FR certain influence; changes in health care practices etc.); Making the connection with public health intervention (setting budget priorities, measures to prevent and / or control; evaluation of new strategies, forecasting evolutionary trends of CVD in the population); check assumptions (establishing the causal role of certain FR, survivals following prevention interventions role or therapeutic effect on the population health technologies implemented to increase capacity or reduce mortality diagnoses, etc.); data archiving manifestation of the disease at the population level.

Increased efficiency of ES is based on achieving their optimal parameters of the relevant characteristics. The system should be: simple, sensitive, flexible, acceptable, desirable; representative, with positive predictive value increased. The stages are the CVD SE: data collection, processing, interpretation and dissemination of the results and putting them in Devices makers and population (13). Representation type iceberg reveals the importance of the SE of CVD prevention that apply especially at the basic levels, respectively in the general population, the groups of persons carrying FR and the stages of preboală. Interventional actions are specific stages of screening patients with clinical forms inapparent or

ignored, and monitoring and hospitalization cases showing overt clinical manifestations and / or in association with complications *(Figure 75).*

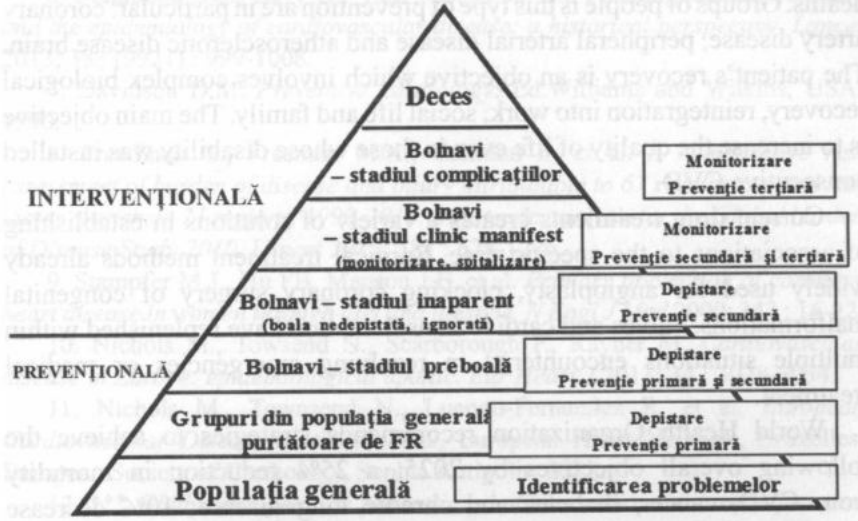

Figure 75. *Levels of epidemiological surveillance system in cardiovascular diseases.*

Prevention measures

CVD prevention is presented as a difficult and complex activity that requires coordination of activities of all stakeholders.

Primary prevention addresses the general population apparently healthy seeks to detect and neutralize FR and protection of the population against their aggression. Among these actions are highlighted as those that develop health policies to fight against tobacco; tax increases for foods high in fat, sugar and salt; reducing the excessive use of alcohol; offering opportunities to ensure healthy eating in schools.

Secondary prevention is aimed at people who have a higher risk in order to reduce the occurrence of heart attack or stroke first. In this regard, in addition to screening for risk monitoring activities, individual measures will be applied by the treatment of diabetes, hypertension or hypercholesterolemia. Interventions aimed at preventive use of aspirin, beta-blockers, ACE inhibitors, angiotensin, together with monitoring of lifestyle in terms of diet, alcohol consumption, smoking and physical activity.

Tertiary prevention includes therapeutic interventions mainly with character development and recovery in order to limit complications and deaths. Groups of people is this type of prevention are in particular: coronary artery disease, peripheral arterial disease and atherosclerotic disease brain. The patient's recovery is an objective which involves complex biological recovery, reintegration into work, social life and family. The main objective is to increase the quality of life even in those whose disability was installed consecutive CVD.

Current drug treatments creates a variety of solutions in establishing of associations to the specific case. Surgical treatment methods already widely used for angioplasty, clocking coronary surgery of congenital malformations, valves and cardiac transplantation have replenished within multiple situations encountered in resolving emergencies or medical treatment.

World Health Organization recommends strategies to achieve the following overall objectives by 2025: a 25% reduction in mortality from CVD, cancer, diabetes and chronic lung disease; 10% decrease excessive alcohol consumption; 10% reduction in the prevalence of insufficient physical activity; reducing by 30% the average population salt consumption; 30% reduction in the prevalence of smokers among people over 15; 25% decrease in the prevalence of hypertension; reducing the prevalence of diabetes and obesity; at least 50% of the eligible persons must receive appropriate medication and counseling for prevention of MI and stroke by developing the National Response Systems (14).

Bibliography

1. Levi F., Chatenoud L., Bertuccio P. et al. *Mortality from cardiovascular and cerebro-vascular disease in Europe and other areas of the world: an update. Eur J Cardivasc. Prev. Rehabil* 2009; 16 : 333-350.

2. World Health Organization, WHO Mortality Database: 1st May 2013 update (http://who.int/healthinfo/statistics/mortality_rawdata/en/index.html) Departament of Health Statistics and Information Systems, Switzerland, 2013.

3. Braunwald's Heart Disease: *A Textbook of Cardiovascular Medicine*, editors Douglas L. Mann, Douglas P. Zipes, Peter Libby, Robert O. Bonow, 10th Edition, edit Saunders, 2015.

4. Omran Abdel R. *The epidemiologic transition theory revisited thirty years later*. World Health Statistics Quarterly 1998; 51 : 2-4.

5. Gaiţă D., Avram C., Avram A. şi col. *Optimizarea stilului de viaţă în prevenţia cardiovasculară. De la ghiduri actuale la practica clinică*. În: *Progrese în cardiologie*, MediaMedPublicis, Bucureşti, România, 2007, II, 361-384.

6. Mahmood S.S., Lavy D., Vasa R.S. et al. *The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet* 2013; 383 (9921) : 999-1008.

7. Davidson D.M. *Preventive cardiology*. Ed.Williams and Wilkins, USA, 1990.

8. Freedman G., Freeman M.K., Gakidou E. et al. *A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clustensin 21 regions, 1990-2010: a systemic analysis for the Global burden of Disease Study 2010. Lancet* 2013; 381 : 628-1276.

9. Stampfer M.J., Hu FB, Manson J.E. et al. *Primary prevention of coronary heart disease in women through diet and lifestyle. N Engl J Med* 2000; 343 : 16-22.

10. Nichols M., Towsend N., Scarborough P., Rayner M. *Cardiovascular disease in Europe: epidemiological update. Eur Heart J* 2013; 34 : 3028-3034.

11. Nichols M., Townsend N., Luengo-Fernandez R. et al. *European Cardiovascular Disease Statistics 2012*, European Heart Network, Bruxelles, European Society of Cardiology, Sophia Antipolis, 2012.

12. *** *Ghidul european de prevenţie a bolilor cardiovasculare în practica clinică (versiunea 2012)*. În *Romanian Journal of Cardiology* 2014; 24 : 4.

13. Goff DC Jr, Brass L., Braun L.T. et al. *Essential features of a surveillance system to support the prevention and management of heart disease and stroke: a scientific statement from the American Heart Association Councils on Epidemiology and Prevention, Stroke, and Cardiovascular Nursing and the Interdisciplinary Working Groups on Quality of Care and Outcomes Research and Atherosclerotic Peripheral Vascular Disease. Circulation* 2007; 115: 127–155.

14. Graham I., Atar D., Borch-Johnsen K. et al. *European guidelines on cardiovascular disease prevention in clinical practice: executive summary.* European Heart J 2007; 28 (19) : 2375-2414.

2. Epidemiology of cancers

Introduction

Cancers are the leading cause of death worldwide and have a significant rate of deaths globally (1). However, mortality, taken separately, does not reflect fully the burden that this pathology generates in society, given that some types of cancers affecting young people more frequently than others (2, 3).

Another way to express the disease burden in the population outside indicators of incidence, prevalence and mortality is evaluating the indicator DALYs (Disability Adjusted Life Years) introduced by the Global Burden

of Disease (GBD), which connects the burden caused by mortality cancer and degrees of illness and disability in patients and long-term survivors. The two components of DALYs are the years of life lost due to premature death (Years of Life Lost = YLLs), ie before the age of 80 years for men and 82.5 years for women before and years lived with disability (YDLs = Years lived with Disability), expressed as a number or as a rate per 100 thousand (4). Estimates show that in 2008, 169.3 million years of healthy life were lost worldwide due to cancer. The most important role in most of the regions of the world have had colorectal cancer, lung cancer, breast cancer in women and prostate cancer, explaining 18-50% of healthy life years lost (5, 6). The economic costs of these DALYs were 895.2 billion dollars (7). Asia and Europe are regions with a major rate (73%) of the global burden of DALYs lost produced as a result of cancer. In 2008, the global men had 6% more DALYs products cancers than women, those from Eastern Europe burden of this pathology is the highest, a situation due to incidence and high mortality in these regions for several types of cancer, including colorectal, lung, pancreatic and renal cancer. Women owe DALYs cancerous diseases were most important in Oceania and sub -Saharan (5).

As can be seen from the data presented above, cancer is an important public health problem, both by the large number of cases and deaths by age increasingly younger that affect them, and the burden it put on „shoulders" of the health system.

There are two great institutions that are considered reference regarding the epidemiology of cancer and whose assessments were worth „gold standard" in this field, namely: International Agency for Research on Cancer (IARC) and the World Cancer Research Fund / American Institute for Cancer Research (WCRF / AICR).

IARC evaluated the scientific evidence related to carcinogenic risk of exposure to smoking, alcohol, radiation, drugs and occupational exposures (8) and WCRF / AICR assess the relationship between cancer and factors such as diet, overweight and obesity and physical activity, in terms of evidence-based medicine (9).

IARC has contributed and continues to contribute to the implementation of the Global Action Plan for Prevention and Control of Chronic Non-Communicable Diseases 2013-2020 (The Global Action Plan for the Prevention and Control of NCDs 2013-2020) by providing credible

scientific evidence and high quality, essential for the development and substantiation of health guidelines and policies by the world health Organization (WHO) to protect human health worldwide (10).

Short history

Some of the earliest evidence of cancer have been identified as: bone tumors fossilized human mummies from Ancient Egypt (which were identified some suggestive osteosarcoma cell growth) and ancient manuscripts. The earliest description of a cancer (though not the name was used) is contained in the Edwin Smith papyrus (which were described in 8 cases of tumors / ulcers of the breast). It dates back to 3000 BC and it was discovered in Egypt.

The origin of the word *cancer* is attributed by Hippocrates (460-370 BC), who used the terms of carcina and carcinoma to describe tumors forming unformatted or ulcer. In Greek, the *crab* word used probably to name this disease because the spread of tumor tissue is similar to the shape of a crab. Later, the Roman physician Celsius (28-50 BC) the Greek term translated into cancer, Latin for crab and Galen (130-200 BC), another Greek physician, used the term *Oncos* (= swelling in Greek) to describe tumors. Today, this word enters the structure of medical term whose specialty is cancer pathology - oncology (11, 12).

In 1761, Giovanni Morgagni of Padua made the first autopsy. This was based on the study of cancers (ie scientific oncology).

Scottish surgeon, John Hunter (1728-1793) suggested that some cancers can be treated with surgery and described how to make a decision about the cancers (those not invaded neighboring tissues and are „mobile"). A century later, it allowed the development of anesthesia for surgery of classical cancer – radical mastectomy.

In the XIXth century, Rudolf Virchow (Father cellular pathology) provide scientific basis for modern pathologic study of cancer after the development of modern microscopy. The method allowed to understand the destruction caused by cancer and allowed an accurate diagnosis. It also, allowed to the pathologist to confirm or deny whether or not surgery removed completely the tumor (11, 12).

The first theories about the causes of cancer were:

- humoral theory. Hippocrates believed that the body has four humors (body fluids): blood, phlegm, yellow bile and black bile. When the humors are balanced, the body is healthy, but an excess of black

bile is believed to be cancer. The theory was taken up by Galen and unchanged ot for 1300 years (until the Middle Ages).

- Lymph theory. Stahl and Hoffman issued a theory that cancer was composed of lymph fermented and degenerate density, acidity and alkalinity variables. The famous Scottish surgeon John Hunter (1700) supported this theory.
- Blastema theory. In 1838, German pathologist Johannes Muller showed that the cancer consists of abnormal cells and there are not in serum. Muller states that cancer cells develop from structural elements (blastema), which are between normal tissues.
- Theory of chronic irritation. Virchow is the one who introduces the concept of chronic irritation – a cause of cancer. In 1860, German surgeon Karl Thiersch shows how cancer metastasis occurs through the spread of malignant cells and not through some unidentified fluid.
- Trauma theory. In the late 1800s until the 1920s, trauma has been considered by some researchers as a cause that can cause cancer.
- Theory of infectious diseases. Zacutus Lusitania (1575-1642) and Nicholas Tulp (1593-1674), two Dutch doctors concluded almost in the same time (1649 or 1652), that the pathology of cancer is contagious, and people affected should be isolated (based on the state their experience with members of families identified with breast cancer). This theory was taken up during the XVII and XVIII centuries. At present, although it is shown the involvement of viruses or bacteria in the etiology of cancer, it is known that this pathology is not contagious.

