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GENOTYPING AND PHYLOGENETIC ANALYSIS OF FRANCISELLA TULARENSIS HOLARTICA STRAINS ISOLATED ON THE TERRITORY OF UKRAINE

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For the first time in Ukraine, a detailed genotypic characterization of a collection of strains (197) of Francisella tularensis holarctica, isolated both against the background of epidemic complications and in the inter-epidemic period, was provided. The sources of the pathogen were: ticks (56.30 %), small mammals (22.30 %) and the aquatic environment (19.30 %), three isolates were isolated from humans (2.03 %). A significant genetic diversity of the pathogen was found on the territory of Ukraine with the dominance of genotypes belonging to genogroup A (81.21 %), genogroups B and C were found in 11.68 % and 7.11 %, respectively, which distinguishes the genetic composition of the pathogen circulating in the territory of the majority European countries. A dendrogram was constructed based on PCR analysis of VNTR loci. the genetic distances between the isolates were calculated, and certain regularities regarding their molecular genetic polymorphism were also revealed. It was established that isolates belonging to genotypes of group C are the most genetically distant from group A. The genetic distance between group A and C isolates is 0.24. The difference in genetic distances between pathogens with genotypes A and B is minimal and amounts to 0.15, which may indicate their close relationship, although they were isolated in different years and in different regions of Ukraine. There is a tendency for the relatedness of isolates depending on belonging to a certain host and geographical origin, which indicates the combined influence of these factors on the processes of microevolution during the formation of the genotype.

Key words: Francisella tularensis holartica, genetic polymorphism, phylogenetic analysis, the risk of activation, Ukraine

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ГЕНОТИПУВАННЯ ТА ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ ШТАМІВ FRANCISELLA TULARENSIS HOLARTICA, ІЗОЛЬОВАНИХ НА ТЕРИТОРІЇ УКРАЇНИ

Вперше в Україні надана детальна генотипова характеристика колекції штамів (197) F. tularensis holarctica, ізольованих як на тлі епідемічних ускладнень, так і в міжепідемічний період. Джерелами збудника були: кліщі (56,30 %), дрібні ссавці (22,30 %) та водне середовище (19,30 %), від людей виділено три ізоляти (2,03 %). На території України виявлено значне генетичне розмаїття збудника з домінуванням генотипів, віднесених до геногрупи А (81,21 %), геногрупи В і С зустрічались у 11,68 % та 7,11 % відповідно, що відрізняє генетичний склад збудника, що циркулює на території більшості європейських країн. За даними ПЛР аналізу VNTR-локусів сконструйовано дендрограму. розраховано генетичні дистанції між ізолятами, а також виявлено певні закономірності стосовно їх молекулярно-генетичного поліморфізму. Встановлено, що найбільш генетично віддаленими від групи А є ізоляти, що належать до генотипів групи С. Генетичні дистанції між ізолятами групи А та С становлять 0,24. Різниця генетичних дистанцій між збудниками з генотипами А та В мінімальна і становить 0,15, що може свідчити про їх близьку спорідненість, хоча вони були ізольовані в різні роки і в різних регіонах України. Спостерігається тенденція до спорідненості ізолятів залежно від належності до певного господаря та географічного походження, що свідчить про сумарний вплив цих факторів на процеси мікроеволюції при формуванні генотипу.

Ключові слова: Francisella tularensis holartica, генетичний поліморфізм, філогенетичний аналіз, ризик активації, Україна

The study is a fragment of the research project "Scientific substantiation and development of sanitary and anti-epidemic measures when studying the role of sea cargo containers in the potential introduction of alien entomozoofauna and dangerous pathogens by ships into Black Sea ports", state registration No. 0123U001635.

Tularemia is one of the most important zoonoses, which have a natural focal nature with a wide range of sources of infection (numerous species of small mammals), many vectors, and a variety of ways of transmission of infection (alimentary, transmissible, aspiration). The epidemiological situation in the world with tularemia is assessed as tense, and the activation of the epizootological process is noted. Large outbreaks and sporadic cases of tularemia are periodically registered in many countries of the world, including Ukraine, and the habitat of the pathogen is expanding [2, 12, 15].

