

**PROTOCOL OF STEP-BY-STEP INSTRUCTIONS FOR IDENTIFYING
THE SOURCE OF LEPTOSPIROSIS PATHOGEN AND PREVENTING ITS
SPREAD FOR JOINT PREVENTIVE AND LIQUIDATION MEASURES
(REFERENCE DOCUMENT)**

Developed under Activity 1.5. “Increasing awareness on emerging diseases and
the prevention of cross-border epidemics”
of the international project

“Development of screening and monitoring systems for emerging diseases and
prevention of cross-border epidemics in the current context” ROUA00136,
co-financed by the EU under the Interreg NEXT Romania – Ukraine 2021–2027
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PREAMBLE

This Protocol of Step-by-Step Instructions for Identifying the Source of the Leptospirosis Pathogen and Preventing its Spread for joint preventive and liquidation measures (hereinafter – the Protocol) was developed by PI "Educational and research institute of biotechnology and biosafety" Agreement No. 151 dated September 22, 2026 (hereinafter – the Agreement), concluded with Odesa State Agrarian University (OSAU) as Partner 3 (PP3) of the international project “Development of screening and monitoring systems for emerging diseases and prevention of cross-border epidemics in the current context” ROUA00136, co-financed by the EU under the Interreg NEXT Romania – Ukraine 2021–2027 Programme (hereinafter – the Project).

The document was prepared in the framework of Activity 1.5. “Increasing awareness on emerging diseases and the prevention of cross-border epidemics” (Deliverable D.1.5.1.).

The purpose of this Protocol is to provide veterinary medicine specialists, healthcare professionals, local authorities, sectoral agencies, research institutions and interested parties with step-by-step practical instructions for:

- identifying sources of the leptospirosis pathogen in the “animal – water – soil – human” chain;
- assessing pathogen transmission routes and risk factors in a cross-border context;
- implementing joint preventive and control measures in line with the One Health principles;
- harmonising approaches to leptospirosis surveillance, monitoring and control between Ukraine and Romania in the Lower Danube region.

This Protocol is based on:

- the results of joint field investigations and laboratory analyses conducted under the Project;
- the Joint Terms of References (TOR) for analysis approved by partners, establishing unified methods for sample collection, delivery, preparation and testing;

- experience and outputs from previous international projects and research activities;
- guidance documents of WHO, WOA (OIE), EFSA and the current regulatory framework of Ukraine and the EU.

This Protocol was prepared in accordance with the requirements of and corresponds to the provisions of the following documents:

- Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases (“Animal Health Law”);
- Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents;
- Law of Ukraine No. 1206-IX of 04.02.2021 “On Veterinary Medicine and Animal Welfare”;
- Law of Ukraine No. 4718-IX of 16.12.2025 “On Amendments to Certain Laws of Ukraine Regarding the Alignment of Regulation in the Fields of Veterinary Medicine, Animal Welfare and Feed with EU Law”;
- Strategy for Biological Safety and Biological Protection on the One Health Principle until 2025 (CMU Order No. 1416-r of 27.11.2019);
- Instruction of the Ministry of Agriculture and Food of Ukraine No. 5 of 15.03.1994 “On Measures for the Prevention and Recovery of Animals from Leptospirosis”;
- European Veterinary Big Data Strategy 2022–2027;
- WHO Technical Report on Leptospirosis.

The implementation of the SMS-RO-UA project supports the EU Strategy for the Danube Region (EUSDR) in the areas of environmental protection and public health, and is aligned with the objectives of the EU Biodiversity Strategy to 2030 and the European Green Deal.

RELEVANCE

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Leptospirosis is a zoonotic disease prevalent worldwide that affects most mammalian species; clinical signs are frequently recorded in dogs and occasionally in cats. All animals infected with leptospirosis, including domestic animals, pose a direct threat to humans, as they can shed the pathogen in urine, creating a high probability of human infection. A particular danger is posed by carrier animals that, without showing clinical signs, serve as a source of the pathogen [1, 2, 3, 4]. According to the World Organisation for Animal Health (WOAH), there has been an upward trend in leptospirosis among domestic animals in recent years, with dogs and cats having the closest contact with humans. WOAH states that leptospirosis is one of the most widespread naturally focal diseases in the world, characterised by a severe course and a high case fatality rate. The problem of leptospirosis in animals and humans has been, remains and will remain relevant for at least several more decades. This is because the disease forms natural foci where rodents, domestic and wild carnivores serve as the source and reservoir of the pathogen. In addition, the etiological spectrum of leptospirosis pathogens periodically changes due to the absence of strong immunity to all serogroups of pathogenic leptospires following disease caused by a single serogroup [5]. Ukraine is among the countries with a complex epizootic situation regarding this disease, as there is a serious problem with stray animals, which are also a source and reservoir of leptospirosis pathogens [6].

