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PRESENT KNOWLEDGE REVIEW ON THE PROBLEM OF LEPTOSPIROSIS

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Abstract

The article presented contains meta-analysis of the published data on the problem of leptospirosis, mainly its epidemiology. The problems of taxonomic and genetic classifications are studied, breadth of extension in the world's different regions analyzed, the relation between leptospirosis causative agents and its hosts is pointed, modern methods of leptospirosis diagnostics are compared. Poor knowledge about the pathology under study has been revealed. The latter is connected with leptospirosis' neglect, lack or just few governmental initiatives on this problem decision, lack of medical and sanitary guidance.

Key words: leptospirosis; epidemic process; taxonomic classification; genetic classification; extension; diagnostics.

Urgency. Due to the wide variety of reservoir hosts and susceptible species, leptospirosis is the world's number one disease due to the prevalence of zoonoses because of widespread distribution of natural and anthropurgical foci. According to modern data, the total number of leptospirosis cases in the world is 1.03 million annually, including 58.9 thousand fatalities, which make up the majority of zoonotic deaths [1, 2].

The objective. To analyze available information and literature reviews on current aspects of leptospirosis' epidemiology.

Materials and methods. On the basis of published data on current studies of the manifestations of leptospirosis epidemic process the meta-analysis was conducted. Internet search resources: English-language database of medical and biological publications - PubMed and the Russian Scientific Electronic Library, integrated with the Russian Scientific Citation Index (RSCI) – eLIBRARY were used.

Results and their discussion

The disease was first discovered and described in Germany in 1886 by A. Weil and, almost simultaneously in 1886 - 1888 in Russia by N. P. Vasilyev. Initially the disease was called "infectious jaundice", later - "Vasilyev-Weil's disease". After the bacteriologists Inada, Ido and others in Japan in 1915. found the pathogen (*Spirocheta Icterohaemorrhagiae*) the disease was called jaundice leptospirosis.

Further study of leptospira was very intensive and in the 1960s, information was collected on 124 serotypes of leptospira pathogenic to humans and animals [3]. In 2010, 202 serotypes were already known [4]. Today serologically leptospira has been classified into 26 serogroups and over 300 serovars (both saprophytic and pathogenic) by microhemagglutination (MHA) and cross-sectional MHA [5, 6].

Unfortunately, serological taxonomy does not correlate with genetic features, and some serogroups include strains of even six different bacterial species. However, since leptospirosis' epidemiology has long been studied using serological instruments, serological taxonomy is still widely used [7]. The previous taxonomic classification, created on the basis of cross-agglutinin-absorption analysis, divided the genus Leptospira into two types: pathogenic - *L. interrogans* and saprophytic - *L. bifleha.* In 1987, based on the results of DNA-DNA hybridization, the species *Leptospira interrogans* was decided to be divided into 7 species. In the following years, new species, both pathogenic and saprophytic, were added to the Leptospira genus. Currently, there are 9 pathogenic species, 5 opportunistic and 7 saprophytic ones [8]. Pathogens: *L. interrogans, L. kirschneri, L. borgpetersenii, L. mayottensis, L. santarosai, L. noguchii, L. weilii, L. alexanderi, L. kmetyi* and *L. alstonii),* intermediate (i. e, species unclear or low pathogenic) : *L. broomii, L. fainei, L. inadai, L. licerasiae, L wolffii* and saprophytic species (i.e. free-living organisms found in water and soil and not infectious): *L. biflexa, L. idonii, L meyeri, L. terpstrae, L. vanthielli, L. wolbachii, L. yanagawae* [9]. (Fig. 1).