The risk of activation of the epizootic process in Ukraine, where outbreaks of the disease were previously registered, is associated with military events on the territory of the country. Thus, during the war in Kosovo (1998–1999), there were two large outbreaks of tularemia, as a result of mass displacement of the population and violations of sanitary and hygienic living conditions. This led to the formation of an endemic zone for tularemia in the territory of this country, where from 25 to 327 cases are registered every year, the incidence is on average 5.2 per 100 thousand population [7].

However, disease cases are detected not only in endemic regions, but also in new geographical areas [3, 4]. A number of authors attribute tularemia to re-emergent infections, the causative agent in some cases has antimicrobial resistance, which becomes too dangerous [3, 5]. On average in Europe, the incidence rate per 100,000 population ranges from 0.1 to 0.3 [9].

On the territory of Europe, isolates of Francisella tularensis subspecies holartica are mainly isolated. In most European countries, the terrestrial life cycle of the pathogen is dominant, with the participation of arthropods as vectors (ticks of the species Ixodes ricinus), to whose bites the hosts are sensitive: small rodents (Microtus arvalis) and hares (Lepus europaeus) [6, 9, 10, 12].

The causative agent of tularemia is one of the most virulent microorganisms. The infectious dose for a person during aerial contact is 10 microbial cells, and susceptibility approaches 100 % [15].

Currently, the genus Francisella includes two species – F. tularensis and F. philomiragia. The species F. tularensis is represented by four subspecies – F. tularensis subsp. tularensis (type A), F. tularensis subsp. holarctica (type B), F. tularensis subsp. mediaasiatica and F. tularensis subsp. novicida, which differ in virulence and geographical distribution, however, have a similar antigenic structure [11]. Human diseases are mainly caused by two subspecies: the highly virulent subspecies F. tularensis tularensis (type A), circulating in North America, and the less virulent F. tularensis holarctica (type B), endemic to all continents of the Northern Hemisphere [4].

To identify the population genetic structure of the causative agent of tularemia, multilocus VNTR analysis is used, in which highly mutagenic variable tandem repeats are used as DNA markers. VNTR analysis is used to study the evolution and molecular epidemiology of the pathogen: genotyping, detection of interstrain differences within a species and subspecies, study of the clonal structure of the genome, explanation of the genetic basis of virulence and phenotypic variability of strains [8, 9, 11, 13]. The method makes it possible to deeply investigate the causes of evolutionary changes in F. tularensis, the influence of ecological niches on the formation of genotypes and adaptation of the pathogen to living conditions, to determine the significance of unique genotypes in the development of the epizootic and epidemic process.

Genotyping of F. tularensis strains isolated during epidemic outbreaks revealed several closely related genotypes persisting for a long period [11]. When combined with the data of phylogenetic analysis, it will be possible to identify and better understand the microevolutionary changes of the causative agent of tularemia, which characterize its epidemic variant [10]. it is the data of genotyping by VNTR loci that can be useful for the epidemiological analysis of disease outbreaks and the prediction of the activation of the epizootological and epidemic process. The data obtained so far indicate a significant variation in the allele composition of VNTR loci of F. tularensis strains, however, the biological meaning of the modulation of the number of tandem repeats of individual loci is unknown [6, 9].

The purpose of the study was identification of the population genetic structure of the causative agent of tularemia to assess the risk of activation of the epizootic process in Ukraine during the war.

Materials and methods. In the work, 197 strains of F. tularensis holarctica were studied, of which 190 were isolated in different regions of Ukraine in 1993–2012, and 7 were isolated in Poltava region in 1967.

DNA was isolated from thermolysates of F. tularensis strains using AmpliSens kits. Thermolysates of F. tularensis were obtained according to the method recommended by WHO [15]. To obtain a suspension of bacteria, 5-10 colonies grown on FT-agar were used, which were emulsified in 1 ml of sterile distilled water, the suspension was inactivated at a temperature of 65° C for 2 hours in a solid-state thermostat. The specific sterility of thermolysates was determined by sowing on Petri dishes with FT-agar, which were incubated at a temperature of 37° C for 7 days.

Purification of bacterial DNA of all studied strains was carried out with the help of 3M sodium acetate and 96 % ethanol, followed by drying of the samples. Dried DNA was stored at -20° C.