To date, the problem of leptospirosis remains pressing despite significant efforts by veterinary and human medicine practitioners. A retrospective analysis of leptospirosis morbidity in animals and humans in Ukraine from 2003 to 2022 was conducted based on reports from regional state veterinary medicine laboratories and the Public Health Centre. Incidence and prevalence of leptospirosis across various animal species, including livestock, domestic and wild animals, were analysed. Seropositivity to leptospirosis was assessed in blood serum samples from various animal species. A total of 6,543,934 samples were analysed, with an overall seropositivity rate of 3.3%. Among cattle, 4.1% of samples were positive. Among pigs, 2.1% were positive. In horses, seropositivity was 7.9%, and in dogs and cats – 17.5% and 13.1% respectively. In wild boar, the positive



rate was 2.2%. The number of seropositive animals across species in Ukraine showed varying dynamics from 2003 to 2022. The highest numbers of positive cases were found in cattle, pigs and horses; however, after 2015 the number of positive cases declined significantly, coinciding with a sharp reduction in livestock numbers. In the period 2003–2022, 7,937 people were infected in Ukraine. Individual regions such as Lviv, Kherson, Kyiv, Mykolaiv and Vinnytsia account for a large share of total cases, ranging from 4.8% to 7.3%. Human leptospirosis incidence fluctuated over the years, peaking at 473 cases in 2014, followed by a decline: 301 cases in 2015, 323 cases in 2016, and 330 cases in 2017, with 142 to 295 cases per year in subsequent years. The study highlights the need to improve preventive measures and raise public awareness of the disease, particularly in rural areas. A comprehensive interdisciplinary approach is essential, involving cooperation between veterinary medicine and public health and environmental authorities, to impact on animal and human health [7].

According to data from the European Centre for Disease Prevention and Control, during 2010–2021 a total of 12,180 confirmed leptospirosis cases were reported across EU/EEA member states, with an overall notification rate that increased by 5.0% per year (95% CI: 1.2–8.8%). Five countries — France, Germany, the Netherlands, Portugal and Romania — accounted for approximately 79% of all reported cases, confirming that Romania bears one of the highest leptospirosis burdens in the European Union. In 2022 alone, 765 confirmed cases were registered across the EU/EEA, with a notification rate of 0.18 cases per 100,000 population; the highest rates were observed in males aged 45–64 years [8].

GENERAL CHARACTERISTICS OF THE PATHOGEN

The classification of leptospires was proposed by Wolff and Broom [9]. This classification was adopted by the WHO International Subcommittee on Taxonomy and Nomenclature and, having been refined, remains in use today [10]. The antigenic

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composition of leptospiral strains serves as the taxonomic criterion for intraspecies classification. The agglutination reaction, assessed by light microscopy with cross-absorption of agglutinins, is used as the standard method for serovar determination. For convenience, serovars with closely related antigenic compositions are grouped into serogroups. Leptospire is a spiral-shaped microorganism. Its length is usually 6–24 µm and its diameter up to 0.2 µm. The ends of the bacterium are bent into hooks, giving it a distinctive appearance. Electron microscopy has established that leptospire consists of a spiral protoplasmic cylinder covered by a cytoplasmic glycopeptide membrane [11]. An essential condition for the survival of leptospire in the environment is high humidity and a pH of 7.0–7.6. They die rapidly under direct sunlight. In the water of open reservoirs, leptospire remains viable for 7 to 30 days, and in moist soil for up to 9 months. They are sensitive to high temperatures: boiling kills them instantly, and heating to 56–60°C kills them within 20–25 minutes. Conversely, these microorganisms are resistant to low temperatures and remain viable after prolonged freezing in ice. Leptospire is sensitive to acids; to disinfect pure cultures, a 2% hydrochloric acid solution with a 24-hour exposure is recommended, and for pathological material a 5% phenol solution with a 2-hour exposure should be applied. In vitro, streptomycin sulphate and enrofloxacin cause the death of leptospire within 20–30 minutes [12].

IMPACT ON HUMAN HEALTH

The problem of leptospirosis is of growing importance. It is registered annually across all administrative territories of the Ukraine and EU. Peak incidence falls between June and September, driven by active outdoor recreation, the swimming season and fishing. In Ukraine, diseases caused by pathogens of 13 serogroups of leptospire are recorded. The infection is transmitted via the following routes: waterborne, alimentary and wound-related. A sick person poses no danger to those around them — the disease is not transmitted between humans. At-risk groups include persons in certain occupations:

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farmers, workers at meat-processing plants, sewage and water treatment workers, hunters, fishermen, persons working on waterlogged meadows (hay and peat harvesting) and veterinarians.

