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Leptospirosis: classification, epidemiology, and methods of detection. A review

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Bacteria of the genus *Leptospira* can cause widespread and potentially lethal bacterial zoonosis called leptospirosis. Considered a neglected tropical zoonotic disease, leptospirosis was recognized as a global public health problem due to its increasing prevalence in developing and developed countries. This review focused on the current knowledge on leptospiral infection, classification, epidemiology, and detection methods. We are also reviewing the data on the study of *Leptospira* in Lithuania available in the literature.

Keywords: *Leptospira*, leptospiral infection, Lithuania

INTRODUCTION

Infectious diseases remain one of the leading causes of high annual morbidity and mortality. Infectious diseases transmitted through animals or vectors (carriers) are called zoonoses. A zoonosis is associated with a specific pathogen that is transmitted from an animal to a human and involves the interaction of humans, animals, and the environment. A meta-analysis from 1940 to the early 21st century showed that 60.3% of new infectious diseases were caused by a rapid spread of zoonotic pathogens (Samrot et al., 2021). Recent outbreaks of SARS and avian influenza demonstrated again the high potential of microorganisms from an animal reservoir to adapt to the human host. Various types of animals, both domestic and wild, are reservoirs of zoonotic pathogens. Considering the high diversity of animal species and the complex life cycles and transmission of pathogens, effective surveillance, prevention, and control of zoonotic diseases pose a public health challenge (Thompson, Kutz, 2019). Echinococcosis, tularaemia, leptospirosis, avian influenza, Hantavirus infection, rabies, and pox viral infections are examples of zoonoses that are transmitted by direct contact with wildlife. These zoonoses are endemic to Europe and occur more often or can emerge again (Rahman et al., 2020). Zoonotic pathogens can circulate in different ecosystems.

Bacteria of the genus *Leptospira* can cause widespread and potentially lethal bacterial zoonosis called leptospirosis. Considered a neglected tropical zoonotic disease, leptospirosis was recognized as a global public health problem due to its increasing prevalence in both developing and developed countries (Vijayachari et al., 2008). It usually affects vulnerable segments of the population, such as rural farmers and urban slum dwellers. Leptospirosis-induced morbidity and mortality rates are the highest in the world's poorest regions

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and the areas where medical care is not regularly carried out (Costa et al., 2015). Leptospirosis is endemic mainly in tropical regions and most often spreads after flooding or heavy rain.

Various wild and domestic mammals can act as reservoir hosts for *Leptospira* species. An increasing number of studies demonstrate detection of *Leptospira* spp. in Carnivora, Didelphimorphia, Rodentia, Cingulata, Cetacea, Chiroptera, Afrosoricida, and Primate orders, as well as in Reptilia and Amphibia classes (Cilia et al., 2021). Rodents, which are abundant in urban and peridomestic environments, especially rats *Rattus norvegicus* and *R. rattus*, are considered the most important known source of *Leptospira* infection (Haake et al., 2015). Reservoir hosts carry the pathogen in their renal tubules and excrete pathogenic *Leptospira* in their urine. Humans and animals can be infected with *Leptospira* spp. through direct or indirect contact with infected animals or contaminated environments such as soil or water.

According to the Annual Epidemiological Report for 2016 of the European Centre for Disease Prevention and Control, 1319 cases of leptospirosis were reported in 26 countries of the European Union in 2016, of which 783 (59%) were confirmed cases.

In Lithuania, leptospirosis was first diagnosed in 1945. Annually, from 14 to 40 cases were registered between 1995 and 2005 and from three to 20 cases between 2012 and 2016 (ECDC, 2021).

This review is focused on the current knowledge on leptospiral infection, its classification, epidemiology, and detection methods. We are also reviewing the available data on the study of *Leptospira* in Lithuania in the literature.

TAXONOMY AND CLASSIFICATION

Leptospires belong to the order Spirochaetales, family Leptospiraceae, genus *Leptospira*. The bacteria belonging to the genus *Leptospira* are Gram-negative, aerobic, slow-growing, thin, flexible, and tightly coiled spirochetes. Leptospires are usually 0.1 μm wide and 6 to 20 μm long. The cells have pointed ends bent into a typical hook-like shape that allows leptospires to be clearly differentiated from other spirochaetes (Levett, 2001; Samrot et al., 2021). *Leptospira* spp. consists of an outer membrane with various functional proteins.