Modern theories about the causes of cancer include: chemical carcinogens and viral. John Hill (London) identified tobacco as a carcinogen and Katsusaburo Yamagiwa and Koichi Ichikawa (University of Tokyo, 1915) evidence of carcinogenicity of coal tar for the animal. Until today, many substances have been identified as being responsible for cancer: coal tar derivatives, and various hydrocarbons, aniline, asbestos etc. Ionizing radiation in the environment, arsenic from drinking water or biological agents (viruses Hepatitis B and C - HBV, HCV, Epstein-Barr virus, Human Immunodeficiency Virus – HIV, Human Papillomavirus – HPV) are also part of the factors causing cancer. By 2014, IARC has identified over 100 carcinogens physical, chemical and biological (11, 12).

Subsequently, the researchers found that radiation, viruses or chemical carcinogens can damage the DNA and they can lead to cancer development.

In the 70s of the XX century were discovered two important families of genes associated to cancer: oncogenes (allowing uncontrolled multiplication of cancer cells) and tumor suppressor genes (which are normal genes that slow down the cell division, repair errors of DNA and are responsible for apoptosis = programmed cell death). In the 90s of XX centery were identified genes of BRCA1 (Breast Cancer) and BRCA 2, which produce some breast cancers of major importance, since it allows the identification of subjects at risk of developing this cancer, promoting prevention measures and early intervention. There were identified genes associated with familial colorectal cancers, ovarian, pancreatic, kidney, thyroid and skin melanoma as well.

Researchers begun studies for the development of targeted gene therapies after the identification of the role of specific genes that increase the risk of cancer.

Three milestones have been identified in the XVIII century in the history of cancer and its epidemiology:

- In 1620, Thomas Venner (London) first demonstrated the role of tobacco. In 1761, John Hill associated smoking to cancer, but only in 1950-1960 demonstrated the relationship between cigarette smoking and lung cancer.
- In 1713, Bernardino Ramazzini, Italian doctor reported no incidence of cervical cancer and breast cancer relatively high nuns, demonstrating the importance of hormonal factors and sexually transmitted infections (STIs) for the risk of developing cancer.
- In 1775, Percival Pott (St. Bartholomew's Hospital, London) described the cancer of the scrotum in chimney sweep (occupational exposure).

Epidemiologists continue to study the effect of risk factors (RF) (smoking, obesity, UV, etc.), and the protection factors (PF) cancer (physical activity, healthy diet, etc.) with the aim of providing the scientific evidence necessary for the development of public health recommendations.

The first screening test widely used for early identification of cancer Pap-test was developed by George Papanicolaou (1923). Although, there were reservations from the beginning, the American Cancer Society (ACS) promoted extensively in the 60s of XX century. It became a widely used

test and helping to decrease the mortality of cervical cancer by 70% in the US of America (USA). Modern mammography was developed in the late 60s and was officially recommended by ACS in 1976. Currently, ACS guidelines include the screening for early detection of cervical cancer, breast, colorectal, endometrial, prostate, lung and recommendations for regular checks, depending on the age and sex of subjects, for thyroid cancer, oral, skin, testicular and ovarian.

The first cancer treatment is surgery, to which were added over time hormone therapy, radiation therapy, immunotherapy, targeted therapy (inhibitory signal for growth, angiogenesis inhibitors, drugs that induce apoptosis) (11, 12).

Risk factors

Any factor that increases the likelihood of an event in a cancer are called a risk factor, and factors that decrease the chance of developing this event are called protective factors. Some of these risk factors can be avoided, while others action can not be influenced, such risk factors are divided into modifiable risk factors (smoking, diet, number of births, etc.) and non-modifiable (genetic factors, age, sex, etc.).

The world scientific study of risk and protective factors are aimed to find ways to prevent new cases of cancer.

According to IARC and WCRF / AICR risk factors (RF) or protective (PF) for cancer fall into the following categories, depending on the type of evidence found in the literature (8, 9):

- Factors that increase the risk (insufficient evidence or convincing);
- Factors that may increase the risk (limited evidence or probable);
- Lowering risk factors (insufficient evidence or convincing);
- Factors that may increase the risk (limited evidence or probable).

Factors that increase the risk of cancer include smoking, infections, radiation, immunosuppressive medicines etc.

Factors that may increase the risk of cancer include diet, alcohol consumption, physical activity, obesity, carcinogens in the environment, etc.

Smoking. Smoking is a factor that increases the risk of many types of cancer. It is the main cause for the following cancers: lung cancer, esophageal the oral cavity, bladder cancer, kidney cancer, gastric, pancreatic, cervical cancer and leukemia acute myeloid leukemia (13). The relationship between smoking and lung cancer vary depending on

the duration and intensity of exposure to smoking, or smoking cessation, cigarette type smoking, lung cancer histologic type and characteristics of the population (14). After 10 years of abstinence, the risk of lung cancer drops by 30-50% compared with the risk they are exposed to those who continue to smoke, and the risk for oral and esophageal cancer is halved to 5 years after smoking cessation. Also, those who quit smoking can lower their risk of developing cervical cancer, gastric or bladder (13).

Most studies demonstrated the relationship between smoking and cervical cancer among women who had infection with Human Papilloma Virus (HPV), a smoker or former smoker, and relative risk compared with non-smokers. Passive smoking is associated with an increased risk for cervical neoplasia, but to a lesser extent (14, 15). This association between exposure to smoking and cervical cancer does not disappear when the risk value is adjusted for factors that are known to influence the occurrence of this cancer: early age at sexual debut, sexual partners multiple socio - economic status or infection HPV (14).

The risk of colorectal cancer is higher in both active smokers (17 to 21%) and to former smokers (17 to 25%) compared with non-smokers. The association is stronger in men than women and is more important for rectal cancer than colon cancer (16, 17). The relative risk highlighted in the literature association of smoking in colorectal cancer that is 1.18 (smokers versus non-smokers) (18). IARC classified smoking as a factor that can increase the risk for breast cancer or probable cause of neoplasia, due to limited scientific evidence (8). The literature shows that the risk in active smokers is by 12% higher compared to non-smokers and 9% higher in former smokers (19, 20, 21), it partly explained the fact that smoking is associated with high levels of sex hormones (22).

Infections. Some viruses or bacteria may increase the risk of cancers (mainly in developing countries of the world).

HPV infection increases the risk of cervical cancer, oropharyngeal, vaginal, anal and penile, depending on the type of HPV involved (23). Oncogenic HPV types 16 and 18 are responsible for approximately 70% of all cases of cervical cancer and nearly 50% of the vaginal cancer, vulvar and penile (15). IARC includes HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 in the category of risk factors proven to cervical cancer, and HPV types 26, 53, 66, 67, 68, 70, 73 and 82 – in the category of probable causes (23, 24). Besides HPV infection, a series of cofactors can act to

increase the risk of cervical cancer by increasing of exposure to HPV, or persistent infection (25): a risk for cervical cancer is approximately 3 times higher in women that had ≥ 6 sexual partners compared to those who had a partner; cancer risk is double the ones who started sexual life at age ≤ 14 years compared with those who started after 25 years (26); halved the risk in women whose partner has undergone circumcision, compared with those whose partner has no circumcision (27). HPV types 16, 33, 31 and 18 are commonly implicated in increasing the risk for cancer of the vulva and vagina. Also, HPV types 16 and 18 are associated with an increased risk for cancer of the penis (24).

Infection with hepatitis B and C viruses (HBV and HCV) is a factor shown to increase the risk for liver cancer, IARC, and probable cause for cholangiocarcinoma (28). Epidemiological studies show that HBV infection is responsible for 50-90% of cases of hepatocellular carcinoma in areas of high endemicity. The literature shows the relationship between HBV infection and liver cancer, the relative risk varies between 9.6 and 71, values reaching 161 where co-infection with HCV exist (24).

Radiation. The radiation exposure is a known cause of cancer. Two types of radiation are associated with increased risk for cancer, namely ultraviolet radiation (the main cause of non-melanoma skin cancer) and ionizing radiation, including medical radiation originating from diagnostic tests for cancer (radiography, CT, fluoroscopy or nuclear medicine) and radon found in the atmosphere of dwellings (houses and increased concentration at the basement). Researchers have documented the relationship between radiation exposure and increased risk of leukemia, thyroid cancer, and breast cancer, and melanoma, lung cancer, gastric, colon, esophageal, bladder and ovarian cancer (29).

Many other factors are associated with an increased risk for various types of cancer. Among these are: diet rich in fat, protein, calories and red meat (colorectal cancer); Alcohol (oral cancer, esophageal, breast, colorectal and hepatocellular); obesity (associated with an increased risk of postmenopausal breast cancer, colorectal, endometrial, esophageal, kidney and pancreas); carcinogens in the environment (passive smoking, air pollution in the external environment and exposure to asbestos, increasing the risk of lung cancer; high concentrations of arsenic in drinking water associated with an increased risk of skin cancer, bladder or lung) and immunosuppressive medication, commonly used in organ transplant patients (30).

Among the risk factors of breast cancer are: age (80% of cases are diagnosed after age 50 years); endogenous hormones (increased levels of estradiol, estriol, testosterone or androsterone and IGF-1 levels – insulin-like growth factor 1) (31, 32, 33); reproductive factors (nulliparity small number of births, age over 35 years at first birth, early menarche – under age 12; late menopause – over 52) (34); use of oral contraceptives (CO) combined (estrogen-progestogen) (31, 35); hormone replacement therapy (HRT) (31, 36, 37); family history (has a major role in this type of cancer) and genetic factors (BRCA1, BRCA2, ATM, p53, CHEK2, PTEN, CDH1, STK11, PALB2) (19, 20, 38); cancer in anamnesis (breast, endometrial, Hodgkin's lymphoma, chronic lymphocytic leukemia, melanoma, adenocarcinoma of the lung); overweight and obesity (39); exposure to ionizing radiation; exposure to carcinogens in the environment (eg. of ethylene oxide); Increased breast density; benign breast disorders (ex. atypical hyperplasia); high fat diet; alcohol; smoking; medical conditions and treatments (diabetes, autoimmune thyroiditis, increased bone mineral density, treatment with digoxin or diethylstilbestrol) (31).

The researchers identified risk factors in ovarian cancer: general factors (age, race / ethnicity, status socio – economic); reproductive factors (increased number of ovulations / ovulatory cycles, age at first menstruation under 12 years of age at menopause over 52, the age at first birth more than 35 years, nulliparity, infertility); exogenous hormones (HRT treatment fertilizer) (40); smoking; occupational exposure to carcinogens; ionizing radiation; family history (important in this type of neoplasia) and genetic (mutations in genes: BRCA1, BRCA2, p-53, HNPCC, OVCA1, CYP1A1, HER-2 / neu, CYP1A2, CHEK2, EMSY, p21, PTEN, CTNNB1, PIK3CA, Akt2, SOD2, MPO, NQC1, B7-H4) (19, 41); overweight and obesity; personal history of cancer (breast or colorectal cancer); perineal use of talc powder; medical conditions and treatments (endometriosis or diabetes) (19, 42, 43).

Factors that may increase the risk of colorectal cancer include factors both modifiable and non-modifiable. Among these are: age (43% of cases over 75 years); gender (more common in men); diet (rich in red meat or processed meat, high in animal fats, use of dietary sugars); Obesity and overweight; alcohol; smoking; occupational exposure to carcinogens (asbestos); ionizing radiation; presence of infection history (H. pylori HPV); various medical conditions (adenomatous polyps, inflammatory

bowel disease – Crohn's disease, ulcerative colitis, gallstones, diabetes, metabolic syndrome); a personal history of cancer (colorectal, esophageal, laryngeal, lung, prostate, endometrial and breast cancer, also in patients with chronic lymphatic leukemia and melanoma); family history and genetic syndromes (familial adenomatous polyposis – FAP, hereditary polyposis colorectal cancer non-; mutations BRCA1) (44, 45).

Protective factors

Besides risk factors exist a category of factors that can influence the risk of cancer, factors which addresses prevention strategies at the global level (eg Global Action Plan for the prevention and control of NCDs 2013-2020) (46) and national (eg program to prevent and control noncommunicable diseases).

Diet. It was studied both as RF and as PF. It is difficult to assess accurately just one of the effects, because a person's diet contains foods that increase the risk of cancer, and foods that decrease risk. Some studies support the hypothesis that non-starch diet rich in vegetables and fruits may protect against oral cancer, esophageal and gastric. Also, eating fruits may protect against colorectal cancer (29).

Physical activity. Research shows that there is a strong relationship between physical activity and decreased risk for colorectal cancer. There is scientific evidence supporting it protects against postmenopausal breast cancer and endometrial cancer (29).