DNA was investigated by PCR [1] and multilocus VNTR analysis. PCR products were analyzed by horizontal electrophoresis in 1.7 % agarose gel and vertical electrophoresis in 6 % non-denaturing polyacrylamide gel with ethidium bromide and silver nitrogen staining, respectively. The results were evaluated by comparing the DNA bands of the tested samples with the positive control and the pUC 19 DNA/Msp I length marker. Video images and sizes of amplified fragments for genotyping were obtained using the Image Master VDS video system (Amersham Pharmacia Biotech, USA) according to the instructions. Calibration of the molecular weight of amplicons was carried out using a standard pUC 19 DNA / Msp I. The number of variable tandem repeats (VNTR), which are non-coding regions of the pathogen's chromosome DNA, was determined using multilocus VNTR analysis. Allelic variations in the 4 polymorphic VNTR loci selected for research were studied in 197 strains of F. tularensis using our own test systems: multiplex PCR test systems for polymorphic VNTR loci FT-M3, FT-M6, FT-M19, FT-M20 [2, 6].

The genotype of an individual strain was determined by the alleles of these loci and registered in the form of a genetic alphanumeric formula. Variability of VNTR loci was assessed using the Ney-Lee diversity index, $Di=1-\Sigma i$, (i – allele frequency). The frequency of the occurring allele was calculated as the

ratio of the number of alleles of a given type to the total number of alleles of all studied strains in the studied locus [1]. The discriminatory ability of the typing system was determined by the Simpson index, which is calculated by the formula D=1-N1(N-1)-Σnj(nj-1), where D – is the index of discriminatory power, N – is the number of different strains, Σ – is the number of types, nj – is the number of strains of type j [1].

Cluster analysis and dendrogram construction were carried out using Trees (Ukraine) computer software based on the unweighted pair-group method on a personal computer.

The genotyping system of F. tularensis holarctica strains circulating in Ukraine is based on multiplex VNTR DNA analysis of microorganism strains. The number of studied VNTR loci for differentiating individual strains can be limited to 4 polymorphic VNTR loci (FT-M3, FT-M6, FT-M19, FT-M20) without reducing the discriminatory ability of the typing system. In this regard, PCR test systems containing specific primers, flanking loci FT-M3, FT-M6, FT-M19 and FT-M20 were designed and obtained for genotypic characterization of individual strains [6].

Methods of determining evolutionary distances based on the comparison of nucleotide sequences of homologous genes are used to identify phylogenetic relationships between strains.

Visualization of phylogenetic deviations is carried out with the help of a constructed dendrogram, which reflects the genetic relationships between strains.

Calculation of genetic distances and phylogenetic cluster analysis of strains was carried out by constructing dendrograms taking into account the polymorphism of the allelic state of the studied VNTR loci of F. tularensis using the Molecular Evolutionary Genetics Analysis (MEGA-4) program package, the method using arithmetic averages (UPGMA) and the Neighbor-joining (NJ) method. This is the most common distance-matrix method [14].

Results of the study and their discussion. 190 strains of F. tularensis isolated on the territory of different regions of Ukraine (Chernihiv, Sumy, Odesa, Poltava, Volyn, Rivne, Lviv, Vinnytsia, Zaporizhzhya, Mykolaiv, Autonomous Republic (AR) of Crimea, Sevastopol) were studied from 1993 to 2012, 7 strains that were isolated in the Poltava in 1967 (Fig. 1).

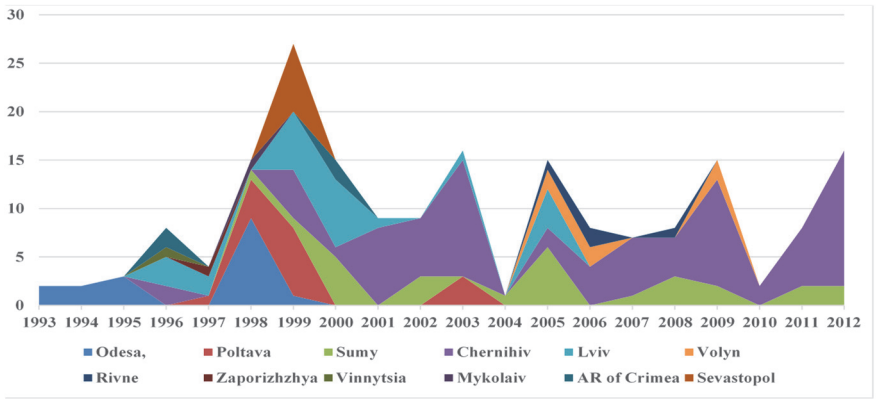

Fig. 1 Dynamics of detection of tularemia pathogen isolates in different regions of Ukraine

The sources of pathogens were mainly ticks (56.3 %), small mammals (22.3 %) and the aquatic environment (19.3 %), three isolates were isolated from humans (2.03 %). All strains were identified by cultural, morphological and biochemical properties as representatives of the subspecies F. tularensis holarctica.