Signs of disease:

The incubation period (from the moment of infection to the appearance of first signs of disease) averages 6–14 days.

The onset of disease in humans most often resembles influenza. The illness begins acutely, with body temperature rising to 39–40°C. From the first day of illness, there is pronounced general weakness, headache, muscle pain (especially in the calves), thighs, lower back, dizziness, nausea, vomiting, and possible loss of consciousness.

Muscle pain is so intense that patients can barely move; it lasts 8–10 days. An unsteady gait is characteristic.

Reddening of the eyes is observed (resembling those of a rabbit); jaundice of the mucous membranes is possible; herpetic rash on the lips and nasal wings often appears; the liver and spleen are enlarged.

Haemorrhagic syndrome manifests as skin rash, haemorrhages, nosebleeds, possible bloody vomiting and diarrhoea with blood.

EPIDEMIOLOGY AND EPIZOOTOLOGY

Understanding the causes and conditions underlying leptospirosis is of practical importance for correct diagnosis and for organising control measures. Leptospirosis is a zoonosis distributed worldwide that causes disease in a large number of animals. The disease is associated with various serovars belonging to the species *Leptospira interrogans*. These serovars persist in nature in many animals (rodents, piglets, horses, cattle and others). Domestic animals (cattle, horses, sheep, pigs, guinea pigs, etc.) as well as wild animals (rodents, hedgehogs, raccoons, foxes, wolves, jackals, leopards, etc.) serve as reservoirs of infection during transmission to humans [13]. The principal



pathogenic serovars include: *L. canicola*, *L. icterohemorrhagiae*, *L. grippotyphosa*, *L. pomona*, and *L. bratislava*. Classical vaccines induce immunoresistance against *L. canicola* and *L. icterohemorrhagiae*, resulting in a decline in the prevalence of leptospirosis caused by these two agents [14, 15]. Transmission of leptospirosis occurs through direct or indirect contact. Direct contact involves infected urine, sexual or transplacental routes, or bites and consumption of infected tissues. During recurrent episodes, infected animals may shed leptospires in urine for months. Indirect transmission occurs via stagnant water, soil, feed or contaminated bedding. Certain environmental conditions can facilitate pathogen transmission. Leptospires survive in stagnant water bodies. Warm weather (0 to +25°C) favours the development of leptospirosis; low temperatures cause the death of this pathogen. Accordingly, leptospirosis often has a seasonal character (late summer – early autumn) [15, 13].

Duration of leptospira carriage in various animal species:

cattle — up to 2.5 years;

horses — up to 7 months;

pigs — up to 2 years;

dogs — up to 2–2.5 years;

foxes — up to 17 months.

Cattle, sheep, pigs, dogs and foxes are the most susceptible to leptospirosis; horses somewhat less so. Cats, rats and mice are relatively resistant. Among laboratory animals, guinea pigs, golden hamsters, rabbits and fox pups are susceptible. Birds play no role in the epizootic process — leptospires do not survive in birds, as the body temperature of birds (41–43°C) lies beyond the temperature limits of leptospires [16].

ROUTES of infection (transmission)

Humans can become infected while swimming in still-water bodies, fishing, during agricultural work, when caring for sick animals, or by consuming food contaminated by

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rodents. *Leptospira* spp. can enter the human body through cuts and scratches on the skin, as well as through intact mucous membranes (nose, mouth, eyes). Infection is also possible through consumption of raw water from natural, unknown sources.