Chemoprevention consists in administeration of some medicines that prevent the occurrence or recurrence of cancer: tamoxifen or raloxifene (administered treatment for a period of five years reduces the risk of breast cancer by 50%) or finasteride to lower the probability of prostate cancer. New substances are continually researched in hopes of identifying effective preparations for the prevention of various types of cancer. Thus, COX-2 inhibitors are being studied for the prevention of colorectal and breast cancer (but increases the incidence of cardiovascular events) and aspirin for the colorectal cancer (47).

Protective factors of breast cancer are: feeding (risk decreases by 4% for every 12 months of breastfeeding) (48); physical activity (49); celiac disease (50); regular intake of aspirin or anti-inflammatory drugs (NSAIDs) (51); diet (eating fruits and vegetables, fiber, carotenoids, soybeans, mushrooms, coffee maker) (19, 20, 32); hysterectomy with oophorectomy (practiced before menopause reduce this risk by 24-41%) (32).

Regarding ovarian cancer, lowering risk factors include multiparity; lactation (decreases risk by 24%); using CO (decreases risk by 25 to 28%); Hysterectomy (decreases risk by 27 to 31%); ovariectomy; Tubal ligation (decreases risk by 30%); statins (decreases risk by 21%); presence of systemic lupus erythematosus (decreases the risk by 34%); non-starch consumption of vegetables (19, 41, 42).

Protective factors in colorectal cancer are: physical activity; HRT (decreases risk by 16%) (52); using CO (decreases risk by 14%) (53); Daily aspirin use (for 5 years or more decreases the risk by 32-49%); Parkinson's disease (decreased the risk by 24%); diet (rich in fiber, garlic, milk, calcium) is a factor which probably lowers the risk for colorectal cancer (44).

The manifestation of epidemiological process / extent of spread / Incidence, prevalence, mortality

The latest data available on cancer were published by the International Agency for Research on Cancer (IARC), which is an agency of the World Health Organization (WHO) specializes in the study of cancers (54). According to GLOBOCAN 2012 the number of new cases of cancer increased to 14.1 million in 2012 (recording an incidence of 182.3 new cases / 100,000 population) and the number of deaths due to cancer reached 8.2 million in the same year (with a mortality of 102.4 deaths / 100,000 papulation). Also, the prevalence increased in people over 15 years, reaching 32.6 million people living with cancer, diagnosed in the last 5 years (2, 54, 55). The risk to develop cancer before the age of 75 was 18.5% and the risk of dying from cancer before age 75 – 10.3% (54).

Over the world, the most frequently diagnosed malignancy was in lung cancer at a rate of 1.8 million cases (13% of all cancers), breast cancer (1.7 million cases, accounting for 11.9% of total), and cancer colorectal 1.4 million cases (9.7%). When referring to the number of deaths, was find that the first place is occupied by 1.6 million lung cancer deaths (19.4% of total), followed by liver cancer with 0.8 million (9.1% of total) and gastric with 0.7 million (8.8% of total). The worrying increase recorded a breast cancer (an increase of over 20% compared to 2008) (54, 55).

Another feature of this pathology, for 2012, is that most commonly affects the low developed countries, both in terms of number of new cases, 8 million (representing 56.8% of all cancers worldwide) to 6 million in more developed countries, and the number of deaths, 5.3 million (64.9% of

the global total) compared to 2.8 million for developed countries. But if we look at the prevalence of five years, it appears that the situation is reversed, the developed countries taking the lead with 16.9 million to 15.6 million in less developed countries.

Also, the overall standardized by age, is 25% higher in men (205 new cases / 100,000 population, with a total of 7.4 million) than females (165 new cases / 100,000 population, with a number 6.6 million). Regarding mortality, the highest rate in men was recorded in Central and Eastern Europe (173 deaths / 100,000 population) and lowest in West Africa (69 / 100,000 population) (2, 54).

In Central and Eastern part of Europe were identified 1.03 million new cases of cancer incidence was 216.1 new cases / 100,000 population (amount above the global average) and 638 187 deaths, mortality is 123.4 / 100,000 population (value, also higher than the average worldwide). The risk of a subject of developing cancer in their lifetime before age 75 years was 25.5% and the risk of cancer death before 75 years of 13.7%. The total number of patients living with cancer in this region is 2.44 million (980.1 cases / 100,000 population). Referring to assess the gender of this pathology in the central and eastern Europe, noted the presence of a higher incidence in men than women (260 new cases / 100,000 population, compared to 193.5 / 100,000 population) and the mortality with higher values in males (173.4 deaths / 100,000 to 91.6 / 100,000). However, the total number of persons living with cancer is higher in women compared to men (1.39 million cases respectively 1.04 million) (54, 56, 57). Following the data presented, it can be concluded that in Central and Eastern part of Europe were diagnosed 7.09% of all new cases of cancer worldwide, and there were 7.68% of all deaths from cancer across world.

In 2012, there were 9,894 of new cases of cancer were recorded in Moldova (incidence being of 194.1 new cases / 100 000 inhabitants) and 6292 cases of cancer deaths (mortality is 120.3 deaths / 100 000). Values were higher than the global average, but below the average recorded in Central and Eastern part of the Europe. The risk of people in this region to develop cancer during their life until the age of 75 years was 21.1%, while the risk of dying from the same disease was 13.9%. And in this area the incidence of cancers was higher in males (230 new cases / 100 thousand) compared to females (170.3 new cases / 100 000). Mortality was almost double malignancies in men and women (163.6 deaths / 100 thousand or

88.4 deaths / 100 000), while 5-year prevalence was higher in women than men (835, 7 cases / 100 000, respectively 733.1 / 100 000).

The prognosis made for 2025, based on estimates of the source (GLOBOCAN 2012) shows that the number of new cases of cancer will continue to increase, reaching 19.3 million (54).

Estimates published by the American Cancer Society shows that 10 million people will die of cancer every year by 2020.

Clinical manifestations

Cancers are a group of diseases that can cause almost any sign or symptom, depending on the location of the malignant process, its size or part of the affected organ or tissue. With the spread of cancer cells in the body (metastasis), the signs and symptoms may occur away from the original location of the cancer. Thus, there is cancer that begins in the critical areas, where even a small tumor produces signs and symptoms (eg the brain), while other malignancies begins in anatomical regions where it will not produce symptoms until they reach a significant size and will cause pressure on neighboring organs or structures (eg pancreatic cancer), usually in an advanced stage. Also, a number of general signs and symptoms (eg, fever, extreme tiredness, weight loss) can occur as a clinical manifestation of cancers, probably the consequence of excessive use of energy resources of the body by cancer cells (58).

The clinical manifestations of cancer are:

- skin changes: a new mole or a mole changes in older lesion that does not heal;
- changes in the breast: changes in the shape and volume of the breast or nipple, breast skin texture changes;
- nodules on the skin or subtegumentar;
- hoarseness and persistent cough that does not heal; dyspnoea;
- changes in bowel (colorectal cancer);
- difficult or painful urination;
- nutrition problems: abdominal discomfort after eating, difficulty swallowing, persistent heartburn or indigestion, changes in appetite (generally, loss of appetite), persistent bloating;
- unexplained weight loss (most common cancer of the pancreas, stomach, esophagus or lung) or gaining weight without explanation;
- unexplained pain (bone or testicular cancer, brain tumors, headaches or back pain in colorectal cancer or ovarian cancer);

- inexplicable night sweats;
- unexplained bleeding: blood in the urine (cancer of the kidney or bladder) or stool, bleeding outside menstrual periods (in cervical cancer or the endometrium);
- weakness or extreme fatigue (leukemia, colon cancer or gastric) (59, 60).

Paraclinically diagnosis (laboratory, imaging, pathology)

Paraclinically diagnosis of cancer is based on various investigations, among which: laboratory testing, imaging processes and pathological examination (biopsy).

Laboratory tests. These include:

a. counting blood elements (the number of red blood cells, white and platelets, hemoglobin, neutrophils or lymphocytes).

b. Evaluation of levels of urea and electrolytes in the blood (allowing assessment of renal function).

c. Making altered liver function tests that occur in liver or gall bladder cancers. These include enzyme assays: transaminases (ALT = alanine aminotransferase, aspartate aminotransferase = AST), which are a marker of liver inflammation; alkaline phosphatase and gamma glutariltransferaza (δ-GT) enzymes with increased levels in the presence of a blockage in the bile duct or liver or as a result of excessive alcohol consumption; and bilirubin and albumin testing.

d. Assessment of tumor markers. These markers can be produced by the tumor or may be produced by the body in response to tumor development. Interpretation of their presence must be done with caution as some are produced within a particular type of cancer, while others appear in different types of cancer or other pathological conditions and non-malignant. For example, CA125 (cancer antigen) is a marker for ovarian cancer with high values, whereas PSA (Prostate Cancer Antigen) has elevated in prostate cancer, and HCG (Human Chorionic Gonadotrophin) in malignant germ cell tumors.

e. The genetic tests. They are those that assess the presence of genetic mutations, such as mutations of the BRCA1 or BRCA2 genes from breast or OVCA gene for ovarian cancer.

Imaging procedures. In this category can be grouped: CT (CT), nuclear imaging assessment (which uses radioactive tracers), MRI (magnetic resonance imaging), X-ray, PET (Positron Emission Tomography), ultrasound, mammography, endoscopy and cystoscopy.

Pathological examination (biopsy). It is the „gold standard" in the diagnosis of cancer and is a test of certainty. Fine needle can be performed by using the endoscope or surgical (61, 62).

Early detection (screening)

Screening is an assessment guidance that allows presumptive identification of unrecognized disease or risk factor, with quizzes, examinations or other investigations. The screening test identifies, in a population apparently in good health, perhaps people affected by the disease, which makes it possible to select them to be subject to further investigation or secondary prevention measures. In general, screening tests are part of a public health program; they seek to detect a pathological condition in the population being different diagnostic tests that are more accurate in determining the type of disease (63).

Cancer screening purposes are: accurate prevalence of cancers in the population composition to health programs; early detection of cancer in secondary prevention measures (before symptoms); determining the natural progression of a cancer; identifying a cancer found in an easier phase that can be treated or cured; decreased risk of cancer death; evaluation of public health programs implemented in the population.

Principles developed by the World Health Organization to establish a screening program include: to address a major health issues; treatment facility to be accepted by the found patient; facilities for diagnosis and treatment; detect disease in its earliest stages; be an adequate test evaluation; to be known elements of the natural history of the disease; be acceptable to the population; be a well-established and accepted strategy on who should be treated as patients; cost detection and treatment of subjects detected by screening does not exceed the total cost of health care needed cancer cases; allowing continuous tracking (to ensure continuity of action) (63, 64).

These examinations and tests for early detection of cancers include: medical history and clinical examination; laboratory tests (blood tests, urine, tissue, etc.); imaging procedures (mammography, ultrasound, colposcopy, colonoscopy, nuclear magnetic resonance - NMR etc.) and genetic tests (to identify possible mutations in genes known to increase the risk of many different cancers). Among the methods used for screening, direct or assisted visual observation method is most easily performed to examine cancer, which is useful for identifying skin lesions, retinal, lips, mouth, larynx, cervix and external genitalia. The second most available

method for early detection of cancers is to identify nodules or tumors of the breast, mouth, salivary glands, thyroid, subcutaneous tissue, anus, rectum, prostate, testes, ovaries and uterus or nodules bulky nodes in the neck, armpit or groin by palpation. Viewing cancers of the internal procedures and imaging tests such as endoscopy (ex. Colorectal cancer), radiography, mammography (ex. Breast cancer), ultrasound or MRI. Laboratory tests (ex. Cytology or Pap test for identifying guaiac fecal occult Chair) can also be used to detect certain cancers. For subjects who are at increased risk of developing cancer, such as those who have a personal or family history loaded (two or more first degree relatives affected) and that have a high probability of being a mutation or genetic polymorphism, using various genetic screening tests (64, 65).

Screening test performance is measured usually in terms of sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV). Sensitivity is the probability that the test is positive the person is really sick (in other words, is the ability to correctly detect people affected by cancer). Specificity is the probability that the test is negative the person is not sick (that is the ability of the test to identify real people without cancer). VPP indicates the probability that the person to have cancer when the test is positive and highlights VPN probability that the person is not sick when the test is negative. A screening test is more powerful as the sensitivity and specificity are closer to 100% (63, 64).

The benefits of screening. Cancers identified by screening are generally discovered in an early stage, they are easier to treat, require less treatment and sometimes it is possible to heal.

