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PREDICTING EPIZOOTIC RISKS OF LEPTOSPIROSIS

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Abstract. Leptospirosis is an infectious disease of humans and animals that is caused by pathogenic spirochetes of the genus *Leptospira*. It is considered the most common zoonosis in the world and is associated with settings of poor sanitation and agricultural occupations involving contact with animals or water. The authors have analyzed the present day world and particular Ukrainian situation with leptospirorsis and pointed out the urgent measures which could prevent its prevalence.

Key words: zoonosis; leptospirosis; path of prevalence; protective measure.

Urgency. Leptospirosis is one of the most severe zoonotic diseases. The main mechanism of transmission of pathogens is fecal-oral, this is realized through the waterway, less often leptospira gets on the surface of food products that are not subjected to heat treatment before consumption [1]. The natural environment, contaminated with the urine of many animal is a natural reservoir, and serves as a transmission factor. At the same time, a significant variety of non-pathogenic leptospira, as well as individual species, for which virulence has not yet been established, is detected in the soil and water of surface water bodies.

According to modern concepts, the genus *Leptospira* is divided into 35 species. The classification was created according to the known bacteria's virulence and they are divided into three phylogenetic clusters [2].

Zoonosis caused by pathogenic spirochetes *Leptospira spp.* is a particularly important public health problem in tropical countries' urban settlements [3]. Rats are one of the main reservoirs of the pathogen in urban areas. In Ukraine, this is one of the most common zoonoses with natural foci in all administrative territories. Particularly favorable for the formation of anthropurgic and natural foci of icterohemorrhagic leptospirosis are the southern and central regions of the country, in the floodplains of the rivers: the Dniester, the Danube, tributaries of the Dnieper, the Southern Bug, and along reclamation canals [1, 4].

Synanthropic rodents play an important role in the formation of anthropurgic foci, which infect domestic animals - pigs, cattle, dogs, cats. Small mammals (common voles, mice, rats and other species), insectivores (hedgehogs, shrews) are the main reservoir of infection in nature. Domestic animals (dogs, pigs, cattle, sheep, less often goats and horses), wild fur animals (foxes, arctic foxes, nutrias) becoming infected with leptospira turn into an additional reservoir of infection for other susceptible animals and humans [5 - 7].

There is an opinion about the self-maintenance of leptospirosis' foci among cattle, due to the fact that in farm animals the disease is often asymptomatic, with a high degree of infection and a high mortality rate, representing a potential danger not only during the illness, but also for a long time after recovery [1].

The main source of infection are small mammals (carriers and sick subjects at all stages of the disease). In their body leptospira persist throughout life and are released into the external environment, then, under certain conditions, enter the body of a healthy person. In natural foci, they release the pathogen into the environment, contaminating the water of open reservoirs, soil, plants, foodstuffs, feed and other environmental objects. Leptospirosis in wild rodents is mild or asymptomatic, the fact of its carriage is considered as a manifestation of commensalism [8].

The prevalence of leptospirosis in the world and Ukraine

Leptospirosis is the leading zoonotic cause of morbidity and mortality in the world's poorest regions and in areas where adequate diagnostic tests and surveillance are not in place. The highest incidence is recorded in Oceania - 150.68 per 100 thousand people. (^{o} / $_{oooo}$), Southeast Asia and the Caribbean - 55.54 and 50.68, respectively. The lowest - in Southern Europe - 1.43 $^{o}/_{oooo}$. Central Europe is characterized by morbidity in the range of 4.02 $^{o}/_{oooo.}$ with lethality - 0.21. The highest mortality rate is observed in Oceania - 9.61, the lowest - in

Southern Europe - 0.09. According to a group of authors, the total leptospirosis' morbidity rate in the world is 1.03 million cases and 58,900 deaths per year [9].

In Ukraine, leptospirosis remains an actual infection, cases of which are registered annually in all administrative territories. The highest intensity of epizootological and epidemic processes in recent years has been noted in the Transcarpathian, Kyiv, Kirovograd, Nikolaev, Odessa, Chernihiv and Chernivtsi regions [3]. Over the past 10-12 years, the largest number of patients in the country as a whole was registered in 2010 - 632 (1.38 $^{o}/_{oooo}$). In 2011 - 2013 there was mortality rate decrease (0.67 - 0.79 $^{o}/_{oooo}$). In 2014, the intensive indicator constituted 1.04 $^{o}/_{oooo}$, and in 2015 it was 0.7 $^{o}/_{oooo}$. [7, 8].

The study of the manifestations of leptospirosis epidemic process in the Odessa region and in territories with similar climatic and geographical features in the North-Western Black Sea region (Kherson, Nikolayev regions) made it possible to establish that in 2010-2017. the incidence in the Odessa region (0.34±0.05) $^{o}/_{oooo}$ was significantly lower than in Kherson (3.62±0.67, $p<0.01$) and Nikolayev (1.89±0.41, $p<0.05$) areas. Among the three regions of the Northwestern Black Sea region, only Kherson one had a tendency to mortality rate increase (the average growth rate was 12.33%) [7].