The identification of *Leptospira* isolates has been traditionally based on serological methods, and species identification was determined by the pathogenicity of the isolate. The taxonomy of *Leptospira* is quite complex due to the high serological diversity. Individual species of *Leptospira* are divided into serological groups, which are further divided into serovars (Samrot et al., 2021). Initially, after the first description by Stimson (1907), *Leptospira* were classified into two species, *Leptospira interrogans* and *Leptospira biflexa*, which clearly distinguished between pathogenic and saprophytic (non-pathogenic) strains, respectively. These species were differentiated by their nutritional requirements and other phenotypic characteristics. They were further subdivided into specific serovars based on the presence of homologous antigens, defined by the structural heterogeneity of their lipopolysaccharide carbohydrate component (FTA) (approximately 60 *L. biflexa* serovars and at least 225 *L. interrogans serovars*) (Levett, 2001). A genotypic classification replaced the phenotypic classification of *Leptospira*, and there was a move from phenotype-based typing methods towards genotype-based methods such as pulsed field gel electrophoresis (PFGE) and PCR-based methods (Cerqueira, Picardeau, 2009). As 16S rRNA gene sequencing became a common technique for identifying unknown bacterial isolates, it was also applied for the identification of *Leptospira* species. The phylogeny of *Leptospira* species is based on a comparative 16S rRNA sequence analysis. Currently, 22 species are distinguished in the genus *Leptospira*, which are divided into three main groups (clades): pathogenic, intermediate, and saprophytic, and more than 300 serovars. Recently, 12 new species of *Leptospira* have been discovered in the soil or water, which indicates insufficient knowledge of the biological diversity of this genus (Bulach, Adler, 2018).

A genotypic classification complicated the identification of leptospires: some species contain both pathogenic and nonpathogenic serovars, and several serovars are found in more than one species. Thus, neither the serogroup nor serovars can reliably predict *Leptospira* species, and the identification of the serovar is no longer sufficient to assign an isolate to its correct species (Morey et al., 2006). In addition, more recent studies have demonstrated the genetic heterogeneity of the serovars. The reclassification of *Leptospira* according to genotypic characters is taxonomically correct and provides a solid basis for further classification of these bacteria (Levett, 2001; Samrot et al., 2021). Other genes have been used to discriminate between species: *rpoB*, *gyrB* and *ligB* (reviewed by Cerqueira, Picardeau, 2009).

METHODS OF *LEPTOSPIRA* DETECTION

Currently, several different methods are used for the diagnostics of leptospirosis: serological tests (microscopic agglutination test, solid phase assay, enzyme linked immuno-sorbent assay, and indirect haemagglutination assay), direct diagnostic methods (microscopy – phase contrast or dark field microscopy; histochemical staining and immunostaining), culture methods and molecular techniques. Other advanced techniques, such as flow cytometry, have also been developed. In epidemiological studies, identification by species level is not informative, except for the identification of *Leptospira* for pathogens and for saprophytes. Since each serovar is usually associated with a specific host, the identification of serovars is necessary for epidemiological studies and the development of appropriate prevention strategies (Cerqueira, Picardeau, 2009).

MOLECULAR DETECTION. PCR-BASED METHODS

Modern technology has dramatically improved laboratory procedures, particularly those for the detection, identification, and typing of epidemiologic strains. Molecular methods for DNA detection, such as PCR, are widely used in the diagnosis of leptospirosis in humans and animals. The main advantage of these methods is high sensitivity and specificity. The molecular methods appear to be very useful for the diagnosis of chronic silent leptospirosis in domestic animals.