The disadvantages of screening are varied, most related to technical or significance of the result, and include:

a. False positive results, which represent the positive results experienced by people who have cancer and can cause anxiety subjects in question and determine the use of additional diagnostic tests for people who have no risk;

b. False negative results are negative outcomes identified in people suffering from cancer, with consequent delay discovery of cancer lesions, those subjects were diagnosed in advanced stages, with more reserved prognosis;

c. Overdiagnoses and overtreatment refers to identify very slow-moving cancers that would never have occurred inconvenience / problems

relevant subjects. Most women with breast cancer, for example, receive the recommendation therapeutic surgery to remove the cancer, accompanied, for most of them, radiotherapy, chemotherapy or hormone therapy. For women who have slow growing cancers may not need treatment to never, but currently can not be a shootout between the two types of malignancies (very slow-moving and rapidly evolving);

d. The side effects of the tests (after sigmoidoscopy or colonoscopy ex.sângerare, exposure to low doses of radiation during mammography) (64, 66)..

Examples of screening.

a. Screening for the early identification of breast cancer include (64, 65, 66, 67, 68):

- breast self-examination, from the age of 20, carried out at 5-7 days after the menstrual period;
- to specialist clinical examination conducted once every three years for women aged between 20 and 40 years and annually for those over 40 years;
- annual mammograms from the age of 40, according to the American Cancer Society;
- for women at high risk (those with family history uploaded those known to have mutations in the BRCA1 and BRCA2, which have first degree relatives with mutations in BRCA1 or BRCA2, people who were exposed to chest radiographs in the age 10-30 years; women with RDS. Li-Fraumeni, RDS. Cowden or those who have first degree relatives with such syndromes) mammography and MRI is recommended annually.

Screening for the early identification of cervical cancer

includes (64, 66, 69, 70):

- for women aged between 21 and 29 years recommended a Pap test every 3 years; HPV testing is not recommended before abnormal Papanicolaou test identification;
- for women aged between 30 and 65 is recommended Pap test and HPV test every five years (this recommendation is preferred), but can also be used Pap test every 3 years;
- in women over 65 who were regularly tested were negative and no longer subjected to the test;
- women vaccinated against HPV screening should continue to age.

According to WHO guidelines, screening tests of cervical cancer

include HPV testing, visual inspection with acetic acid, Papanicolaou cytology and colposcopy for early detection. Screening strategies including various combinations of these different tests, depending on the existence or absence of previously implemented screening programs and resources available in this population (70).

Epidemiological surveillance

Epidemiological Surveillance (ES) of cancers represent collection and systematic analysis and interpretation of data essential for planning, implementation and evaluation of public health measures, closely linked to the dissemination of these data to those interested (source data and factors decision). ES is the application of data in prevention and disease control.

Most countries have national programs for surveillance of diseases, both communicable and non-communicable.

Steps ES of cancers include identifying the priorities, using standardized case definitions to define the problems, develop an algorithm for reporting, data analysis, personnel involved, feedback actions. It were illustrated on cervical cancer.

Th identification of the priorities can be done using several methods: gathering prior information regarding the prevalence of cancers and FR at regional level; conducting a cross-sectional study to assess the prevalence FR for cancers in the population assisted; conducting an analytical cohort or case-control for ranking FR after power association with malignancy: relative risk (RR) or odds ratio (OR)> 2.

Using standardized case definitions for defining problems include:

a) Check that each FR identified to the definition.

examples:

1. early menarche = menstruation before the age of 12;
2. Early onset of sexual = sexual contact before age of 18;
3. The first pregnancy after age of 30 = the first term pregnancies, premature or stopped evolving after the age of 30;
4. Menopause = late last period of life of women aged over 52;
5. Hormonal treatments with high-dose oral contraceptives with estrogen doses of estrogen => 0,625mg / pill;
6. Tobacco:

- Tobacco active => 5 cigarettes / day for ≥ 3 years
- Passive smoking: Exposure> 3 hours / day in a confined space for ≥ 3 years.

b) accurate evaluation of diagnosis and classification of cancers after the case definition.

Example: Staging the International Federation of Gynecology and Obstetrics (FIGO) cervical cancer (71).

Data analysis. It will create a database and check-TA registration correction information. It also will analyze and report the following indicators:

- Monthly: number of patients treated in stages; number of patients treated by type of therapy;
- Quarterly: number of persons examined by screening; the incidence rates of cases diagnosed in stages; number of deaths by cancer.

Personnel involved. Problems related to this issue include: motivating staff involved through the contract with CNAS, medical training of staff involved in supervising cancers (epidemiology descriptive and analytical recommendations of practice guidelines, using PC), using a system of control and competition to increase quality benefit (46, 72).

Develop an algorithm for reporting. The algorithm of reporting for supervision of cancers include communications from the primary (physician, physician or physician) to the intermediate level (Directorate of Public Health, Institute of Public Health and the Cancer Registry), from the data being directed to the central (represented by the Ministry of Health), which report international level represented by international organizations (World Health Organization - wHO and the European Centre for Disease Prevention and control - ECDC).

Feedback actions represent a very important step that allows evaluating the effectiveness of the surveillance system cancer prevention and control, with improvement actions implemented in the population.

ES of cancers objectives include determining the prevalence of cancers in the population; detection of risk factors for cancer (examples: sexual life early onset births and abortions repeated oral contraceptives with higher doses of estrogen administered> 5 years in women very young or very old ~ for cervical cancer) and cancer screening early by various methods (laboratory, imaging).

SE modalities of cancers are multiple and include:

- epidemiological triage (screening) for early detection of cervical cancer (Pap test citodiagnostic);
- epidemiological triage (screening) for early detection of breast cancer (breast self-examination, clinical examination, mammography);

- epidemiological triage (screening) for the detection of other cancers with high incidence (colon, lung, prostate, etc.);
- training and retraining of family doctors and other specialists;
- Health education, individual and group of patients with risk factors.

Prevention measures

An important aspect is the fact that at least one third of all cancer cases are preventable, and prevention offers the most cost-effective long-term strategy for cancer control (WHO).

Although our genes influence the cancer risk, most often the difference to cancer risk in the population is due to factors that are not hereditary.

General prevention addressing individuals apparently healthy exposed to the risk factors and includes measures to avoid and / or limiting the action of modifiable risk factors (medical evaluation regular, avoiding smoking, limiting alcohol consumption, avoiding excessive exposure to ultraviolet solar use in beauty salons, tanning, etc.) and promoting protective factors (the use of a diet rich in fruits and vegetables, maintaining a healthy body weight and physical activity making) (73, 74). These measures include epidemiological surveillance, health education etc. Options for adopting a healthy lifestyle can be made by each of us, but they can be sustained or slowed by the social, physical and economic environments in which we live and the existing legal regulations. To create an enabling environment to facilitate adoption by the population of these healthy behaviors requires sustained efforts of that community (46).

Prevention of special addresses population groups identified with an increased risk of developing cancer and include measures such as oophorectomy, prophylactic bilateral prevention of breast and ovary in carriers of mutations BRCA or OVCA (ovarian cancer) or prophylactic mastectomy to reduce the risk for cancer breast mutations in BRCA carriers (67, 75).

Prevention includes specific measures targeted to certain types of cancer or FR: vaccination or screening. For example, vaccination against infections with oncogenic types 16 and 18. HPV can prevent over 90% of persistent infections with this organism and thus may prevent cervical cancer. There are several that can be used in vaccine preparations: the bivalent vaccine (which contains two of the most common oncogenic strains involved in the production of cancer of the cervix: 16 and 18); quadrivalent vaccine (includes two oncogenic strains - 16, 18 and two

other strains non-oncogenes - 6:11, responsible for producing warts skin) and, most recently, approved this year by the European Medicines Agency (EMA) vaccine nonavalent (which includes, in addition to the above mentioned four strains, four strains oncogene 31, 33, 45, 52, 58) (76). Another example of a vaccine that prevents the occurrence of a type of cancer is one against HBV, which can also prevent the development of hepatocellular carcinoma.

Screening for early detection of cancers is a secondary prevention measure that allows detection of pre-cancerous changes, those which are in a reversible stage or in an early stage of disease (72, 73, 76).

Therapeutic measures include: chemotherapy, radiation therapy, surgery, gene therapy.

Bibliography

1. Burnet N.G., Jefferies S.J., Benson R.J., Hunt, D.P., Treasure F.P. *Years of life lost (YLL) from cancer is an important measure of population burden--and should be considered when allocating research funds. Br J Cancer* 2005; 92 (2) : 241-245.

2. Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F. GLOBOCAN 2012 v1.0, *Cancer Incidence and Mortality Worldwide: IARC CancerBase* No. 11, 2013 [Internet]. Lyon, France: International Agency for Research on Cancer. (http://globocan.iarc.fr.).

3. Brustugun O.T., Møller B., Helland Å. *Years of life lost as a measure of cancer burden on a national level. BJC* 2014; 111:1014-1020. doi: 10.1038/bjc.2014.364.

4. Aoun S., Pennebaker D., Pascal R. *To what extent is health and medical research funding associated with the burden of disease in Australia? Aust N Z J Public Health* 2004; 28 (1) : 80-86.

5. Soerjomataram I., Lortet-Tieulent J., Parkin D.M., Ferlay J., Mathers C., Forman D. et al. *Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. Lancet* 2012; 380 (9856) : 1840-1850.

6. Soerjomataram I., Lortet-Tieulent J., Ferlay J., Forman D., Mathers C., Parkin D.M., Bray F. *Estimating and validating disability-adjusted life years at the global level: a methodological framework for cancer. BMC Med Res Methodol* 2012; 12 : 125.

7. *** *The global economic cost of cancer.* Report of American Cancer Society and LIVESTRONG, 2010.

8. *** *International Agency for Research on Cancer. List of Classifications by cancer sites with sufficient or limited evidence in humans*, Volumes 1 to 112.

(http://monographs.iarc.fr/ENG/Classification/index.php). Accesat în aprilie 2015.

9. *** World Cancer Research Fund/American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective.* Washington DC: AICR, 2007.

10. Stewart B.W., Wild C.P. *World Cancer Report 2014*, IARC, WHO Press, 2014.

11. *** American Cancer Society. *History of cancer*, 2014.

12. Faguet G.B. *A brief history of cancer: age-old milestones underlying our current knowledge database. Int J Cancer* 2015; 136 (9) : 2022-2036. Doi: 10.1002/ijc.29134.

13. *** PDQ database of National Cancer Institute. *Cigarette Smoking: Health Risks and How to Quit* - Aprilie 2015.

(http://www.cancer.gov/cancertopics/pdq/prevention/control-of-tobacco-use).

14. *** *IARC. Tobacco smoke and involuntary smoking.* In: *IARC Monographs on the evaluation of carcinogenic risks to humans*, vol. 83; Lyon, France, 2004. Disponibilr on-line. Accesatr on Aprilie 2015.

(http://monographs.iarc.fr/ENG/Monographs/vol83/mono83.pdf).

15. Schiffman M., Castle P.E., Jeronimo J., Rodriguez A.C., Wacholder S. *Human papillomavirus and cervical cancer. Lancet* 2007; 370 (9590) : 890–907.

16. Liang P.S., Chen T.Y., Giovannucci E. *Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. Int J Cancer* 2009; 124 (10) : 2406-2415.

17. Huxley R.R., Ansary-Moghaddam A., Clifton P., et al. *The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. Int J Cancer* 2009; 125 (1) : 171-180.

18. *** PDQ database of National Cancer Institute. *Colorectal prevention* – aprilie 2015.

(http://www.cancer.gov/cancertopics/pdq/prevention/colorectal/HealthProfessional/page2).

19. *** Centre d`Expertise Collective de l`Inserm. *Cancer de l`ovaire.* In: *Cancer et environment.* Les ăditions Inserm, Paris, France, 2008, 511-555.

(www.inserm.fr/content/download/.../cancer+environnement.pdf).

20. *** PDQ database of National Cancer Institute. *Breast cancer prevention* – April 2015.

(http://www.cancer.gov/cancertopics/pdq/prevention/breast/HealthProfessional/page2)

21. Gaudet M.M., Gapstur S.M., Sun J. et al. *Active Smoking and Breast Cancer Risk: Original Cohort Data and Meta-Analysis. J Natl Cancer Inst* 2013; 105 (8) : 515-525.

22. Endogenous Hormones Breast Cancer Collaborative Group. *Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. Br J Cancer* 2011; 105 (5) : 709-722.

23. *** PDQ database of National Cancer Institute. *Cervical cancer Prevention* – Aprilie 2015

(http://www.cancer.gov/cancertopics/pdq/prevention/cervical/HealthProfessional)

24. *** *IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents.* In: (IARC monographs on the evaluation of carcinogenic risks to humans, vol. 100, part B; Lyon, France, 2009. Disponibilr on-line – accesatr on aprilie 2015.

(http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B.pdf)

25. Rositch A.F., Koshiol J., Hudgens M.G. *Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. Int J Cancer* 2013; 133 (6) : 1271-1285.

26. International Collaboration of Epidemiological Studies of Cervical Cancer. *Cervical carcinoma and sexual behaviour: collaborative reanalysis of individual data on 15,461 women with cervical carcinoma and 29,164 women without cervical carcinoma from 21 epidemiological studies. Cancer Epidemiol Biomarkers Prev* 2009; 18 (4) : 1060-1069.