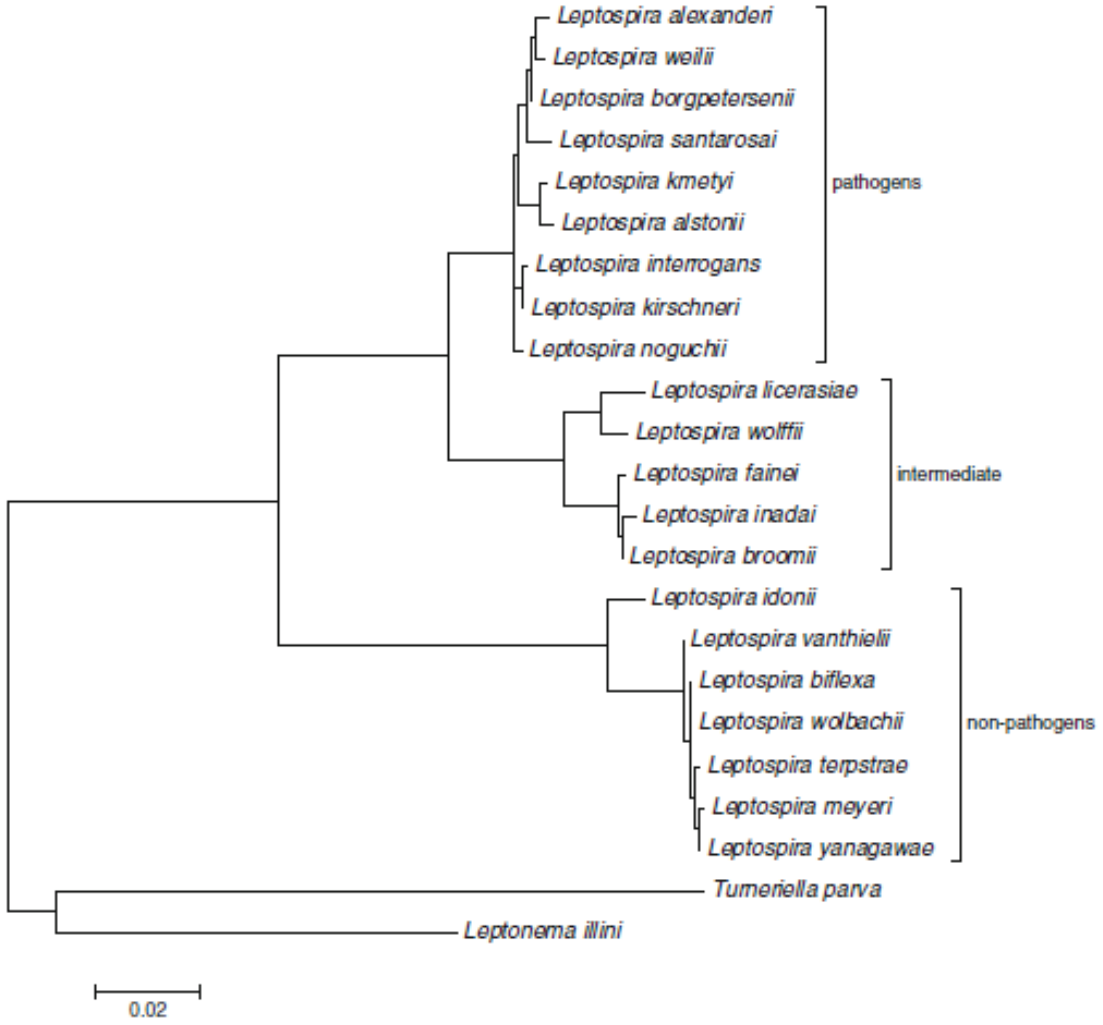

Fig. 1. Phylogenetic tree of *Leptospiraceae* family.

After conducting a total genomic analysis of 20 Leptospira species, a team of scientists showed the evolutionary relationship of genetic features associated with the pathogenicity and virulence found in different Leptospira species. Pathogenic strains of leptospira have revealed genetic features of mammalian parasite adaptation: sialic acid biosynthesis, pathogen-specific porphyrin metabolism, ability to synthesize vitamin B12 from L-glutamine. A new large family of virulence-modifying proteins has also been identified due to specific adhesins that are unique to pathogenic leptospores. Comparative genomic analysis of the genus outlined new insights into the general evolutionary processes by which bacteria shape their pathogenicity [10].

It has been revealed that extracellular proteases of leptospires exhibit proteolytic activity against the proteoglycans of the host and plasma proteins, with the possible

involvement of metalloproteases. It has been found that species with attenuated pathogenic and saprophytic properties do not exhibit proteolytic activity, indicating that the ability to degrade host molecules is a leading sign of leptospir virulence. It has also been shown that extracellular Leptospira proteins promote the pathogenic mechanisms required for infection [11].