The study of DNA of F.tularensis strains by the PCR method made it possible to detect allelic variations of VNTR loci FT-M3, FT-M6, FT-M19 and FT-M20, to determine the molecular weight of the alleles of the studied VNTR loci and the number of repeats in them. The identified genotype of the isolates was expressed by the name of the allele of the studied loci.

Comparative VNTR DNA analysis of F. tularensis strains isolated in different regions of Ukraine demonstrated genotypic heterogeneity of the pathogen. This heterogeneity was determined mainly due to the polymorphism of the FT-M3, FT-M6, FT-M20 loci, which were used for genotypic characterization of the pathogen (Table 1).

The conducted study established a large variability of the number of repetitions in the studied loci of the genomic material, which indicates their high variability. This was proven by calculating the Ney-Lee diversity index (Di) for all studied isolates by three markers, which varied from 0.15 to 0.91, and the frequency of registration of detected allelic variants of these loci varied widely – from 0.005 to 0.92.

The most variable was the FT-M3 locus, for which the presence of 19 types of alleles was registered, the detection frequency of which was from 0.005 to 0.141. The Nye-Lee diversity index (Di) was quite high at 0.91. The FT-M6 locus was less variable and was represented by six types of alleles, the frequency of which ranged from 0.015 to 0.72. At the same time, the majority of cultures (144 isolates) contained an allele carrying 4 repeats (detection frequency 0.72), and the 9 allele was determined in 24 strains (the frequency of which was 0.121). The variability of the FT-M6 locus (Di) was 0.42. When analyzing the FT-M20 locus, 2 allelic variants of three and four repeats were identified, the frequency of which was 0.92 and 0.07, respectively. The presence of two types of alleles indicates its low polymorphism (Di=0.15).

Table 1

Variability and frequency of detection of alleles of VNTR loci in the genomic material of F. tularensis isolated on the territory of Ukraine

Locus	Allel	Number of strains	The frequency of allele formation	Di (Ney-Lee index)
FT-M3	9	4	0.020	0.91
	10	3	0.015	
	11	4	0.020	
	12	6	0.030	
	13	20	0.101	
	14	25	0.126	
	15	19	0.096	
	16	15	0.076	
	17	28	0.141	
	18	21	0.106	
	19	16	0.081	
	20	10	0.051	
	21	2	0.010	
	22	4	0.020	
	23	9	0.045	
	24	3	0.015	
	26	1	0,005	
	28	7	0.035	
	29	1	0.005	
FT-M6	4	144	0.722	0.42
	5	9	0.045	
	6	10	0.051	
	7	8	0.045	
	8	3	0.015	
	9	24	0.121	
FT-M20	3	184	0.920	0.15
	4	14	0.071	

The obtained data indicate the circulation of a heterogeneous population of tularemia microbes on the territory of Ukraine and the presence of a tendency for strains to be related depending on the main host and geographical origin. The cyclical course of periods of subsidence and activation of natural foci of tularemia has a direct effect on the population structure of the pathogen. The revealed diversity of the genotypic structure of circulating isolates of F. tularensis reflects the epidemic situation in specific natural foci of tularemia.

At the same time, the specificity of the variability of loci FT-M3 (19 types of alleles), FT-M6 (6 types of alleles), FT-M20 (2 types of alleles) in 197 isolates of F. tularensis holarctica found in different landscape and geographical zones of Ukraine, made it possible to identify 44 variants of individual genotypes with a frequency from 0.005 to 0.096, including 28 genotypes of group A, 9 genotypes of group B and 7 genotypes of group C (Fig. 2).