Several molecular typing techniques have been used for the characterisation of *Leptospira* strains: bacterial typing methods based on insertion sequence (IS) elements; ribotyping (determination of the restriction fragment length profiles of digested chromosomal DNA probed with rRNA); restriction endonuclease analysis of total genomic DNA and pulsed-field gel electrophoresis (PFGE); randomly amplified polymorphic DNA (RAPD) and arbitrarily primed PCR (AP-PCR); amplified fragment length polymorphism (AFLP). Later, genome analysis identified numerous repeated sequences and many short repetitive DNA sequences (Variable Number of Tandem Repeats) in Leptospira genomes. These sequences have been used for fingerprinting of *Leptospira*. Conventional PCR is still the most commonly used method for diagnosing leptospirosis in animals, followed by real-time PCR (qPCR), which allowed faster and more sensitive diagnosis compared to conventional PCR (Levett et al., 2005). qPCR is less sensitive to contamination.

Several pairs of primers were described to detect leptospirosis by PCR, some of which were based on specific gene targets, usually 16S or 23S rRNA genes and repeating elements, while others were made from genomic libraries. The 16S rRNA gene was the first molecular marker applied for Leptospira identification, and it is still used today in many studies, mainly for an initial screening. This marker has a good capacity to discriminate between pathogenic, intermediate, and saprophytic leptospires. However, it is ineffective in distinguishing between Leptospira species within a clade. Other universal genes present in bacteria, such as gryB and secY, and *Leptospira* surface protein genes *lipL21*, *lipL32*, *lipL41* and *ligB* are used in PCR-based genotyping (Guernier et al., 2018). The *lipL32* gene is currently the most common target used for *Leptospira* detection, with 48% of

studies using this genetic marker (Di Azevedo, Lilenbaum, 2021). Multilocus sequence typing (MLST), a method based on partial sequences of several housekeeping genes (such as *adk*, *icdA*, *lipL32*, *lipL41*, *rrs*, *secY*, *pntA*, *sucA*, *pfkB*, *tpiA*, *mreA*, *glmU*, and *fadD*), has also been applied to *Leptospira* spp.

EPIDEMIOLOGY IN THE EUROPEAN UNION

In the past decade, the highest number of confirmed leptospirosis cases in the EU was in 2019, when it reached 1049 cases (ECDC, 2021). In 2020, 22 EU countries reported 565 confirmed leptospirosis cases. Cyprus, Finland, Iceland, Lithuania, Luxemburg, Malta, and Sweden had no confirmed cases in 2020. The countries with the highest number of confirmed cases were Germany, France, and Portugal. The notification rate was 0.14 cases per 100,000 population in the EU. Four countries – Estonia, Portugal, Slovenia and Ireland – had a notification rate of above 0.5 confirmed cases per 100,000 population. In 2020, leptospirosis caused six deaths , compared with ten deaths reported in 2019; in 2018, the fatality rate reached 18. In 2020, almost 90% of people with confirmed cases were hospitalised. As for the Baltic countries, 24 confirmed cases of human leptospirosis were reported in Estonia, 37 in Lithuania, and 24 in Latvia between 2016 and 2020 (Table).

Table. **Cases of human leptospirosis in the European Union, 2016-2020 (ECDC)**

	Reported cases				
Country	**2016**	**2017**	**2018**	**2019**	**2020**
Austria	14	68	24	24	11
Belgium	19	17	20	18	11
Bulgaria	9	5	15	7	1
Cyprus	0	0	0	0	0
Czechia	18	21	10	24	27
Germany	91	129	117	160	118
Denmark	15	22	19	13	14
Estonia	**3**	**5**	**6**	**5**	**10**
Greece	19	24	18	27	17
Spain	16	19	65	49	20
Finland	1	0	0	0	0
France	79	134	129	201	127
Croatia	11	24	7	22	4
Hungary	15	14	19	14	3
Ireland	26	19	19	17	25
Iceland	0	0	0	0	0
Italy	54	32	41	34	18
Lithuania	**18**	**16**	**3**	**0**	**0**
Luxembourg	0	0	0	0	0
Latvia	**5**	**8**	**4**	**4**	**3**
Malta	1	2	2	4	0