Leptospira of 10 serogroups were the most widespread among animals and people in the Northwestern Black Sea region: *Icterohaemorrhagiae, Grippotyphosa, Pomona, Bataviae, Canicola, Hebdomadis, Tarassovi, Australis, Autumhalis, Javanica.* The role of leptospira from serogroups *Grippotyphosa, Pomona, Canicola, Icterohaemorrhagiae* in the general population's morbidity has been proven. At the same time, differences in the dominant serogroup were established (2016-2017): in the Odessa region, antibodies to *L. Icterohaemorrhagiae* (71%) were detected, in Nikolayev region, in 80.00% of cases, antibodies to pathogens from several serogroups and from rare groups were simultaneously detected; in Kherson - the main ones were *L. Hebdomadis* (21%), *L. Grippotyphosa* (15%), *L.Icterohaemorrhagiae* (14%). When examining environmental objects, a direct correlation of high strength ($r = 0.97$) was obtained between the composition of leptospira serogroups isolated from the external environment and from leptospirosis patients dwelling in the areas of water bodies localization [7].

The development of new natural complexes, climate change, urbanization modify the previous natural distribution areas of leptospira, which leads to the formation of new anthropurgic foci of the disease. Genetic variants of 16 identified genotypes of leptospirosis pathogens explain the variety of clinical manifestations of the disease: from asymptomatic or mild influenza-like forms (60–90%) to severe, with the development of multiple organ

lesions, and in cases of *L. Icterohaemorrhagiae* infection, mortality up to 20%. On the example of the Western Ukrainian region, the effectiveness of hospital surveillance for leptospirosis has been shown: if a febrile condition is detected in a patient and impossibility to exclude the disease, verification is carried out by determining IgM in paired blood sera [8, 10].

The role of molecular diagnostic studies in predicting the epidemic process of leptospirosis. The identification of new species in environmental samples collected around the world indicates their wide diversity and requires changes in the existing classification. The study of the set of main genes and pangens makes it possible to establish evolutionary links between representatives of the genus *Leptospira.* A number of studies indicate the ongoing evolution of a group of species with atypical genomic features that are often associated with human infection, and this requires further study [2, 11].

The most important direction of epidemiological surveillance of leptospirosis in the world is genomic surveillance of isolates circulating in different territories, combined with the analysis of clinical and epidemiological data. Molecular genetic monitoring is a promising method for predicting the epizootological process of leptospirosis, which makes it possible to clearly determine the source of the pathogen, the natural reservoir in a particular area, and successfully study the virulence factors and species characteristics of leptospirosis. In recent years, a bank of mutant strains has been created, and replication plasmids have been constructed, which contributes to the further study of mutations. The need to study various manifestations of the pathogenesis of the disease puts the genetic diagnosis of the pathogen into a routine category [11].

Studies of the genomes of 90 Leptospira isolates found in different countries of the world (Japan, Malaysia, New Caledonia, Algeria, France, Indian Ocean islands) made it possible to establish their nucleotide identity. When compared with representative genomes of known species, 30 new Leptospira species were identified [2].

The results of the study indicate a significant diversity of Leptospira genotypes circulating in Laos. During a 12-year period (from 2006 to 2017), 75 Leptospira isolates were isolated from patients, and *L. interrogans* was identified. The most common genotype was CG272 (n=18; 26.8%). This genotype was identical to that isolated in neighboring Thailand, where it caused many fatalities [12]. The identified strains of *L. Interrogans* CG272 belong to a unique clonal group capable of evolving through clonal expansion.

In Japan, the prevalence of leptospirosis among domestic cats has been conducted. Antibodies were found in 16.6% (40/241) of the animals investigated. In most seropositive

objects, antibodies against the Javanica serogroup were determined. In 7.1% (3/42) of urine samples from infected cats, DNA with sequences identical to *L. Borgpetersenii* was found in the fla B gene. A genetic relationship was established at the whole genome sequencing and multilocus typing of *L. borgpetersenii* of the *Javanica* serogroup isolates' sequence. The latter were isolated from cats, black rats, mongooses, and humans.

The role of cats as a host and source of infection for humans L. borgpetersenii of the Javanica serogroup has been proven [2].

In Vietnam, a serological study of the blood of urban rats (*R. argentiventer, R. norvegicus and R. rattus*) was carried out. 12.6% of them had antibodies to *L. interrogans* of the *Bataviae* serogroup were found. The possibility **of carriage** of *Leptospira spp.* by detecting DNA in the urine of animals. Sequencing of Leptospira DNA samples isolated from *R. norvegicus* kidney tissue revealed three different fla B gene sequences in 23 out of 81 (28.4%), identical to *Bataviae* and *Pomona* serogroups. Among rats living in urban areas of Vietnam, a wide circulation of *L. interrogans* has been established [13].