Due to recent genetic studies, the original transcriptional genomic site (OTGS) and promoter maps for pathogenic *L. interrogans* were first deciphered. In the analysis of bacterial RNA, the authors proved the possibility of OTGS culturing at 30° and 37°C, without disturbing its morphological structure. More than 500 RNAs with regulatory functions (rRNA) have been identified. According to the results of RNA sequencing of the most common transcripts of *L. interrogans,* it is established that most of the lipoproteins in pathogenic strains are encoded by the following genes: lipL32, lipL21, lipL41, 1a22 and lip36; 30S and 50S ribosomal subunit genes and flagellin-encoding genes, consistent with previous transcriptional and translation assays. The data obtained are the basis for a modern understanding of adaptive mechanisms that have evolved to form Leptospira, which can only be established through genetic studies [12].

Leptospirosis is recorded worldwide, both in rural and urban areas of temperate and tropical climates. The number of human cases has not been clearly documented. It varies from 0.1 to 1 per 100000 per year in temperate regions and up to 10 or more per 100000 per year in wet tropics. During outbreaks and among high-risk groups, 100 or even more people per 100000 may be infected. For a number of reasons, leptospirosis in many regions of the world is neglected and, as a consequence, the incidence of the disease is underestimated. In endemic areas the incidence of leptospirosis can be as high as possible during the rainy season, and in the event of floods it can reach the level of epidemy [13].

The highest incidence rate is observed in Oceania - 150.68 per 100 thousand of population, Southeast Asia and Caribbeans - 55.54 and 50.68, respectively. The lowest incidence rate is recorded in Eastern Europe (where Ukraine is located) - 1.43 per 100 thousand. The highest mortality rate is in Oceania - 9.61, the lowest in Eastern Europe - 0.09. Mortality is highest in Sub-Saharan Africa (7.35-9.92%), lowest in Australia, Central Europe and at the south of Latin America - 4.17%. It is noteworthy that the highest rates of morbidity and mortality are recorded in the poorest regions of the world and in areas where there is insufficient surveillance and diagnostic tests are unavailable [1].

The main burden of this infection, which affects vulnerable population groups - peasants, farmers, subsoil dwellers, servicemen, public servants - falls on developing countries [14, 15]. The group of authors emphasizes that the highest leptospirosis burden evaluation is in tropical regions of the world on the Asian, American and African continents [15]. In many tropical countries, leptospirosis is often under-diagnosed, especially in areas where there is high endemicity of Dengue fever, malaria, and Coxiella burnetti infection [16].

Researchers note that leptospirosis is increasingly observed when travelers return from trips to tropical regions, and recommend to consider this diagnosis with any febrile condition in travelers [17, 18].

Due to the fact that leptospirosis is an important public health problem, knowledge of the sources, pathways, symptoms and complications of leptospirosis in population are crucial for the prevention, early diagnosis, early treatment and reduction of mortality. However, studies conducted on Trinidad, in India, Sri Lanka and Malaysia have shown lack of knowledge about leptospirosis among general population. According to their results 48.0-87.2% of respondents had a low level of knowledge about leptospirosis [19 - 22] while knowledge about disease is a prerequisite for effective prevention. However, the low level of knowledge found in the respondents is attributed to the neglect of the disease, lack or low number of government initiatives addressed to the solution of health care need, lack of medical education, including the use of non-specialized terms in population [23].

Due to the severe course of the disease, need for a long-term inpatient treatment and high mortality rate among the able-bodied population, the economic losses caused by this infection are considerable, even if the disease occurred in mild forms or was recorded under another diagnosis.

As a result of the disability or death of people, society loses years of productive activity annually. In 2015, the analysis of adjusted years of disability (DALY) lost as a result of leptospirosis was estimated at 2.90 million of DAILYs per year. It is interesting to note that men accounted for approximately 80.0% of the overall burden of the disease [15].

Leptospirosis is also widespread in farm animals, especially in cattle and pigs, leading to great economic losses. An analysis of the data from the international epizootic bureau shows that of the 130 countries that have officially reported the leptospirosis epizootic, 56 countries have been recognized as unfavorable for this infection; in 9 - the disease was registered in limited territories; in 19 - showed only seropositivity; 11 countries did not provide reliable information and 35 did not register the disease [24].