27. Albero G., Castellsagué X., Giuliano A.R., Bosch F.X. *Male circumcision and genital human papillomavirus: a systematic review and meta-analysis. Sex Transm Dis* 2012; 39 (2) : 104-113.

28. Cogliano V.J., Baan R., Straif K., et al. *Preventable exposures associated with human cancers. JNCI* 2011; 103 : 1827-1839.

29. *** National Cancer Institute. *Cancer Prevention PDQ Overview.*

(http://www.cancer.gov/cancertopics/pdq/prevention/overview/HealthProfessional/page2#section_2.5).

30. Engels E.A., Pfeiffer R.M., Fraumeni J.F. Jr. et al. *Spectrum of cancer risk among US solid organ transplant recipients. JAMA* 2011; 306 (17) : 1891-1901.

31. Anothaisintawee T., Wiratkapun C., Lerdsitthichai P., Kasamesup V., Wongwaisawan S. et al. *Risk factors for breast cancer: a systematic review and meta-analysis. Asia Pac J Public Health* 2013; 25 (5) : 368-387.

32. *** Cancer Research U.K. *Breast cancer risk factors* – aprilie 2015

(http://www.cancerresearchuk.org/cancer-info/cancerstats/types/breast/riskfactors/breast-cancer-risk-factors).

33. *The Endogenous Hormones and Breast Cancer Collaborative Group. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol* 2010; 11 (6) : 530-542.

34. Collaborative Group on Hormonal Factors in Breast Cancer. *Menarche,*

menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet 2012; 13 : 1141-1151.

35. Moorman P.G., Havrilesky L.J., et al. *Oral contraceptives and risk of ovarian cancer and breast cancer among high-risk women: a systematic review and meta-analysis. J Clin Oncol* 2013; 31 (33) : 4188-4198.

36. Chlebowski R.T., Manson J.E., Anderson G.L., et al. *Estrogen plus progestin and breast cancer incidence and mortality in the Women's Health Initiative Observational Study. J Natl Cancer Inst* 2013; 105 (8) : 526-535.

37. Thorbjarnardottir T., Olafsdottir E.J., Valdimarsdottir U.A., et al. *Oral contraceptives, hormone replacement therapy and breast cancer risk: A cohort study of 16 928 women 48 years and older. Acta Oncol* 2014; 53 (6) : 752-758.

38. Turnbull C., Rahman N. *Genetic Predisposition to Breast Cancer: Past, Present, and Future. Ann Rev Genom Hum Genet* 2008; 9 (1) : 321-345.

39. Cheraghi Z., Poorolajal J., Hashem T., et al. *Effect of Body Mass Index on Breast Cancer during Premenopausal and Postmenopausal Periods: A Meta-Analysis. PLoS ONE* 2012;7(12):e51446.

40. Vo C., Carney M.E. *Ovarian cancer hormonal and environmental risk effect. Obstet. Gynecol Clin N Am* 2007; 34 : 687-700.

41. *** Cancer Research UK. *Ovarian cancer risk factors* – aprilie 2015. (http://www.cancerresearchuk.org/cancer-info/cancerstats/types/ovary/riskfactors/uk-ovarian-cancer-risk-factors#source19).

42. *** PDQ database of National Cancer Institute. *Ovarian, Fallopian Tube, and Primary Peritoneal Cancer Prevention* – aprilie 2015 (http://www.cancer.gov/cancertopics/pdq/prevention/ovarian/HealthProfessional)

43. Jervis S., Song H., Lee A., et al. *Ovarian cancer familial relative risks by tumour subtypes and by known ovarian cancer genetic susceptibility variants. J Med Genet* 2014; 51 (2) : 108-113.

44. *** Cancer Research UK. *Bowel cancer risk factors* – aprilie 2015 (http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/riskfactors/bowel-cancer-risk-factors)

45. Johnson C.M., Wei C., Ensor J.E. et al. *Meta-analyses of colorectal cancer risk factors. Cancer Causes Control* 2013; 24 (6) : 1207-1222.

46. *** World Health Organization. *Global Action Plan for the prevention and control of noncommunicable diseases*. WHO Press, Geneva, Switzerland, 2013.

47. Rothwell P.M., Fowkes F.G., Belch J.F., et al.: *Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet* 2011; 377 (9759) : 31-41.

48. Collaborative Group on Hormonal Factors in Breast Cancer. *Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast*

cancer and 96 973 women without the disease. Lancet 2002; 360 (9328) : 187-195.

49. Wu Y., Zhang D., Kang S. *Physical activity and risk of breast cancer: a meta-analysis of prospective studies. Breast Cancer Res Treat* 2013; 137 (3) : 869-882.

50. Ilus T., Kaukinen K., Virta L.J., et al. *Incidence of malignancies in diagnosed celiac patients: a population-based estimate. Am J Gastroenterol* 2014; 109 (9) : 1471-1477.

51. Luo T., Yan H.-M., He P., et al. *Aspirin use and breast cancer risk: a meta-analysis. Breast Cancer Res Treat* 2012; 131 (2) : 581-587.

52. Tsilidis K.K., Allen N.E., Key T.J., et al. *Menopausal hormone therapy and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer* 2011; 128 (8) : 1881-1889.

53. Luan N.N., Wu L., Gong T.T., et al. *Nonlinear reduction in risk for colorectal cancer by oral contraceptive use: a meta-analysis of epidemiological studies. Cancer Causes Control* 2015; 26 (1) : 65-78.

54. ***GLOBOCAN 2012: *Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012.* (http://globocan.iarc.fr/Pages/fact_sheets_population.aspx)

55. *** International Agency for Research on Cancer. *Latest world cancer statistics*. Press release 2013; nr. 223.

56. Ferlay J., Steliarova-Foucher E., Lortet-Tieulent J., Rosso S., Coebergh JWW, Comber H., Forman D., Bray F. *Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer* 2013; 49 (6) : 1374-1403.

57. *** EUCAN (http://eco.iarc.fr/EUCAN/Reference.aspx).

58. *** American Cancer society. *Signs and symptoms of cancer* (http://www.cancer.org/cancer/cancerbasics/signs-and-symptoms-of-cancer).

59. *** National Cancer Institute. *Symptoms of cancers* (http://www.cancer.gov/cancertopics/diagnosis-staging/symptoms)

60. *** Cancer Research UK. *Key signs and symptoms of cancer* (http://www.cancerresearchuk.org/about-cancer/cancer-symptoms).

61. *** National Cancer Institute. *Diagnosis.* (http://www.cancer.gov/cancertopics/diagnosis-staging/diagnosis).

62. ***CancerResearchUK.*Cance tests.* (http://www.cancerresearchuk.org/about-cancer/cancers-in-general/tests/blood-tests?script=true).

63. Azoicăi D., Boiculese L., Pisică-Donose G. *Noţiuni de metodologie epidemiologică şi statistică medicală*. Editura Edit. DAN, Iaşi, 2001.

64. *** National Cancer Institute. *Cancer Screening Overview (PDQ®).* (http://www.cancer.gov/cancertopics/pdq/screening/overview/).

65. *** American Cancer Society. *American Cancer Society Guidelines for the Early Detection of Cancer.*

(http://www.cancer.org/healthy/findcancerearly/cancerscreeningguidelines/american-cancer-society-guidelines-for-the-early-detection-of-cancer).

66. *** Cancer Research UK. *Understanding Cancer Screening.* (http://www.cancerresearchuk.org/about-cancer/screening/understanding-cancer-screening).

67. *** American Cancer Society, 2014. *Breast Cancer Prevention and Early Detection.* (http://www.cancer.org/acs/groups/cid/documents/webcontent/003165-pdf.pdf).

68. *** American Cancer Society, 2014. *Cervical Cancer Prevention and Early Detection* (http://www.cancer.org/acs/groups/cid/documents/webcontent/003167-pdf.pdf).

69. *** American Cancer Society, 2014. *Cervical Cancer Prevention and Early Detection* (http://www.cancer.org/acs/groups/cid/documents/webcontent/003167-pdf.pdf).

70. *** WHO guidelines. *WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention.* WHO Press, Geneva, Switzerland, 2013.

(http://apps.who.int/iris/bitstream/10665/94830/1/9789241548694_eng.pdf).

71. *** FIGO Committee on Gynecologic Oncology: FIGO staging for carcinoma of the vulva, cervix, and corpus uteri. *Int J Gynaecol Obstet* 2014; 125 (2) : 97-98.

72. *** WHO. *National cancer control programmes: policies and managerial guidelines.* 2nd ed. Health & Development Networks, Italy, 2002.

(http://www.who.int/cancer/media/en/408.pdf).

73. *** Kushi L H, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, Gapstur S, Patel AV, Andrews K, *Gansler T and the American Cancer Society 2010 Nutrition and Physical Activity Guidelines Advisory Committee. American Cancer Society Guidelines on nutrition and physical activity for cancer prevention. CA: A Cancer Journal for Clinicians* 2012; 62 : 30–67. doi: 10.3322/caac.20140.

74. dos Santos Silva I. *Cancer Epidemiology: principles and methods.* International Agency for Research on Cancer, Lyon France, 1999.

75. *** American Cancer Society, 2014. *Ovarian cancer.*

(http://www.cancer.org/acs/groups/cid/documents/webcontent/003130-pdf.pdf).

76. Nelson R. *EU Recommends Approval of Gardasil 9 Vaccine. Medscape.* March 27, 2015.

3. Epidemiology of obesity

Introduction

Obesity is a complex of conditions that requires the existence of an excessive amount of body fat. It is not only an individual thar suffer, but it is a real public health problem with multiple causes. Obesity induces the risk of heart disease, diabetes mellitus (DM) or hypertension (HTA) as well (1, 2, 3, 4, 5, 6).

Worldwide, the prevalence of obesity is doubled in the period 1980-2014. In 2014, this disease has affected 11% of men aged 18 and over and 15% of women of the same age, and the number of overweight children aged under 5 years reached 42 million in 2013 (7).

Obesity can be prevented by applying a series of measures to promote physical activity and use a healthy diet throughout life, at the population, the community (institutional) and individual level (7).

Short history

The term of *„obesity"* comes from the Latin *„obesitas"*, which means „robust", „fat" or „fattening" is first mentioned by Randle Cotgrave in his dictionary published in 1611 (8).

Obesity was detected in the human population since the earliest times by numerous figurines, statuettes of the most popular by 23000-25000 years ago in the Paleolithic period, the „Venus of Willendorf" found in Austria today, or „Mother Goddess" Neolithic (8000-5500 BC) on the current territory of Turkey. Typical of many such statues was a major abdominal obesity. Such statues *„obese"* from the same period were found in several sites of Europe and other continents. Anthropological studies shown that obesity was uncommon in the population at that time. In ancient times, the obesity was rare and considered „unacceptable", however, historians shown that Inhapi queen and the pharaoh Ramesses III suffer of obesity. Risks associated with obesity were well known in Hippocratic school, which notes that „Sudden death is more common in the obese than in those weak" and that „obesity is a cause of infertility in women." 500 years later, Galen's from Roman medical school classified the obesity in „moderate" and „immoderate" or morbid, and the school's Arabic Avicenna describes obesity as „a threat" to health. In India, doctors Susruta and Charak (500-400 BC) observed the link between „the sweet taste of diabetic urine" and that occurs frequently in obese people who eat excessively, especially the „sweets and fats." In 1679, Bonetus was first that performed the dissections

on the obese dead. Later, descriptions of obesity were made by Morgagni and Haller. In the 50s XIX centery, Adipocytes highlighted the increased body fat. It was described by Hass in 1849, which suggested that certain types of obesity may result from the increased number of fat cells, thus becoming the forerunner of the concept of obesity „hyperplastic" concept that was further developed by Bjurulf, Hirsch and Björntorp. In 1727, the first monograph on obesity, treatment and recovery indicated that the body's natural balance can be achieved through exercise and diet through a „moderate". In 1760 interesting four essential causes of obesity were mentioned by Flemyng: first, „too much food rich in fat" (noting that not all obese people are „big eaters"); Second, „too much laxity of the membrane of fat cells"; Third, „an abnormality of blood, fat deposition"; Fourth, „a faulty exhaust." Over time, there have been numerous attempts to classify obesity (English doctor Sydenham in the eighteenth century, doctor and chemist English Cullen and French physician Sauvages, a century later), and statistician Belgian Quetelet was one of the first they have developed and validated mathematical methods of measuring the obesity, suggesting that the ratio of the weight of the individual, and the square of the waist may be used as a measure of body fat. This report, known as the Body Mass Index (BMI) is currently named in some European countries „Quetelet index" (QI). It has been shown that IMC is correlated with the content of fat and that can be predicted risk for obesity-related co-morbidities. In the XVIV century was formulated the concept of macronutrients and their role in various pathologies, including the link between increased consumption of carbohydrates and obesity with increased susceptibility to type II diabetes and sudden death. Thus, life insurance emphasizing in the early twentieth century, that „overweight, especially around the abdomen, was associated with a decreased life expectancy." This risk was subsequently proven by numerous epidemiological studies worldwide and achieve the WHO in 1995, the first classification of obesity with risk stratification, with increasing BMI values. Other important findings related to the pathology of obesity are metabolic syndrome (syndrome „X") related to the research of Vague (1947) and Reaven (1980), obesity neuro-endocrine and research of Babinski, Fröhlich and Cushing, adipostatul and studies Kennedy (1953), leptin and Friedman's research team in 1983 (the name coming from the Greek „Leptos" - „weak", „thin"). Davenport, in 1923, observed a family

transmission of elevated BMI and current molecular genetic studies bring new data on obesity, common' common in contemporary populations (9).