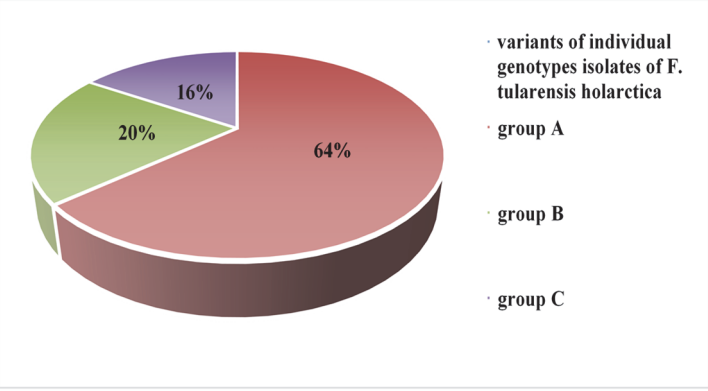

Fig. 2. Variants of individual genotypes isolates of F. tularensis holarctica, in different landscape and geographical zones of Ukraine

When typing isolates by determining the frequency of repetitions in the specified loci, the discriminatory power (ability to differentiate isolates of the same species) was 0.956, which allows us to consider the obtained results as reliable.

According to the results of our research, the dominant genotype was 12, which occurred with a frequency of 0.096 and included 19 isolates (9.60 % of all isolates in the collection). 10 genotypes were represented by single cultures with a high number of tandem repeats in the FT-M3 VNTR locus (from 17 to 29).

The results of the research made it possible to determine the phylogenetic relationships of F. tularensis strains isolated in Ukraine, taking into account the polymorphism of the allelic composition of the studied VNTR loci of these strains. According to the PCR analysis of VNTR loci (molecular weight

of amplification fragments and alleles of polymorphic VNTR loci), a dendrogram was constructed (Fig. 3), genetic distances between isolates were calculated, and certain regularities regarding their molecular genetic polymorphism were also revealed. As can be seen from the dendrogram, there is a tendency towards the relatedness of isolates depending on belonging to a certain host and geographical origin, which indicates the combined influence of these factors on the processes of microevolution during the formation of the genotype. It was established that isolates belonging to genotypes of group C are the most genetically distant from group A. The genetic distance between group A and C isolates is 0.24. The difference in genetic distances between pathogens with genotypes A and B is minimal and amounts to 0.15, which may indicate their close relationship, although they were isolated in different years in Ukraine.