Table 1

Leptospirosis pathogens and their main hosts (in the territory of Russian Federation)

Genome	Serogroup	Serovar	The main hosts
L.interrogans	Icterohaemorrhagiae	Copenhageni icterohaemorrhagiae	Grey, black rat
	Canicola	Canicola	Dog
	Australis	Bratislava	European hedgehog
	Bataviae	Bataviae	Harvest-mouse
L. interrogans L. kirschneri	Pomona	Pomona	Cattle
		Monjakov	Pigs
		Mozdok	Field mouse
L. interrogans	Sejroe	Saxkoebing	Meadow mouse
L. interrogans		Hardjo	Cattle
L. borgpeterseni		Sejroe	House mouse
L. borgpeterseni	Javanica	Poi	Common shrew
L. borgpeterseni	Tarassovi	Tarassovi	Pigs, cattle
L. kirschneri	Autumnalis	Erinacei auriti	Eared hedgehog
	Grippotyphosa	Grippotyphosa	Common field vole

To date, the issues of the epidemiology of leptospirosis have been studied in sufficient detail. The source of leptospirosis infection are wild animals (rats, mice, hedgehogs, etc.), domestic animals (pigs, cattle, dogs), and industrial animals (foxes, minks, foxes, and many other species) (Table 1). Human being is a random host of the pathogen [4, 7, 25-28].

Leptospira enter the environment with infected urine. Survival in nature is ensured by long-term carrier of leptospira in animals (Table 2) [29, 30].

Rodents are the most significant source of infection because carriers can secrete leptospires throughout life, both with urine [7, 31] and with milk [32]. 12.0 - 25.0% of small mammals are carriers of pathogenic leptospira [33].

A matter of dispute is the question of cats' role as a source of infection, the infection of which occurs when eating murine rodents and rats. Like other mammals, their body responds with the formation of antibodies that are detected in *MAR*. However, the group of authors argues that the presence of antibodies is only evidence of contact of the animal body

of any kind with the pathogen leptospirosis. Thus it is impossible to consider an animal as sick. Despite the cases of leptospira isolation of different serogroups from cats, the authors emphasize that cats are not able to maintain the pathogen's circulation, because regardless of the close contact between cats and human beings, no cases of human infection from the cat have been established, and no cases of infection of any other animals from the cat [28].

Table 2

The timing of leptospira secretion by different species of animals with urine

Animal	Duration of pathogen urination
Rodents	All Life
Pigs	Up to two years
Sheep	Up to nine months
Cattle	Up to twenty months
Dogs	Up to three years
Cats	Up to one hundred nineteen days
Foxes	Up to five hundred fourteen days
Chickens, ducks, geese	Up to one hundred and fifty-eight days

Humans may be infected in the following ways: aqueous - occupies a leading place, outbreaks of disease are possible; nutritional if a human does not follow eating hygiene rules after caring for animals (sporadic morbidity); contact transmission - through mucous membranes, cuts and scratches on the skin. Infection occurs after water or soil contaminated with animals' urine get on the mucous membranes and damaged skin. After penetration into the bloodstream, spirochetes multiply in the organs: CNS, kidney, liver. With the help of the immune response, the body is released from leptospira, but the pathogen can persist and multiply in the renal tubules [30, 31].

The representatives of occupational risk group has direct contact with potentially infected animals. They are: veterinarians, farm workers (during hay, harvesting), milkmaids, shepherds, hunters and security guards, animal shelters, scientists, technologists and meat processing plants, fishermen, miners [26, 31, 34].

Often, indirect contact with water or soil contaminated with leptospires can be associated with recreational and professional activities. In addition to the external risks mentioned above, sewerage, military training and agriculture in areas with significant rainfall

are considered. These activities are related to the ones that can cause soil and water contaminated with the urine of rodents and other animals [34] contacts with damaged skin or wounds.

It was noted that in the period of higher rainfall there was a pronounced tendency to morbidity increase, while in the period of insignificant ones there was a tendency to its stability [35]. This phenomenon was confirmed in other studies, when scientists observed a direct relationship between the increase in rainfall and the number of leptospirosis patients with a lag of 2 weeks (duration of the average incubation period) [36].

According to modern data, social, sanitary and behavioral risks of infection include not only poor sanitary conditions, close contact with sewage, the presence of rodents, but also collecting firewood, walking barefoot, outdoor recreation [37] bathing in fresh water [38], water sports in fresh water [34] and trips to exotic places [17, 18].