Risk factors

Obesity is a complex diseases, whose appearance are determined by the combined actions of several factors (behavioral, genetic and environmental), which include: lack of energy balance, inactivity, environment, genetic factors and family history, diet and eating habits unhealthy some medical conditions, pregnancy, lack of sleep, some medications, age, emotional factors, smoking, and cultural factors (6, 10, 11, 12, 13, 14).

Lack of energy balance. Although there are genetic and hormonal factors that influence body weight, overweight and obesity are most often caused by lack of energy balance. Obesity is installed in time when, the amount of calories brought into the body through food and drink (energy produced) are greater constantly than the amount of calories burned to support daily processes of respiration, digestion and physical activity (energy consumed) (11).

Inactivity. Sedentary lifestyle promotes excess of calories to the body, which fail to be burned completely through daily physical activity. The reason why some people are sedentary is that they spend long hours in front of the TV or computer, working, making or having fun. Studies show that 2 hours spent daily watching television increases the risk of overweight and obesity. Other causes are: car use instead of walking, less physical activity at work or at home due to the use of modern technologies and lack of physical classes in schools. Inactive people are more likely to gain weight and an increased risk of developing coronary artery disease, hypertension, diabetes and colorectal cancer (10, 11, 12, 13).

Environment (socio-economic). Our environment lead to the obesity rather than promoting or supporting a healthy lifestyle: lack of sidewalks for pedestrians, parks or recreational places; lack of affordable gyms; loaded work program (lack of time for physical activity); big food portions in restaurants, „fast food" in supermarkets or home; lack of access to healthy food (not close or are expensive); advertisements for food (these advertisements have frequently targeted children, to promote calorie products) (11).

Genetic factors and family history. Epidemiological studies show that genes have a strong influence on a person's body weight, overweight and obesity tend to manifest themselves in the family. The obesity risk is

greater when one or both parents are overweight or obese. Also, genes may influence the amount of fat that a person can store in his body and where this fat will be distributed.

In the family, there are common genes but also common behaivors (in terms of diet and physical activity), so the risk of overweight or obese is the result of complex interaction of genes with environmental factors. For example, if a child is overweight or obese with parents who have bad eating habits (high calorie diet) and are inactive have a higher risk of becoming overweight or obese because children adopte parents' behavior. However, if the child's parents adopt a healthy lifestyle (diet and physical activity), its risk of getting obese will be reduced (10, 11).

Diet and unhealthy eating habits. A diet rich in calories, low in fruits and vegetables full of „fast food", no breakfast, big portions of food contributes to gain of body weight (6, 10, 11, 12, 13, 14).

Some medical conditions. Obesity can sometimes be secondary to the diseases like syndromes: Prader-Willi, sdr. Bardet Biedl, sdr. Cushing or SDR. polycystic ovary syndrome (affecting 5-10% of women of childhood age). Arthritis decrease physical activity and gain weight. Also, a slow metabolism (such as in hypothyroidism) increases the risk of body weight, with the development of obesity (10, 11).

Pregnancy. Weight gain during the pregnancy is normal, but some women fail to return to weight after the pregnancy, thus creating developing of obesity (10, 12).

Insufficiant sleep. Sleep disturbances can cause changes in hormones responsible for appetite. The person eating foods rich in calories and carbohydrates contributes to weight gain. Persons that do not sleep cause hormonal imbalance by increasing ghrelin (which is responsible for hunger) and lowering leptin (responsible for satiety), which promote obesity (10, 12).

Some drugs. Some therapies (some antidepressants, anticonvulsant medication, diabetes, antipsychotic, corticosteroids and beta-blockers) can lead to weight gain (by increasing appetite, decreasing the rate of burning calories, increasing of water retention in the body), if not excluded by diet or physical activity (11, 12, 13, 14).

Age. Muscle mass is lost with age (especially in those less active) and rate of calorie burning in the body decreases. If calorie intake is not restricted to seniors, then they can easily gain weight. Also during menopausal women often earn a few extra kilos in body weight (10, 11).

Emotional Factors. Some people have an increased appetite when they are bored, angry or stressed. This behavior leads to weight gain and obesity (11).

Smoking. Some individuals gain weight when they stop smoking. One explanation may be that food tastes better and it has a more pleasant smell after excluding smoking (11).

Cultural factors. The culture of a particular person may influence their weight. Some cultures use foods with a high fat or sugar, which leads to difficult maintainance of normal body weight. Other cultures have a number of family risk at which individuals consume increased amounts of food (6).

Protective factors

Protective factors are those characteristics of individual or extrinsic behaviors, which are designed to reduce the effects of events causing stress or illness and also increase a person's ability to avoid risks, promoting social and emotional competence (15). So category of protective factors include factors that decrease the risk of obesity.

Protective factors against obesity are: regular physical activity, increased consumption of non polysaccharides or high level of fiber in food, favorable family and school environment (for children) or social (young adults) and lactation *(Table 8)* (16).

Table 8

Protective factors in obesity (according 16)

Factors of protection	Scientifical proov
Regular physical activity	Convincing
A diet rich in fiber or non-starch polysaccharides	Convincing
Breastfeeding	Probable
Family and school environment / social support for healthy eating choice	Probable
Diet with low glycemic index	Probable
Increasing the number of daily meals	Insuficient

Regular physical activity. Protection is one of the factor important in prevention of weight gain. Most studies suggest that moderate levels of daily physical activity during 40-60 minutes can prevent obesity (17). Moderate exercise, since childhood, represents an attractive to prevent the

greater when one or both parents are overweight or obese. Also, genes may influence the amount of fat that a person can store in his body and where this fat will be distributed.

In the family, there are common genes but also common behaivors (in terms of diet and physical activity), so the risk of overweight or obese is the result of complex interaction of genes with environmental factors. For example, if a child is overweight or obese with parents who have bad eating habits (high calorie diet) and are inactive have a higher risk of becoming overweight or obese because children adopte parents' behavior. However, if the child's parents adopt a healthy lifestyle (diet and physical activity), its risk of getting obese will be reduced (10, 11).

Diet and unhealthy eating habits. A diet rich in calories, low in fruits and vegetables full of „fast food", no breakfast, big portions of food contributes to gain of body weight (6, 10, 11, 12, 13, 14).

Some medical conditions. Obesity can sometimes be secondary to the diseases like syndromes: Prader-Willi, sdr. Bardet Biedl, sdr. Cushing or SDR. polycystic ovary syndrome (affecting 5-10% of women of childhood age). Arthritis decrease physical activity and gain weight. Also, a slow metabolism (such as in hypothyroidism) increases the risk of body weight, with the development of obesity (10, 11).

Pregnancy. Weight gain during the pregnancy is normal, but some women fail to return to weight after the pregnancy, thus creating developing of obesity (10, 12).

Insufficiant sleep. Sleep disturbances can cause changes in hormones responsible for appetite. The person eating foods rich in calories and carbohydrates contributes to weight gain. Persons that do not sleep cause hormonal imbalance by increasing ghrelin (which is responsible for hunger) and lowering leptin (responsible for satiety), which promote obesity (10, 12).

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obesity. This depends on several areas of the community and not only those that promote health (18).

Diet rich in polysaccharides without of amidine/ fiber. Because often they are confused, that sugars are predominantly mono or disaccharide and polysaccharides are with or without amidine (the without amidine are called „dietary fiber"). A high intake of these non- amidine polysaccharides, or „fiber" amidine was associated with low BMI in many epidemiological studies (19, 20). The mechanisms of action that explain the effects of fiber-rich diet are a lot. Among these include hormonal effects, including actions on glycemia and insulinemie, or actions on colon (fermentation production of short chain fatty acids and influencing the satiety) (21).

Breastfeeding. A significant number of studies in large groups of people for extended periods have shown that breastfeeding is a potential protective factor against obesity occurring in childhood. This is important because weight gain by children and adolescents leads to adult obesity (22, 23). In an extensive study, von Kries et al showed that the prevalence of obesity in children breastfed group was 2.8% compared to 4.5% in the group who had never been breastfeded. This link barrier depends on the duration of breastfeeding. So, a cohort study conducted in the USA, a number of over 15,000 boys and girls aged of 9 - 14, reported a significant reduction (approximately 20%) of the risk to become overweight / obese associated with breastfeeding in the first 6 months of life infant (24).

BMI of the mother is an important determinant of obesity in childhood, overweight or obese mothers tend to breastfeed less and for a short period of time (25).

Family and social environment promoter of a healthy lifestyle through regular consumption of fruits and vegetables, avoid eating of «fast food» and consumption of „energizing"sweet drinks, performing physical activity daily are protective factors against obesity in children and adult (16).

Glycemic index. High level of carbohydrates increase the blood glucose and insulinemie with effect on subsequent food intake, increasing of their effect on satiety level. So eating foods and beverages with a low glycemic index is a protective factor (perhaps after some studies), by increasing satiety (26).

Increasing the number of daily meals is a protective factor with insufficient scientific evidence to date. However, some studies claim that

portioning food intake into several meals daily helps maintain optimal weight (16).

The manifestation of epidemiological process / extent of events / Incidence, prevalence, mortality

In the last four decades, the prevalence of obesity had a significant increase, being described as a global pandemic, but with marked variations from one region to another and with distinct characteristics. In developed countries, increased prevalence began in the 80s, but subsided in the last 8 years. In developing regions, where two-thirds of obese today's world, it continues to grow. Populations of Pacific Islands and the Caribbean Islands and the Middle East countries and Central America have been also increased rates of obesity (27, 28, 29).

In 2008, it was estimated that 35% of adults over 20 years are overweight (BMI ≥ 25 kg / m2) (34% of men and 35% of women). Worldwide, the prevalence of obesity values almost doubled between 1980 and 2008, so that 10% of men and 14% of women were obese (BMI ≥30 kg / m2) compared with 5% of men and 8% of 80s women, ie an estimated 205 million men and 297 million women aged over 20 years were obese, with a total of over half a billion obese adults worldwide. The prevalence of overweight and obesity has increased in most regions values America (62% overweight and 26% obese), while the lowest values in the Southeast Asian (14% overweight and 3% obese). In parts of Europe, the Eastern Mediterranean and the Americas, more than 50% of women were overweight (of which 23% in Europe, 24% in the Eastern Mediterranean and 29% in the Americas). According to reports, worldwide, women prevalence showed higher values of obesity than men. In regions of Africa, the Eastern Mediterranean and South-East Asia, the prevalence of obesity in women record doubles values compared to men. The epidemic of obesity has expanded in recent decades, in such as Asian and Pacific regions (30).

In 2010, overweight and obesity have caused 3.4 million deaths, 4% of yll (years of life lost) and 4% of DALYs (years of disability-adjusted life by estimating the burden of disease in the population) (29, 31). In 2013, 42 million children younger than 5 years were overweight or obese, and in 2014, according to statistics of the World Health Organization (WHO), more than 1.9 billion adults over 18 were overweight (39%) and of these, over 600 million were obese (13%) (32).

In Europe, the prevalence of obesity in children has tripled in the last two decades: children 6-11 years, from 6.5% in 1980 to 19.6% in 2008

to adolescents in the age group 12 to 19 years of from 5% to 18.1% and preschoolers 2-5 years from 5% in 1980 to 10.4% in 2008.

Lifestyle changes with the adoption of „western", urbanization and mechanization extended to all regions of the Earth are associated with increases in prevalence and mortality complications of obesity (diabetes type II, heart attack, stroke, sudden death) (33).

Clinical manifestations and complications / risks of obesity

Weight gain is a phenomenon that usually occurs in time and is manifested by following signs and symptoms: clothes become tight and the person requires clothing with a larger size than usual; shows a higher weight scales; presence of fat mass around the waist (36).