Table. **(Continued)**

	Reported cases				
Country	**2016**	**2017**	**2018**	**2019**	**2020**
Netherlands	95	77	45	111	60
Poland	4	2	7	4	1
Portugal	101	117	69	82	70
Romania	65	44	51	66	10
Sweden	1	4	3	7	0
Slovenia	17	24	18	59	12
Slovakia	10	7	2	5	3
EU	783	932	803	1049	565

LEPTOSPIROSIS IN LITHUANIA

Relatively few comprehensive studies on leptospirosis have been reported in Lithuania. Leptospirosis was studied in some administrative districts of Lithuania in 1972–1973. Eight species of rodents and one insectivore species were confirmed to be involved in the epizootic cycle of this disease (Burakauskas, Danilevičius, 1985; Motejunas et al., 1974). The effectiveness of treatment and prevention measures against leptospirosis in cattle and pigs were investigated by researchers at the Veterinary Institute of the Lithuanian Veterinary Academy from 1986 to 1990.

In several regions of the country, different *Leptospira* serogroups have been isolated from the internal organs and urine of wild (small rodents, wild boars) and domestic (cattle, pigs, horses) animals (Šiugždinienė et al., 2007; Buitkuvienė et al., 2012; Stankevichienė et al., 2016; Jeske et al., 2022).

Šiugždinienė et al. (2007) investigated the prevalence of *Leptospira* serovars among cattle in Lithuania. The microscopic agglutination test (MAT) was used for the diagnosis of *Leptospira* serovars. The results of the study showed that the antibodies against *L. grippotyphosa* (32.46%), *L. hebdomadis* (25.42%) ser. Kabura, and *L. sejroe* (18.98%) ser. Poland were the most prevalent in cattle blood sera. The highest serological diversity of different *Leptospira* serogroups was observed in the Central and Northern counties of Lithuania.

Stankevičienė et al. (2013) investigated the prevalence of leptospirosis in Lithuanian swine farms in 2010. Blood serum samples were randomly collected from 1266 pigs in 28 swine farms in 19 districts. The samples were tested by the microscopic agglutination method. Scientists detected 542 positive reactions to *Leptospira* in pigs sampled from 17 swine farms. The serovar *L. bratislava* was the most common in blood samples. Other detected serovars were *L. pomona*, *L. copenhageni*, and *L. tarassovi*.

Four-hundred-and-forty blood serum samples were tested to determine the spread of leptospirosis amongst equine family animals (horses, Shetland ponies, donkeys) in six Lithuanian counties from 2011 to 2015 (Stankevičienė et al., 2016). This study was carried out using a standard serological method, the microscopic agglutination test. Of the tested horses, 18.63% were found serologically positive for leptospirosis. Most of the positive samples (31.8%) were found among horses in Panevėžys County, which was followed by Utena County (19.7%), and Vilnius and Kaunas counties, with about 16.0% in each. Antibodies against serogroups of *L. canicola* (33.0%), *L. copenhagen* (26.1%), and *L. grippotyphosa* (20.9%) were identified in the samples analysed.

Seroprevalence of *Leptospira* antibodies was also investigated in wild boars. Blood sera

samples were collected from 659 healthy wild boars from 42 locations throughout Lithuania during the autumn-winter hunting seasons between 2008 and 2010. The sera of wild boars were analysed using the microscopic agglutination (MA) test. From the examined wild boar sera, 4.6% were positive for different serovars of *Leptospira* spp. Most frequent were the findings of the antibodies to *L. bratislava* (3.1%) and *L. grippotyphosa* (2.1%). Antibodies to *L. pomana*, *L. copenhageni*, mixed *L. copenhageni – L. bratislava* serovars, and *L. sejroe* were also detected.