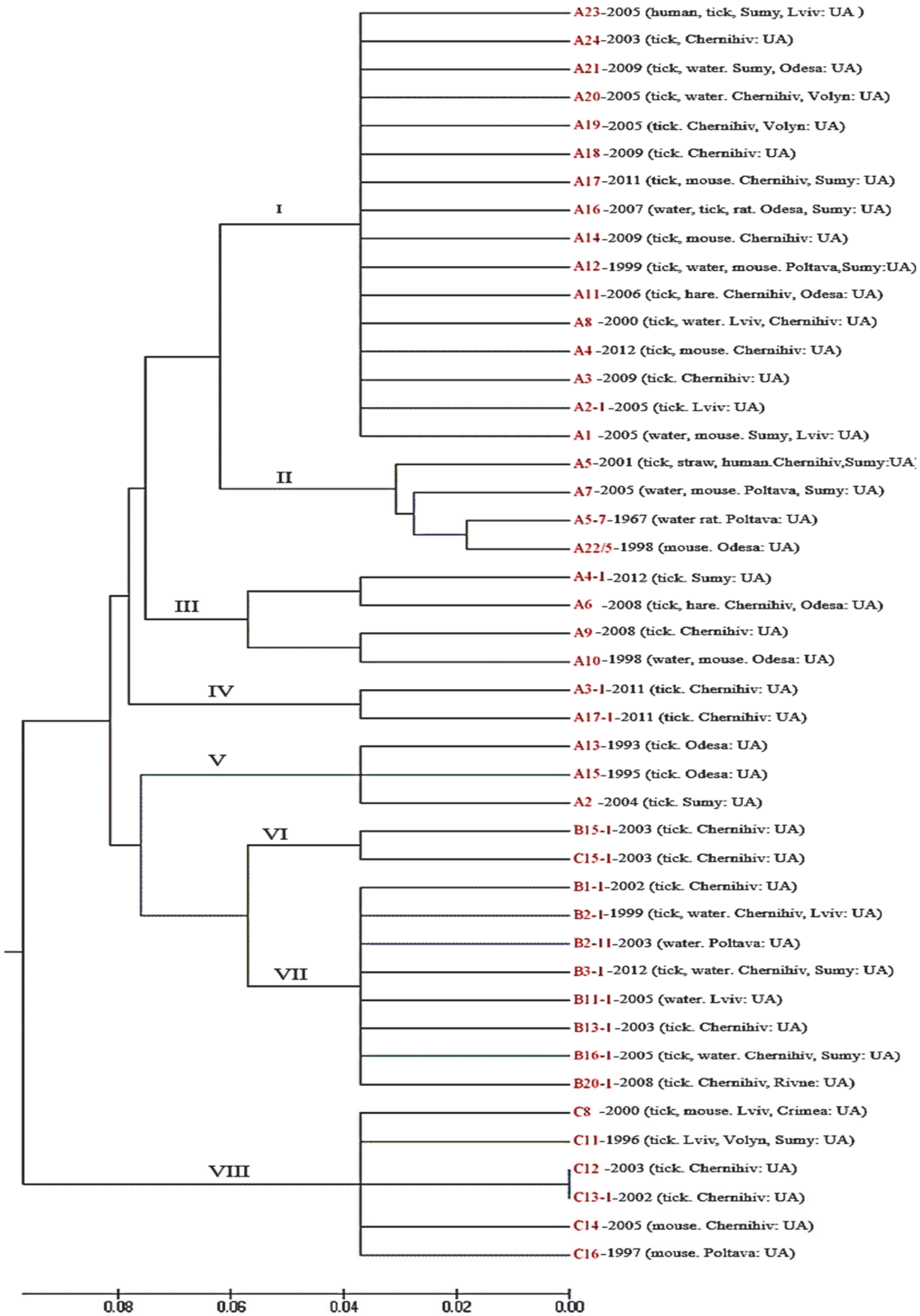

Fig. 3. Dendrogram of F. tularensis holarctica genotypes isolated in different regions of Ukraine in 1967 and 1993-2012 (according to VNTR analysis data).

The dendrogram, built on the basis of MLVA data for 4 polymorphic VNTR loci, made it possible to group all isolates into two clusters and eight subclusters. The distribution took place according to their belonging to certain genotypes and their variants. This made it possible to determine their origin, establish genetic relationships, study the population structure, and calculate genetic distances between clusters and subclusters.

Isolates belonging to the first cluster, which forms 7 subclusters (genotypes from groups A and B with a different number of components) turned out to be dominant. Genogroup A was the most numerous and comprised 160 strains (81.21 %), genogroup B – 23 strains (11.68 %). The second cluster includes 14 isolates of genotype C (7.11 %).

Subcluster 1: the largest in number, represented by 115 isolates belonging to group A (with genotypes A1-A23), which in the VNTR-locus FT-M6 contained an allele carrying 4 repeats.

The majority (53.91 %) of isolates of this subcluster were isolated from ticks (62), 22.61 % of isolates from rodents (26), 20.86 % from water (24 isolates), 2.61 % (3) isolated from people living in adjacent areas of Sumy and Chernihiv regions (Desna River basin), which may indicate the existence of a natural center with similar ecological conditions (Fig. 4). This subcluster included the vaccine strain F. tularensis 15 Gajskyi.

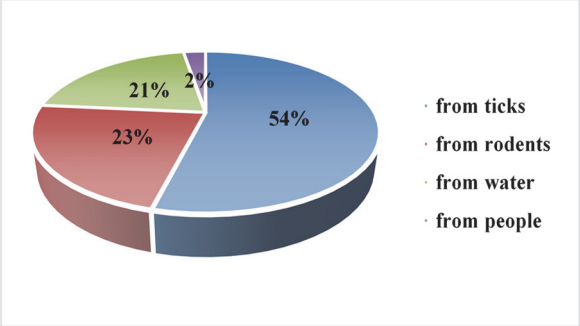

Fig. 4. Sources of detection of the causative agent of tularemia for subcluster 1.

Subcluster 2: contains 20 isolates (genotypes A5, A7, A5/7, A22/5). 8 strains (40.00 %) were isolated from ticks, 7 strains (35,00 %) from rodents, 4 (20.00 %) from aquatic environments, 1 strain (5,00 %) from humans.

Isolates with genotypes A5/7 and A22/5 were isolated from rodents in 1967 (Poltava region) and in 1998 in Odesa region during the period of an epidemic rise in morbidity and are characterized by an unusual set of alleles in the VNTR locus FT-M6 (additional 7 alleles for both) and additional 14 – in the VNTR-locus FT-M3 (strain 146, Odesa region). This is probably explained by the infection of a susceptible animal with two pathogens of different genotypes. Pathogens with common genotypes (A12 and A16) circulate in some regions of Ukraine.