In Malaysia, a study of *Leptospira spp.* prevalence among urban stray dogs, which play an important role in the transmission of the pathogen. A serological study of the presence of antibodies to 20 serological variants of Leptospira has been conducted. In the presence of antibodies, blood, urine, kidney and liver tissue samples were cultured on nutrient media. Isolates were identified by PCR using 2 primers (16s rRNA and LipL32) and hyperimmune serum in a microagglutination reaction. The prevalence of antibodies was 32%, serovars were found: *Javanica, Bataviae, Icterohaemorrhagiae, Autumnalis, Canicola, Copenhageni* and *Australis.* Six species of *Leptospira spp.* were isolated from urine (n=2), kidneys (n=2) and liver (n=2). All 6 isolates belonged to *L. interrogans.* The presence of vaccine serovars (*Icterohaemorrhagiae* and *Canicola)* indicates the presence of post-vaccination antibodies in animals, but the predominance of non-vaccinal serovars (*Javanica* and *Bataviae)* indicates the possibility of natural infection or post-exposure infection. Isolation of *Leptospira spp.* directly from a urine sample does not only indicate an active infection, but also indicates the potential danger of stray dogs in the transmission of the pathogen, which is associated with their constant movement. Polluting the environment with infected urine, dogs pose a serious threat to public health [14].

In the course of molecular genetic studies of plasma, blood serum and urine samples of patients who died of a febrile illness in Tanzania, with serologically confirmed leptospirosis, Leptospira's DNA was obtained. To identify potential sources of the pathogen for humans, the genotypes obtained of circulating isolates were compared with the genotypes

found in animals. Leptospira's DNA belonging to *L. borgpetersenii*. *L. interrogans* and *L. kirschneri* were found in 3.6% of blood samples. In a phylogenetic comparison, the sequences of the genotypes of *L. borgpetersenii* and *L. kirschneri* identified in patients were similar to the genotypes identified among local livestock species. It is necessary to obtain more genetic material to determine the dominant Leptospira species and genotypes [15].

In Cambodia a PCR study of the urinary tract of rats living in urban areas was carried out. The pathogen was found in 20 of 163 rodents (12.3%) and classified as *L. interrogans* and *L. noguchii*. The most infected were *R. Norvegicus* (17.5%), *Rattus sp.* (16.7%) and *R. argentiventer* (9.4%), which proves the important role of rats as a reservoir of highly virulent leptospira [16].

Sequencing of DNA detected in the blood of leptospirosis patients was carried out in 2011-2019. 244 out of 444 samples (55%) have been investigated. The sequences of the sequenced streches were compared with those presented in the international database and previously isolated from patients and reservoir animals (n = 79). It has been established that the main source of *L. borgpetersenii* of the *Ballum serogroup* and *L. interrogans* of the *Icterohaemorrhagiae* serogroup are rats, *L. interrogans* of the *Australis* serogroup are dogs, *L. interrogans* of the *Pomona* and *Canikula* serogroups are pigs. It was found that the most severe cases leading to death were associated with infection with *L. interrogans Icterohaemorrhagiae* (10 cases) and *Australis* (5). The mortality rate significantly increased with the age of patients ($p < 0.001$) [12].

Valuable information for the diagnosis of leptospirosis is the increase in antibody titer in the reaction of microagglutination with paired sera, PCR analysis of blood and urine of sick people and animals. The developed rapid tests for diagnosis at early stages of the disease can prevent the development of its severe course [17].

Between 2013 and 2019 in northeastern Italy, blood samples from dogs with leptospirosis were analyzed. Identification of leptospira was carried out in 1631 samples, 193 of which contained DNA and were subjected to multilocus genotyping. In 135 Leptospira isolates, genotypes were identified. Sequence types characteristic of *L. interrogans* (ST17, ST198 and ST24), *L. kirschneri* (ST117 and ST289), and *L. borgpetersenii* (ST155) were found, this demonstrated a wide circulation of leptospira from the serogroups *Icterohaemorrhagiae, Australis, Sejroe* and *Pomona*. Thanks to a comparative analysis of the obtained genotypes with those presented in the database, possible chains of transmission from rats, mice, hedgehogs, and pigs were established [18].

Thus, molecular methods for the detection and identification of circulating Leptospira strains are an important tool for epizootological surveillance, including the development of vaccines for the immunoprophylaxis of dogs and pigs against the most common Leptospira genovariants [2, 12, 16, 18]. The development of basic preventive strategies against leptospirosis is based on the results of molecular epidemiological studies of infectious agents identified among wild rodents.

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