Infection is possible even after bites of murine rodents. However, data on the degree of infestation of rodents differ.

According to the data of one group of authors, about 19.0 % of bitten patients develop leptospirosis [39]. According to others, in the study of saliva of wild rats for the presence of leptospira (with a positive result in urine), only 1.23% of cases obtained a positive result and 2.47% doubtful [40].

The United States studies indicate that more than 70.0% of leptospirosis cases can be attributed to physical contact with contaminated water [41], where leptospires can remain alive for several months [31]. The ability to survive for long periods in environmental conditions indicates that when contacted with any source of contaminated water, there is a high risk of infection with leptospirosis [42].

Pathogenic leptospires in freshwater reservoirs maintain viability from 7 to 30 days or more, and in seawater, peat swamps they die quickly. In dry soil, they are stored for no more than 2-3 hours, in moist with pH of 6.7-7.2 and humidity of 15.0-31.0% for up to 2.5 months, and in moistened - for up to 7 months. A group of authors found that leptospires remain viable in river soils for a long period (before drying) [43].

The acidic environment affects them adversely, and alkalization to pH 9.8 is well tolerated. Sensitive to drying, in direct sunlight they die in 30-120 minutes. When boiling, they die instantly, at a temperature of 45 - 56° C in 45-30 minutes, at 70° C - in 10 seconds. Leptospires are resistant to low temperatures and can remain viable even after prolonged freezing. They are rapidly inactivated under the action of disinfectants [30].

When study leptospirosis clinical course, the majority of researches note that the main symptoms and clinical manifestations are fever, headache, nausea, vomiting and abdominal pain and these symptoms are observed in about 60% of patients [44, 45]. In half of the cases symptoms of myalgia and conjunctival hemorrhage were noted [38].

The able-bodied population is affected most often, among which men make up 70.0 - 98.0% [15, 44 - 46]. A similar situation was observed in the study of infant leptospirosis - about 90.0% of the persons affected are male patients aged 10 - 20 y.o. In all cases, the likely route of the children infecting was related to the recreational impact of river water [38].

According to scientific observations, leptospirosis mortality rate remains high and ranges from 3.5 to 15.0% [44, 47, 48]. The average mortality rate for untreated leptospirosis is about 2.0% for mild forms, and 12.0 - 40.0% for the patients with more severe disease (jaundice, renal failure) [49].

The mortality rate was significantly higher in patients when the diagnosis of leptospirosis was established on the basis of clinical symptoms without confirmation in the microagglutination reaction (15.13% vs. 5.43%; $p < 0.01$) [47]. The most common complication is acute renal failure, which develops in 79.2% of cases [48].

Non-specific manifestations of leptospirosis include undifferentiated fever, aseptic meningitis, pulmonary bleeding, clinical similarity to certain diseases (viral hepatitis, malaria, viral hemorrhagic fever, Dengue's fever, typhoid fever, paratyphoid fever). Although several methods can be used to diagnose leptospirosis (bacteriological, serological reactions, microagglutination, complement binding reaction, hemagglutination, latex agglutination, immunoassay, MGA, PCR), but each method has its specifications (Table 3) [50, 51].

Bacteriological analysis is not always successful, complicated and quite time consuming due to the need to prepare special nutrient media immediately before sowing, capricious leptospira and the duration of their growth, mainly in liquid nutrient media. The biological sample method is highly effective in isolating clean crops, but is only performed in laboratories that can handle animals. With a small number of bacteria, the microscopy method in the dark field is not informative, but relatively simple and gives the fastest result with a sufficient amount of biological material. Serological methods for detection of antibodies are retrospective, their use is possible only from the second week of the disease. The PCR method is highly diagnostic but not always available [50, 51].

Table 3

Methods of laboratory diagnostics

Method of investigation	Target	Period of disease	Specificity of the method
Bacteriological method	Pathogen	First week (leptospiemia)	Pathogenic leptospira
MAR	AB	Second week - up to several months	Serogroup
Cross MAR	Pathogen	When culture is selected	Serogroup
ELISA, ICA	AB (IgG)	End of the 2nd week - up to several months	Pathogenic leptospira
ELISA, ICA	AB (IgM)	Acute period	Pathogenic leptospira
Real-time PCR	16S rRNA	First week	Pathogenic leptospira
Nested PCR	LipL 32	First week	Pathogenic leptospira
MFA, ELISA, ICA, MIS, LA	AH	First week(leptospermia)	Depending on the tasks
MAR with the panel of monoclonal antibodies	AH	Identification of a culture	Serovariant
Multilocus sequencing (MLS)	gDNA	Identification of a culture	Species(genomic species)
Direct protein profiling with mass spectrometer	Proteins of a cell	Identification of a culture	Species (genomic species)
Hydrogenation multiplex ligasereaction	gSNP	Identification of a culture	Species (genomic species)