Subcluster 3: all 13 strains included in this cluster were isolated in the territory of Chernihiv, Sumy, Odesa and Lviv regions and belong to genotype A, the number of repeats in the VNTR locus FT-M6 is 6. 8 strains were isolated from ticks (61.50 %), rodents – 3 strains (23.10 %) and water 2 strains (15.40 %).

Subcluster 4: 3 strains of genotype A (the number of repeats in the VNTR locus FT-M6 – 8) were isolated in 2011. in the Chernihiv region from ticks (100 %) from one cell.

Subcluster 5: 9 strains of genotype A, the number of repeats in the VNTR locus FT-M6 – 5, isolated from ticks – 8 strains (88.90 %), rodents – 1 strain (11.10 %). This cluster mainly included strains isolated from ticks in Odesa region in 1993-1999. and one strain isolated in Sumy region in 2004.

Subcluster 6: this subcluster includes 6 strains containing 9 repeats in the FT-M6 VNTR locus. All 5 strains of genotype B and 1 strain of genotype C were isolated in Chernihiv region from ticks (100 %) in 2001-2003. Among these strains, genotype C151 (strain 203, 2003) with the allelic formula 18, 9, 1, 4, was unique which contained 9 repeats in the VNTR locus of FT-M6 and 4 repeats in the VNTR locus of FT-M20.

Subcluster 7: 18 strains (the number of repeats in the FT-M6 VNTR locus is 9) from different regions of Ukraine. 10 strains (55.60 %) were isolated from ticks, 1 (5.56 %) from rodents, 7 (38.80 %) from water. This subcluster combines strains of genotype B (B11 – B21).

Subcluster 8 of the second cluster unites strains of genotypes of group C (C8-16), which in the VNTR-locus FT-M20 contained an allele carrying 4 repeats, a total of 13 strains: isolated from ticks – 6 strains (46.16 %). rodents – 6 (46.10 %), from water – 1 (7.69 %). It should be noted that 2 strains with genotypes C12 and C13 were isolated in 2002-2003 from an outbreak in the Chernihiv Region. from ticks, are on one undivided branch, which may indicate their identity by molecular genetic structure.

Study of the genomes of 599 isolates of F. tularensis subsp. holarctica isolated from common voles and Iberian hares conducted recently in Spain [8] revealed new genomes of this pathogen, which are assigned to genogroup B, and various subclusters (B.49, B.51 and B.262) described in most European countries. The isolated genomes show a high phylogenetic closeness to other genomes circulating in Spain and other European countries.

Our study showed that a significant genetic diversity of the pathogen was found on the territory of Ukraine with the dominance of genotypes assigned to genogroups A, B, and C. Most of them are assigned to genogroup A (81.21 %), which distinguishes the genetic composition of the circulating pathogen on the territory of Spain.

In Germany, the genetic structure of 305 isolates isolated from hares (260), ticks parasitizing hares (11), from humans (27), pigs (2), foxes (2), honey bees (2), 1 isolate isolated from grape must [10]. Genotyping assigned all detected isolates to genogroup B, subclusters 6 – B.6 (n=199) and 7 – B.12 (n=102). In our study, isolates with a similar genetic structure were isolated in 23.60 % of cases and were isolated in different regions of the country: from mice (15 isolates), rodents (1) and from aquatic environments (7).

In Switzerland, in the last 10 years, there has been an increase in the incidence of tularemia among people due to the expansion of the habitat of various biotopes of the causative agent, and the rate of infection of ticks with the causative agent of tularemia has increased significantly [3]. Mosquitoes are considered the main carriers of the bacterium, so F. tularensis ssp. holarctica was detected in 11 out of 14 selected mosquito species. According to our research, cases of tularemia pneumonia among military personnel have increased in Ukraine due to hostilities.

In France, a study of the genomes of 350 strains of F. tularensis subsp. holarctica of human and animal origin discovered from 1947 to 2018. All isolates from France (except four) belonged to genogroup B, subcluster B.44, previously described in Europe. Genome-wide analysis revealed 87 new gene variants at the level of the same subcluster. The results show little genetic diversity among of this pathogen from France, the study found that new genovariants arose from clonal expansion of a single population. No connection was established between the severity of the clinical course of the disease and the genovariant of the causative agent. The results of the study indicate signs of persistence of the pathogen in the environment, associated with the slow rate of replication of F. tularensis found in Western Europe. The presence of identical genotypes that were detected over a long period of time (1947-2018) and over long distances (the territory of France and neighboring countries) confirms the hypothesis about the relative genetic stability of the pathogen, which was also obtained in our study, but indicates the spread of the bacterium over large areas distances [9].