The consequences of obesity are both medical, and emotional and socio-economic. Obese people have a greater likelihood of developing a variety of health problems, such as lowering triglycerides and raising HDL-cholesterol (HDL = density lipoprotein); Type II diabetes, which is an important cause of premature death, heart disease, stroke (stroke), kidney disease and blindness; HTA; sdr. metabolic (abdominal obesity, hyperglycemia, hypertension, high triglycerides and low HDL-cholesterol) that increase the risk for cardiovascular disease, diabetes and stroke; heart diseases (angina pectoris, myocardial infarction or heart failure); AVC; cancers (endometrial, cervical, ovarian, breast, colorectal, oesophageal, liver, gall bladder, pancreatic, renal or prostate cancer); breathing disorders (sleep apnea); gallbladder disease (cholelithiasis); Gastro-oesophageal reflux; gynecological disorders (infertility, irregular menstruation); medical problems during pregnancy (gestational diabetes, hypertension); erectile dysfunction; steatohepatitis non-alcoholic; osteoarthritis (including damage articular knee, hip and spine); skin disorders (difficult healing lesions); sdr. obesity hypoventilation (hypoventilation with hypoxemia); children and adolescents overweight or obesity are showing an increased risk of developing type II diabetes and becoming overweight or obese adults (6, 34, 35, 36, 37, 38).

Also, the quality of life at obese person is lower, being influenced by the following factors: the depression, disability, sexual problems, shame and sense of guilt or social isolation (the person avoids public places), feeling discriminated (in employment, regarding wages, and rental housing opportunities or chances to marry) (36, 37).

The diagnosis

The methods used to diagnose overweight and obesity include: history, clinical examination and tests (6, 34, 35, 39, 40, 41, 42).

Anamnesis assesses the body weight history, efforts to lose weight, physical activity (exercise), dietary behaivor, presence of other comorbidities, existing medication (which could lead to weight gain) or stress levels. It also evaluates and family history to identify possible predisposing conditions.

General clinical examination

Performing tests to identify associated health problems (hypertension, diabetes) blood tests (glucose, cholesterol, liver function tests, etc.); electrocardiogram (EKG).

Calculating BMI

The diagnosis of obesity is most likely when the body mass index (BMI) of an individual is greater than 30 kg / m2.

BMI is defined as the ratio of weight in kilograms and the height in squared meters.

BMI = G (kg) / T2 (m2).

Depending on the BMI indicate a person's weight status *(Table 9)*.

BMI represents a reasonable estimate of body fat, but has some limitations. In fact, BMI is not body fat, so some people like athletes may have a BMI that would place them in the category of obese and have no excess of body fat (overstatement) (35) or underestimate the fat corporal in the elderly or those who have lost muscle mass (41).

Table 9

Weight status according to BMI

BMI (Kg/m²)	Weight status
< 18,5	underweight
18,5 - 24,9	Normal weight
25 – 29,9	Overweight
30 – 34,9	***Obezity I grade***
35- 39,9	***Obezity II grade***
≥ 40	***extreme Obezity***

This measurement should be performed at least once a year.

Measuring of waist circumference.

A better assessment of the excess fat is obtained by measuring the

circumference of the waist measurement that can be used for additional BMI correct diagnosis of overweight (BMI = 25 to 29.9 kg / m2) or with moderate obesity (BMI = 30 - 34.9 kg / m2).

In general, men who have a waist circumference greater than 94 cm and women over 80 cm has a high probability of developing health problems associated with obesity. Values greater than 88 cm for women and 102 cm for men are considered to be representative for the diagnosis of obesity and increase the risk of heart disease and diabetes.

This measurement should be performed at least once a year (39, 40, 41).

Early detection (screening)

Overweight and obesity screening, both in adults and in children, include BMI measurement. Other measurements such as waist-hip ratio, waist circumference, central obesity were independent predictive value of BMI on risk quantification of future morbidity and mortality associated with this pathology (43).

In children, screening programs of nutritional status is assessed using BMI to identify those at risk of becoming overweight or obese and provide information to help them to work properly. After determining the child's BMI, the values obtained are compared with reference values in nomogram respecting appropriate growth corridor age and sex of the child. Benchmarks of nomograms are expressed in percentiles or standard deviations (Z score). By comparison, identifies percentile or standard deviation that investigated the child. Z score is a quantitative measure that determines BMI deviation of a particular child's BMI to a reference population composed of children of the same age and sex. The number of Z-score is the number of standard deviations from the mean. Thus, a Z score of 1.0 is the standard deviation above the average, and a score of 1.0 represents a standard deviation below the average. A score of 0 is equivalent to Z median or 50th percentile value and a Z score of 2.00 corresponds to approximately 95 percentile value (44).

Genetic screening for obesity includes sequencing genes MC4R (Melanocortin 4 receiver - for obesity autosomal dominant) FTO (Fat Mass and Obesity Associated - gene associated with obesity), LEP (leptin - for severe obesity), PCSK9 (Proprotein convert Subtilisin / Kexin type 9 gene associated with the syndrome familial hypercholesterolemia with autosomal dominant inheritance), LDLR (Low-Density Lipoprotein

Receptor associated with familial hypercholesterolemia with autosomal dominant) Apolipoprotein E - ApoE (ApoE-e4 - gene associated atherosclerosis; Apo-e2 - hyperlipoproteinemia associated with the gene III). FTO is the most relevant gene associated with obesity and research conducted on a sample of over 40,000 people showed that individuals who carry a copy of the FTO mutant risk 30% more likely to become obese, while individuals with both alleles mutants have a 70% risk (45, 46, 47, 48, 49).

Epidemiological surveillance

In children and adolescents, surveillance programs include the assessment of nutritional status in a population, for example, students in a particular school in a particular region, etc., to identify the percentage of students who are at risk of developing problems improper weight-related health age and sex. Currently, it pays a particular attention to monitoring the nutritional status to identify overweight and obese children, considering that early problem identification and intervention can improve long-term outcomes. To prevent obesity in the child and possible complications, it is recommended that family doctors and health professionals in school activities include calculating BMI usual child health surveillance (44).

Initiative of the WHO European Supervisory Child Obesity (WHO European Childhood Obesity Surveillance The Initiative - COSI) provide measures and data on overweight / obesity in preschool and school-age children. Member states have recognized the need for harmonized surveillance systems, essential for health policies at European level. In this context, the European Action Plan was developed for Food and Nutrition 2015-2020 (eng. European Food and Nutrition Action Plan 2015-2020). COSI target system does not replace existing national surveillance systems, but rather to integrate as much as possible in them. Countries that have joined so far protocol COSI are number 24 (Albania, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Greece, Hungary, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Malta, Norway, Poland, Portugal Moldova, Romania, Slovenia, Spain, Sweden, the former Yugoslav Republic of Macedonia and Turkey). The main targets of this Action Plan are to reduce inequalities related to access to healthy food, to ensure the right of every individual to have access to food, to promote a healthy lifestyle, to enable people and communities to live in an environment to improve their health status and to use in this regard, evidence-based strategies (50, 51).

Prevention measures

Measures of obesity prevention include primary, secondary and tertiary prophylaxis.

Primary prevention aims to identify risk factors, limiting the action of modifiable factors (environmental, behavioral) to prevent obesity and promote the action of protective factors (sanogenic).

Secondary prevention includes a series of measures to limit the irreversible evolution of the disease. It addresses to homeostatic imbalances people, but are in a reversible early state.

Tertiary prevention is aimed to people already obese, in order, to prevent complications due to obesity, premature death and increase the quality of life for people suffering from overweight and obesity.

Worldwide there are a number of strategies and action plans that include objectives and measures to prevent obesity and its control.

These strategies are grouped into three categories and aimed at all those involved in the development of obesity, and those that influence the evolution of this condition in particular population: population-wide policies; interventions at the community and individual level interventions (7).

Policies at the population level interventions include a range of multi and simultaneous production, distribution and marketing of food and ensure an environment that promotes and facilitates physical activity (7):

- Changing agricultural subsidies granted to encourage the production of vegetables and fruits would be beneficial for increasing fruit and vegetable consumption and improve your diet;
- Establishing healthy food tax, allowing permissive setting of prices for these products, favoring the accessibility to them;
- Establishing additional fees for unhealthy foods and sweetened drinks (which favors the appearance of overweight and obesity);
- The legislation governing trade in foodstuffs can be effective in reducing the availability of unhealthy foods and beverages and changing food habits of the population;
- WHO has established a series of recommendations on advertising food and soft drinks for children, knowing that these advertisements influence children's preferences and attitudes;
- Food education measures (through educational and media campaigns supported) dietary changes can help consumers;

- Campaigns to promote physical activity.

Interventions in the community are addressed to family and community in which individuals live, work and play (schools, universities, workplaces, communities, hospitals, churches etc.) (7) school is an important institution for promoting healthy diet and activity physical (eg. supply of fruit to school pupils can influence their eating behavior outside school); Workplace interventions on diet and physical activity can be effective in changing the behavior of individuals; increasing the availability of healthy eating options at work; Posts that promote the healthy food in cafeterias / restaurants.

Interventions are addressed to both individual persons at risk of becoming obese, those who are already overweight or obese, and those with a normal weight. These same measures include weight loss, namely: conducting daily exercise, a healthy diet and a long-term commitment surveillance of everything they eat and drink (7, 52, 57).

Recommendations of Centers for Disease Control and Prevention (eng. Centers for Disease Control and Prevention - CDC, USA) for preventing obesity include 24 community strategies that local governments can implement (53). They have been divided into six categories:

1. Strategies for promoting the availability of healthy food and at affordable prices;
2. Strategies to support healthy food and drink choice;
3. Strategies to encourage breastfeeding;
4. Strategies to encourage physical activity or limit sedentary lifestyle among children and young people;
5. Strategies to create safe communities that support physical activity;
6. Strategies to encourage communities to organize themselves to facilitate change.

Another action plan aimed, the prevention and control of obesity „Global Action Plan for Prevention and Control 2013-2020" (eng. „Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020") WHO, which aims to provide a range of policy options for member states and other bodies concerned regarding the establishment and implementation of coordinated and coherent measures at all levels, locally and globally, to achieve the new targets global set (target 7 - to stop the increasing number of cases of diabetes and obesity) (54).

„Comprehensive Plan for implementation of maternal nutrition, infant

and young child" (eng. „Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition"), developed by WHO, was approved worldwide (global) in 2012. This action plan presents a number of priority actions that should be implemented by member states and international partners, of course, adapted to the local regional and national authorities in order to achieve six goals on diet (nutrition) overall (56). The objectives to be achieved by 2025 include:

1. A 40% reduction in the overall number of children under 5 are malnourished;
2. A 50% reduction of anemia among women of child age;
3. A 30% reduction in low birth weight;
4. No increase in the prevalence of overweight in childhood;
5. Increase the percentage of children exclusively breastfed for the first six months to at least 50%;
6. The reduction of cachexia in childhood to 5% and kept at this level.

To achieve the objective 4 - „No increase in the prevalence of overweight in childhood" Stakeholders should set as a priority following (55): development of coherent public policies that address relevant sectors involved, from production to consumption of food and beverages; Food issuing guidelines for all age groups; measures to prevent exposure during the first years of life, improve nutritional status and influence the pattern of growth and development of the body; supporting research related to the causes of overweight and obesity basic and preventive and control measures; creating an environment that promotes physical activity to prevent physical inactivity in the first years of life.

Control measures include obesity (55, 57):

Weight loss by:

1. diet (low calorie): rich in fruits and vegetables; rich in potatoes, rice, pasta; sometimes milk and milk products; sometimes meat, fish, beans, eggs; Small amounts of food and no rich in fat (58);
2. exercise (running, swimming, tennis etc).

Medication. Treatment with orlistat prevents weight gain, but not necessarily help weight loss.

Surgery. Extreme obesity can be treated using bariatric surgery (59).

Bibliography

1. *** Mayo clinic. *Obesity. Definition.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/definition/con-20014834). Accesat la 11 Mai, 2015.

2. *** Centers for Disease Control and Prevention. *Defining overweight and obesity.* (http://www.cdc.gov/obesity/adult/defining.html) Accesat la 3 Mai, 2015.

3. *** National Heart, Lung and Blood Institute. *What Are Overweight and Obesity?* (http://www.nhlbi.nih.gov/health/health-topics/topics/obe). Accesat la 11 Mai, 2015.

4. *** American Medical Association House of Delegates. *Recognition of obesity as a disease;* pag. 78-84 (http://www.ama-assn.org/assets/meeting/2013a/a13-addendum-refcomm-d.pdf.) Accesat la 2 Mai, 2015.

5. *** Report of the Council on Science and Public Health. *Is Obesity a Disease?* pag. 19-32 (http://www.ama-assn.org/assets/meeting/2013a/a13-addendum-refcomm-d.pdf.) Accesat la 2 Mai, 2015.

6. *** National Institute of Diabetes and Digestive and Kidney Diseases. *Understanding adult overweight and obesity.* (http://win.niddk.nih.gov/publications/understanding.htm). Accesat la 13 Mai 2015.

7. *** World Health Organisation. *Global status report on noncommunicable diseases 2014.* WHO Press, Geneva, Switzerland, 2014.

8. *** *Oxford English Dictionary*. Oxford University Press, 2015. (www.oed.com). Accesat la 17 Mai 2015.

9. Bray, G.A. History of Obesity. In: G. Williams and G. Fruhbeck (eds). *Obesity: Science to Practice.* Chichester: Wiley-Blackwell 2009, pp 2-18.

10. *** Mayo clinic. *Obesity. Risk factors.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/risk-factors/con-20014834). Accesat la 11 Mai, 2015.