In Europe, several ELISA variants are used to determine the titers of Ig G and Ig M antibodies to different leptospira serovars. The test detects Ig M to leptospira within 7 days after infection, when specific agglutinins do not yet reach the diagnostic titer. When using ELISA antibody titer 1: 320 is considered diagnostic. The sensitivity and specificity of Ig M are respectively 86.5% and 97.0%. The use of recombinant LipL32 allows to detect specific antibodies to leptospira with sensitivity (96.4%) and specificity (90.4%) and is considered as a screening test in the study of a large number of serum samples [52].

The multilocus sequencing method provides high accuracy in the results of the strain characteristics. At the present stage, a 3-locus typing scheme is used, using a limited amount of genetic material available in clinical specimens, which may also be proposed for epidemiological monitoring [53].

Due to the fact that more than half of the infections occur in contact with contaminated water [41], studies of environmental factors are important for the development of adequate

prevention measures. But detection of leptospires in water samples is rarely carried out using molecular methods [42]. Detecting pathogenic leptospores in water samples has several difficulties. First, it is necessary to filter large volumes of water and the concentration of leptospires in the sample; the presence of other potential bacteria contained in the water samples which may contaminate the culture media and complicate the procedure.

Currently, there is no conventional method for testing water samples for the presence of leptospires based on DNA detection. Recently, a group of authors developed a DNA - microchip to detect microbial agents in drinking water samples [54]. In the study of drinking water, DNA pyrosequencing was used to detect the virulence of isolated *Leptospira interrogans,* as well as a technique using smaller volumes of water with centrifugation of samples and the detection of pathogenic leptospira by LipL32 gene [55, 56]. According to the same gene, pathogenic leptospiral protein can be detected in ecological aqueous biofilms [57].

To date, most studies aimed at identifying ecological reservoirs of pathogenic leptospires have focused on the study of water samples: sewage, water taken from puddles, wells, fresh water bodies. However, some studies investigate the content of pathogenic leptospires in soil from endemic regions, and confirm that soil is an additional ecological reservoir in the life cycle of pathogenic leptospires and a source of leptospirosis. During heavy rains, floods, or excavations, leptospora are raised from the subsurface soils, which causes the accumulation of a dose sufficient to infect humans in the environment. Leptospira concentrations detected in soil by PCR with sequencing of 16S gene regions were more than 2-fold higher than those detected by LipL32. This is explained by the fact that 16S is detected in pathogenic and intermediate species, whereas LipL32 is detected only in the presence of pathogenic leptospora, but does not detect the presence of saprophytic species. Thus, intermediate species of Leptospira were found to be widespread and present in much higher soil concentrations than pathogens. Soil moisture significantly affects the survival time of leptospores: with moisture content above 20% in 62% of the samples pathogens were found, and with humidity below 20% - in 21%. The results obtained should be taken into account in the development of anti-epidemic measures, including the elimination or reduction of access of population of endemic areas to sites of potential leptospira infection [58, 59].

Conclusions:

1. Leptospirosis is recorded worldwide. The number of cases is 0.1 to 1.0 per 100.000 of humans in temperate regions and up to 10.0 or more in the humid tropics. In endemic areas, incidence of leptospirosis can be as high as possible during the rainy season, and in the event

of a flood, it reaches epidemic proportions. According to current data, leptospirosis ranks first in the world in prevalence, morbidity and mortality among zoonoses.

2. Leptospirosis, according to the DALY index, is a significant cause of the loss of quality, productive and fulfilling life for society and causes significant social and economic damage.

3. Contact with freshwater bodies, both for recreational and consumer purposes and with soil, in endemic areas, is a significant risk factor for leptospirosis.

4. The detection of LipL32 in water or soil samples is a sign of the presence of pathogenic leptospora and indicates the need to limit public contact with these environmental sites.

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