A study conducted in Poland revealed a high genetic diversity of F. tularensis strains [6]. A total of 53 samples were tested, including 16 F. tularensis strains isolated from humans and animals in Poland in 1953-1962, 5 DNA samples isolated from clinical samples of human tularemia cases in 2012-2013 in Poland, and 32 F. tularensis isolated in other countries of Europe, the USA, Japan and China in the 1960s. Among 21 F. tularensis strains isolated in Poland in the period 1951-2013, 14 unique MLVA genotypes were identified. The variability of Polish strains was limited to two loci Ft-V_1 and Ft-V_4, which varied in the number of repeats from 3 to 5 and from 7 to 19, respectively. Arthropods were not the main source of infection, although cases of tularemia associated with their bites were recently described in Poland.

Conclusion

On the territory of Ukraine, a wide genetic diversity of pathogens of F. tularensis subsp. holarctica in contrast to the countries of Eastern and Western Europe. The phylogenetic analysis of isolates of the pathogen circulating in Ukraine showed the genetic affinity of the strains and the processes of microevolution that occur over time in the formation of the genotype of the pathogen. Closely related pathogen genomes found in both humans and animals are found in different, often geographically distant regions of the country. These features are explained by the relative stability of the genome of F. tularensis subsp. holarctica, in the new genetic variants of which only single nucleotide substitutions and deletions are detected, in contrast to the genome of F. tularensis tularensis, which has high genetic variability, which affects the virulence of the pathogen.

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MODERN TRENDS IN THE ERUPTION OF PERMANENT TEETH DURING THE LATE MIXED DENTITION PERIOD

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The study was devoted to establishing the regional terms of permanent teeth eruption in 9–12-year-old children of the city of Odesa. Dental examination was performed according to the generally accepted methodology using standard dental instruments included in the examination kit. Children were divided into groups according to their age and sex. Patients were examined on the basis of the values of age and period of teeth eruption. The results of the study indicate an earlier formation of a permanent bite in the majority of the examined children. In the examined girls, the formation of a permanent bite was completed earlier than in boys. The obtained research data should be taken into account when planning and developing a program for the prevention of major dental diseases, as well as used to establish biological maturity and determine the overall development of the child.

Key words: eruption of teeth, jaws, permanent teeth, children, oral health.

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СУЧАСНІ ТЕНДЕНЦІЇ ПРОРІЗУВАННЯ ПОСТІЙНИХ ЗУБІВ У ПЕРІОД ПІЗНЬОГО ЗМІННОГО ПРИКУСУ

Дослідження присвячене встановленню регіональних термінів прорізування постійних зубів у дітей 9–12 років міста Одеси. Стоматологічне обстеження проводили за загальноприйнятою методикою з використанням стандартного стоматологічного інструментарію, що входить до набору для обстеження. Діти були розподілені на групи відповідно до віку та статі. Пацієнтів обстежували на основі значень віку та періоду прорізування зубів. проведених досліджень свідчать про більш раннє формуванні постійного прикусу у більшості обстежених дітей. У обстежених дівчат формування постійного прикусу завершувалось раніше, ніж у хлопчиків. Отримані дані дослідження необхідно враховувати під час планування та розробки програми профілактики основних стоматологічних захворювань, а також використовувати при встановлені біологічної зрілості та визначені загального розвитку дитини.

Ключові слова: прорізування зубів, щелепи, постійні зуби, діти, здоров'я порожнини рота.

The work is a fragment of the research project "Improvement of diagnostics, prevention and treatment of teeth hard tissues mineralization processes violations in children", state registration No. 0121U114421.

Tooth eruption is a complex physiological process, which is the movement of a tooth from the place of its insertion in the alveolar bone to the occlusal surface in the oral cavity [8]. The analysis of modern literature data indicates the absence of a single universal theory of tooth eruption that can provide comprehensive explanations for numerous factors obtained in the study of the stages of tooth development and its possible disorders. However, the described mechanisms often complement each other, which makes teething a complex multifactorial process that combines the action of several mechanisms [1].