A recent study reported the cocirculation of *Leptospira* spp. and multiple orthohantaviruses in rodents in Lithuania (Jeske et al., 2022). Captured in 23 sites in the country, 1617 rodents and insectivores were screened for zoonotic (re-)emerging *Leptospira* and orthohantaviruses. Molecular detection methods were used for *Leptospira* spp. identification. A real-time polymerase chain reaction targeting the *lipL32* gene (*lipl32*-qPCR) was performed for the initial screening of bacteria. Genomospecies identification was done by *secY*-PCR and multiple locus sequence typing (MLST). *Leptospira* DNA was found in six rodent species (the striped field mouse *Apodemus agrarius*, the yellow-necked mouse *Apodemus flavicollis*, the bank vole *Myodes glareolus*, the common vole *Microtus arvalis*, the field vole *Microtus agrestis*, and the root vole *Microtus oeconomus*) with an overall mean prevalence of 4.4%. *L. kirschneri* was identified in the yellow-necked mouse, the striped field mouse, the common vole, and the bank vole. The authors concluded that the detection of re-emerging human pathogenic *Leptospira* and orthohantaviruses in rodent reservoirs in Lithuania requires increased awareness of public health institutions (Jeske et al., 2022).

CONCLUSIONS

This review summarises the taxonomy, classification, and epidemiology of leptospirosis. The classification of leptospirosis changed over the years, with the genotypic classification replacing the phenotypic classification. Furthermore, as we can see from epidemiology reports, leptospirosis cases in the EU fluctuate from year to year, and the infection still affects hundreds of people across Europe. The review of the studies conducted in Lithuania leads to the conclusion that wild animals such as small rodents and wild boars are natural reservoirs of leptospirosis in particular regions of Lithuania and represent a significant potential source of leptospirosis for other wild and domestic animals as well as for humans. Thus,further studies on leptospirosis and the improvement of molecular diagnostics for pathogen identification are necessary to better understand it and, possibly, even find a way to reduce the numbers of cases and fatalities.

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References

1. Altizer S, Bartel R, Han BA. Animal migration and infectious disease risk. Science. 2011; 331: 296–302. doi: 10.1126/science.1194694.
2. Boey K, Shiokawa K, Rajeev S. *Leptospira* infection in rats: A literature review of global prevalence and distribution. PLoS Negl Trop Dis. 2019;13:e0007499. doi: 10.1371/journal.pntd.0007499.
3. Bulach D, Adler B. Leptospiral genomics and pathogenesis. Curr Top Microbiol Immunol. 2018; 415: 189–214. doi: 10.1007/82_2018_87.
4. Burakauskas A, Danilevičius E. [Natural outbreaks of diseases in domestic animals and birds]. Vilnius, 1985. p. 19–20. Lithuanian.
5. Buitkuvienė J, Valančiūtė J, Čepulis R, Stankevičius A. Prevalence of antibodies to *Salmonella* spp., and *Leptospira* pathogens in Lithuanian wild boar (*Sus scrofa*) population. The 4th European Symposium of Porcine Health Management (ESPHM): 25–27 April 2012, Bruges (Belgium). Abstract book. 199: 178.

6. The 4th European Symposium of Porcine Health Management (ESPHM): 25–27 April 2012, Bruges (Belgium): Programme & Abstract book. 199: P178.

7. Cilia G, Bertelloni F, Albini S, Fratini F. Insight into the epidemiology of Leptospirosis: A review of *Leptospira* isolations from 'Unconventional' hosts. Animals (Basel). 2021; 11: 191. doi: 10.3390/ani11010191.

8. Cerqueira GM, Picardeau M. A century of *Leptospira* strain typing. Infect Genet Evol. 2009; 9: 760–8. doi: 10.1016/j.meegid.2009.06.009.

9. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, Stein C, Abela-Ridder B, Ko AI. Global morbidity and mortality of Leptospirosis: A systematic review. PLoS Negl Trop Dis. 2015; 9: e0003898. doi: 10.1371/journal.pntd.0003898.

10. Di Azevedo MIN, Lilenbaum W. An overview on the molecular diagnosis of animal leptospirosis. Lett Appl Microbiol. 2021; 72: 496–508. doi: 10.1111/lam.13442.

11. European Centre for Disease Prevention and Control. Leptospirosis. In: ECDC. Annual epidemiological report for 2016. Stockholm: ECDC; 2021.

12. Haake DA, Levett PN. Leptospirosis in humans. Curr Top Microbiol Immunol. 2015; 387: 65–97. doi: 10.1007/978-3-662-45059-8_5.