11. *** National Heart, Lung and Blood Institute. *What Causes Overweight and Obesity?*(http://www.nhlbi.nih.gov/health/health-topics/topics/obe/causes). Accesat la 10 Mai, 2015.

12. *** Mayo clinic. *Obesity. Causes.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/causes/con-20014834). Accesat la 11 Mai, 2015.

13. *** Centers for Disease Control and Prevention. *Causes and Consequences.* (http://www.cdc.gov/obesity/adult/causes/index.html). Accesat la 9 Mai, 2015.

14. McAllister E.J., Dhurandhar N.V., Keith S.W., Aronne L.J., Barger J., Baskin M. et al. Ten Putative Contributors to the Obesity Epidemic. *Crit Rev Food Sci Nutr.* 2009; 49 (10) : 868–913. doi:10.1080/10408390903372599.

15. *** Centers for Disease Control and Prevention. *Protective Factors*. (http://www.cdc.gov/healthyyouth/protective/). Accesat la 17 Mai 2015.

16. Swinburn B.A., Catərson I., Seidell J.C., James W.P. Diet, nutrition and the prevention of excess weight gain and obesity. *Public Health Nutr* 2004; 7 (1A) : 123-146.

17. Wareham N.J., van Sluijs EM, Ekelund U. Physical activity and obesity prevention: a review of the current evidence. *Proc Nutr Soc* 2005; 64 (2) : 229-247.

18. Steinbeck K.S. The importance of physical activity in the prevention of overweight and obesity in childhood: a review and an opinion. *Obes Rev* 2001; 2 (2) : 117-130.

19. Pereira M.A., Ludwig D.S. *Dietary fiber and body-weight regulation.* Observations and mechanisms. *Pediatric Clinics of North America* 2001; 48 (4) : 969-980.

20. Aller EEJG, Abete I., Astrup A., Martinez J.A., van Baak M.A. Starches, Sugars and Obesity. *Nutrients* 2011; 3 (3) : 341–369.

21. Burton-Freeman B. Dietary fiber and energy regulation. *J Nutrition* 2000; 130 (2S Suppl.) : 272S–275S.

22. Shields L., Mamun A.A., O'Callaghan M., Williams G.M., Najman J.M. Breastfeeding and obesity at 21 years: a cohort study. *J Clin Nurs* 2010; 19 (11-12) : 1612-1617.

23. Arenz S., Rückerl R., Koletzko B., von Kries R. Breast-feeding and childhood obesity--a systematic review. *Int J Obes Relat Metab Disord* 2004; 28 (10) : 1247-1256.

24. Wadsworth M., Marshall S., Hardy R., Paul A. Breast feeding and obesity. Relation may be accounted for by social factors. *BMJ* 1999; 319 (7224) : 1576.

25. Gillman M.W., Rifas-Shiman S.L., Camargo C.A. Jr., Berkey C.S., Frazier A.L., Rockett H.R., et al. Risk of overweight among adolescents who were breastfed as infants. *J Amer Med Assoc* 2001; 285 (19) : 2461–2467.

26. Brand-Miller J., Holt SHA, Pawlak D.B., McMillan J. Glycemic index and obesity. *Amer J Clin Nutrit* 2002; 76: 281S–285S.

27. *** World Health Organization. *Obesity and overweight.* (http://www.who.int/mediacentre/factsheets/fs311/en/). Accesat la 19 Mai 2015.

28. Roth, J., Qiang, X., Marbán, S.L., Redelt, H., and Lowell, B.C. The obesity pandemic: where have we been and where are we going?. *Obes Res* 2004; 12: 88S–101S.

29. Ng M., Fleming T., Robinson M., et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; 384 (9945) : 766–781.

30. *** World Health Organization. *Obesity. Global Health Observatory (GHO) data*. (http://www.who.int/gho/ncd/risk_factors/obesity_text/en/). Accesat la 20 Mai 2015.

31. Eclemea I., Mincă D.G. Analiza influenței ajustorilor utilizați în calculul DALY asupra estimării poverii bolii. *Acta Medica Transilvanica* 2013; 2 (2) : 1-6.

32. *** World Health Organization. *Obesity and overweight. Situation and trends.* (http://www.who.int/mediacentre/factsheets/fs311/en/). Accesat la 20 Mai 2015.

33. The Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: a pooled analysis of 97 prospective cohorts with 1,8 million participants. *Lancet* 2013; 383 (9921) : 970–983.

34. Baron R.B. Nutritional disorders. In: Papadakis M.A., McPhee S.J. (eds.), Rabow M.W. (assoc. ed). *Current Medical Diagnosis & Treatment 2014*. 53nd ed, The McGraw-Hill Companies, New York, 2014.

(http://www.accessmedicine.com/resourceTOC.aspx?resourceID=1). Accesat la 12 Mai 2015.

35. *** Mayo clinic. *Obesity. Symptoms.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/symptoms/con-20014834). Accesat la 11 Mai, 2015.

36. *** National Heart, Lung and Blood Institute. *What Are the Signs and Symptoms of Overweight and Obesity?*

(http://www.nhlbi.nih.gov/health/health-topics/topics/obe/signs). Accesat la 10 Mai, 2015.

37. *** Mayo clinic. *Obesity. Complications.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/complications/con-20014834). Accesat la 11 Mai, 2015.

38. *** National Heart, Lung and Blood Institute. *What Are the Health Risks of Overweight and Obesity?*

(http://www.nhlbi.nih.gov/health/health-topics/topics/obe/risks).

39. *** NHS choises. *Diagnosing obesity*.

(http://www.nhs.uk/Conditions/Obesity/Pages/Diagnosis.aspx).

40. *** Mayo clinic. *Obesity. Tests and diagnosis.*

(http://www.mayoclinic.org/diseases-conditions/obesity/basics/tests-diagnosis/con-20014834). Accesat la 10 Mai, 2015.

41. *** National Heart, Lung and Blood Institute. *How Are Overweight and Obesity Diagnosed?*

(http://www.nhlbi.nih.gov/health/health-topics/topics/obe/diagnosis). Accesat la 10 Mai, 2015.

42. Moyer V.A., et al. Screening for and management of obesity in adults:

U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2012; 157 : 373-378.

43. LeBlanc E., O'Connor E., Whitlock E.P., Patnode C., Kapka T. *Screening for and Management of Obesity and Overweight in Adults.* Evidence Report No. 89. AHRQ Publication No. 11-05159-EF-1. Rockville, MD: Agency for Healthcare Research and Quality; 2011.

44. *** Ministerul Sănătății din România. Institutul pentru Ocrotirea Mamei și Copilului „Prof. Dr. Alfred Rusescu". Îndrumar privind screeningul obezității la copil. Editura Oscar Print, București, 2010.

45. Wang C., Gordon E.S., Stack C.B., Liu C.T., Norkunas T., Wawak L., Christman M.F., Green R.C., Bowen D.J. A randomized trial of the clinical utility of genetic testing for obesity: design and implementation considerations. *Clin Trials* 2014; 11 (1) : 102-113.

46. *** The National Center for Biotechnology Information. *MC4R melanocortin 4 receptor [Homo sapiens (human)]. Gene ID: 4160.* (http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=4160)Accesat la 23 Mai 2015.

47. *** The National Center for Biotechnology Information. *PCSK9 proprotein convertase subtilisin/kexin type 9 [Homo sapiens (human)]. Gene ID: 255738.* (http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=255738). Accesat la 23 Mai 2015.

48. Alicezah M.K., Razali R., Rahman T., Hoh B.P., Suhana N.H., Muid S., Nawawi H.M., Koshy M. Homozygous familial hypercholesterolemia. *Malays J Pathol* 2014; 36 (2) : 131-137.

49. *** The National Center for Biotechnology Information. *APOE apolipoprotein E [Homo sapiens (human)]. Gene ID: 348.* (http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=348). Accesat la 23 Mai 2015.

50. *** World Health Organization. *WHO European Childhood Obesity Surveillance Initiative (COSI).* (http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/activities/monitoring-and-surveillance/who-european-childhood-obesity-surveillance-initiative-cosi) Accesat la 20 Mai 2015.

51. *** World Health Organization. *European Food and Nutrition Action Plan 2015–2020.* Regional Committee for Europe, 64th session. Copenhaga, Danemarca, 15–18 Septembrie 2014. (http://www.euro.who.int/__data/assets/pdf_file/0008/253727/64wd14e_FoodNutAP_140426.pdf?ua=1).

52. *** Mayo clinic. *Obesity. Prevention.* (http://www.mayoclinic.org/diseases-conditions/obesity/basics/prevention/con-20014834). Accesat la 10 Mai, 2015.

53. *** Centers for Disease Control and Prevention. Recommended Community Strategies and Measurements to Prevent Obesity in the United States. *MMWR* 2009; 58 (RR-7) : 1-29. (http://www.cdc.gov/mmwr/pdf/rr/rr5807.pdf). Accesat la 10 Mai, 2015.

54. *** World Health Organisation. *Global action plan for the prevention and control of noncommunicable diseases 2013-2020.* WHO Press, Geneva, Switzerland, 2013.

55. *** World Health Organisation. *Global Nutrition Targets 2025: Childhood overweight policy brief.* WHO Press, Geneva, Switzerland, 2014 (WHO/NMH/NHD/14.6) (http://apps.who.int/iris/bitstream/10665/149021/2/WHO_NMH_NHD_14.6_eng.pdf?ua=1).

56. *** World Health Organisation. *Comprehensive implementation plan on maternal, infant and young child nutrition.* Geneva, Switzerland, 2014 (WHO/NMH/NHD/14.1) (http://apps.who.int/iris/bitstream/10665/113048/1/WHO_NMH_NHD_14.1_eng.pdf).

57. Jensen M.D., Ryan D.H., Apovian C.M., Ard J.D., Comuzzie A.G. et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. *JACC* 2014; 63 (25) : 2985–3023. http://content.onlinejacc.org/article.aspx?articleid=1770219. Accesat la 3 Mai, 2015.

58. Sacks F., Bray G.A., Carey V.J., Smith S.R., Ryan D.H., Anton S.D. et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* 2009; 360 (9) : 859-873.

59. Nelson D.W., Blair K.S., Martin M.J. et al. Analysis of obesity-related outcomes and bariatric failure rates with the duodenal switch vs. gastric bypass for morbid obesity. *Arch Surg* 2012; 47 (9) : 847-854.

Selective bibliography

1. Bocșan Ioan Stelian. Epidemiologie practică pentru medicii de familie. Cluj-Napoca, 1999, 710 p.

2. Boli infecțioase la copii. Sub redacția conf. univ., dr. Galina Rusu. Chișinău, 2012, 290 p.

3. Buiuc Dumitru, Neguț Marian. Tratat de microbiologie clinică. Ediția a III-a, București, 2009, 1250 p.

4. Ciufecu Constantin, Ciufecu Elvira-Sînziana. Viroze transmisibile umane supravegheate în Uniunea Europeană. București, 2011, 165 p.

5. Cârstina Dumitru, Ciutică Ionel. Infecția cu virusuri hepatitice. Cluj-Napoca, 2002, 191 p.

6. Ciufecu Constantin, Prisacari Viorel. Vibrionul holeric – holera. Chișinău, 1995, 150 p.

7. Iarovoi Petru. Combaterea bolilor infecţioase în Republica Moldova. Chişinău, 2012, 97 p.

8. Pântea Victor. Hepatite virale acute şi cronice. Chişinău, 2009, 221 p.

9. Prisacaru Viorel. Epidemiologie generală. Bazele medicinei prin dovezi. Chişinău, 2012, 379 p.

10. Tratat de epidemiologie a bolilor transmisibile. Sub redacţia prof. univ. dr. Aurel Ivan, Polirom, 2012, 837 p.

11. Баранов А.А., Брико Н.И., Намазова-Баранова Л.С., Ряпис Л.А. Стрептококки и пневмококки. Ростов-на Дону „Феникс", 2013, 301 с.

12. Брико Н.И., Зуева Л.П., Покровский В.И., Сергиев В.П., Шкарин В.В. Эпидемиология. Том I. М., 2013, 832 с.

13. Брико Н.И., Зуева Л.П., Покровский В.И., Сергиев В.П., Шкарин В.В. Эпидемиология. Том II. М., 2013, 654 с.

14. Зуева Л.П., Яфаев Р.Х. Эпидемиология. Санкт-Петербург, 2005, 745 с.

15. Частная эпидемиология. Том 2. Под редакций академика РАМН, профессора Б.Л. Черкасского. М., 2002, 272 с.

16. Частная эпидемиология. Том 1. Под редакций академика РАМН, профессора Б.Л. Черкасского. М., 2002, 385 с.

17. Шкарин В.В., Ковалишена О.В. Новые инфекции: Систематизация, проблемы, перспективы.

18. Шляхов Э.Н. Практическая эпидемиология. Издание пятое. Издательство „Штиинца", 1991, 566 с.

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