13. Jeske K, Schulz J, Tekemen D, Balčiauskas L, Balčiauskienė L, Hiltbrunner M, Drewes S, Mayer-Scholl A, Heckel G, Ulrich RG. Cocirculation of *Leptospira* spp. and multiple orthohantaviruses in rodents, Lithuania, Northern Europe. Transbound Emerg Dis. 2022; 1–6. doi: 10.1111/tbed.14470.

14. Levett PN, Morey RE, Galloway RL, Turner DE, Steigerwalt AG, Mayer LW. Detection of pathogenic leptospires by real-time quantitative PCR. J Med Microbiol. 2005; 54: 45–9. doi: 10.1099/jmm.0.45860-0.

15. Levett PN. Leptospirosis. Clin Microbiol Rev. 2001; 14: 296–326. doi: 10.1128/CMR.14.2.296-326.2001.

16. Morey RE, Galloway RL, Bragg SL, Steigerwalt AG, Mayer LW, Levett PN. Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. J Clin Microbiol. 2006; 44: 3510–6. doi: 10.1128/JCM.00670-06.

17. Motejunas LI, Kovaleva LI, Ezerskiene EP. [Spontaneous infection of a population of murine rodents with pathogenic agents for humans in the Lithuanian SSR]. ZHMEI: Moscow. 1974; 9: 122–3. Russian.

18. Rahman MT, Sobur MA, Islam MS, Ievy S, Hossain MJ, El Zowalaty ME, Rahman AT, Ashour HM. Zoonotic diseases: etiology, impact, and control. Microorganisms. 2020; 8: 1405. doi:10.3390/microorganisms8091405.

19. Samrot AV, Sean TC, Bhavya KS, Sahithya CS, Chan-Drasekaran S, Palanisamy R, Robinson ER, Subbiah SK, Mok PL. Leptospiral infection, pathogenesis and its diagnosis. A review. Pathogens. 2021; 10: 145. doi: 10.3390/pathogens10020145.

20. Stankevičienė M, Juknius T, Steponavičienė A, Buitkuvienė J. The prevalence of leptospirosis in Lithuanian swine farms. Veterinarija ir zootechnika. 2013; 63: 71–5.

21. Stankevičienė M, Buitkuvienė J, Bartaševičiūtė N, Adomkienė R, Statkevičiūtė J. Seroepizootic survey of leptospirosis in horses. Veterinarija ir Zootechnika. 2016; 74: 64–8.

22. Stimson AM. Note on an organism found in yellow-fever tissue. Public Health Reports (1896–1970). 1907; 22: 541. DOI: 10.2307/4559008

23. Šiugždinienė R, Ružauskas M, Virgailis M, Buitkuvienė J. The prevalence of *Leptospira* serovars among cattle in Lithuania. Veterinarija ir zootechnika. 2007; 37: 86–90. Lithuanian.

24. Thompson A, Kutz S. Introduction to the special issue on 'Emerging Zoonoses and Wildlife'. Int J Parasitol. Parasites Wildl. 2019; 9: 322. doi: 10.1016/j.ijppaw.2019.07.002.

25. Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. J Biosci. 2008; 33: 557–69. doi: 10.1007/s12038-008-0074-z. PMID: 19208981.

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LEPTOSPIROZĖ: KLASIFIKACIJA, EPIDEMIOLOGIJA IR APTIKIMO METODAI

Santrauka

Leptospira genties bakterijos gali sukelti plačiai paplitusią ir potencialiai mirtiną bakterinę zoonozę – leptospirozę. Dar nepakankamai įvertinta ši sparčiai plintanti atogrąžų zoonotinė liga buvo pripažinta pasauline visuomenės sveikatos problema tiek besivystančiose, tiek išsivysčiusiose šalyse. Straipsnyje apžvelgėme informaciją apie leptospirozės infekciją, klasifikaciją, epidemiologiją ir aptikimo metodus, taip pat literatūrą apie Lietuvoje atliktus *Leptospira* tyrimus.

Raktažodžiai: *Leptospira*, leptospirozės infekcija, Lietuva