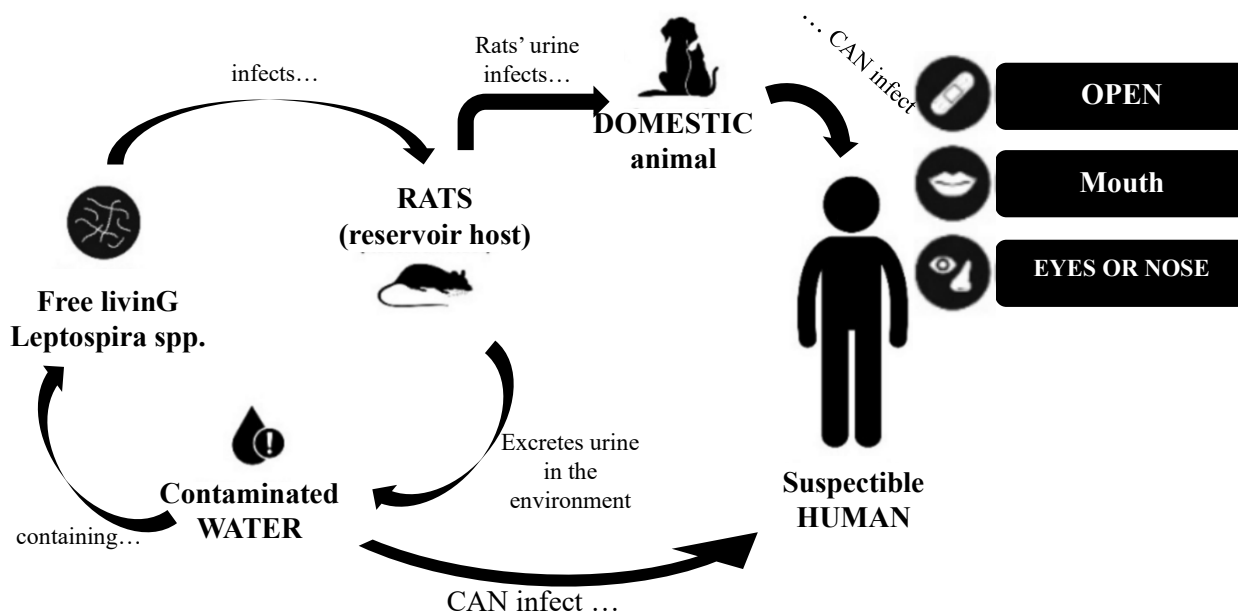


Fig. 1. Transmission of *Leptospira* spp. at the human-animal-environment interface, with emphasis on different reservoir hosts [18].

Domestic animals (e.g. dogs) can become infected by sniffing the markings of other animals, by consuming contaminated feed or raw meat, or during hunting of sick rodents. Infection is transmitted from mother to foetus.

According to epidemiological investigations [17], in 2023 leptospirosis infection occurred:

- through contact of damaged skin areas with contaminated objects and surfaces — 57% of cases;
- in water (swimming in open water bodies, fishing) — 38% of cases;
- through consumption of contaminated food — 5% of cases.

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IDENTIFYING THE SOURCE OF THE LEPTOSPIROSIS PATHOGEN (LABORATORY RESEARCH)

Diagnosis of leptospirosis in humans, livestock and wild animals is based on the totality of epizootological-epidemiological, clinical and pathomorphological data, with mandatory laboratory confirmation of the diagnosis [18]. The diagnosis of leptospirosis is established comprehensively, but laboratory diagnostics are of decisive importance. Leptospirosis is characterised by the “iceberg phenomenon”. The visible tip above the waterline consists of clinically ill animals and those that have aborted — easy to identify and respond to. The much larger submerged part consists of carrier animals shedding leptospire in urine and infected animals with anti-leptospiral antibodies in their blood serum. This group shows no external signs of disease but represents the main source of leptospirosis and the principal threat of infection for healthy animals and humans.

The predominance of subclinical cases, the complexity of differential diagnosis and the absence of pronounced clinical signs in carrier animals place laboratory methods at the forefront of leptospirosis diagnostics. The search for new methods of leptospirosis diagnosis and leptospire identification based on more specific molecular biology and genomic systematics approaches remains topical [19, 20].

The principal methods (Table 1) of laboratory diagnosis of leptospirosis include: bacteriological; serological: MAT, macroagglutination reaction, complement fixation test, haemagglutination reaction, latex agglutination reaction, immunoadsorption reaction, enzyme-linked immunosorbent assay (ELISA); molecular-genetic methods, polymerase chain reaction (PCR).

Table 1. Methods of laboratory diagnosis of leptospirosis

Method	Target	Disease period	Method specificity
Bacteriological method	Pathogen	First week (leptosiraemia)	Pathogenic leptospire

MAT	Ab	Second week — up to several months	Serogroup
Cross MAT	Pathogen	Upon culture isolation	Serogroup
ELISA, RIA (IgG)	Ab (IgG)	End of second week — up to several months	Pathogenic leptospires
ELISA, RIA (IgM)	Ab (IgM)	Acute period	Pathogenic leptospires
Real-time PCR	16S rRNA	First week	Pathogenic leptospires
Nested PCR	LipL32	First week	Pathogenic leptospires
IFA, ELISA, RIA; Magnoimmunosorption; Latex agglutination	Ag	First week (leptosiraemia)	Depending on objectives
MAT with monoclonal antibody panel	Ag	Culture identification	Serovariant; Netherlands, WHO Reference Centre for Leptospires
Multilocus sequence typing (MLST)	Genomic DNA	Culture identification	Species (genomic species); International resource mist.net
Direct protein profiling by Microflex LT mass spectrometer	Cell proteins	Culture identification	Species (genomic species)
Multiplex ligation-dependent hybridisation reaction	Genomic DNA	Culture identification	Species (genomic species)

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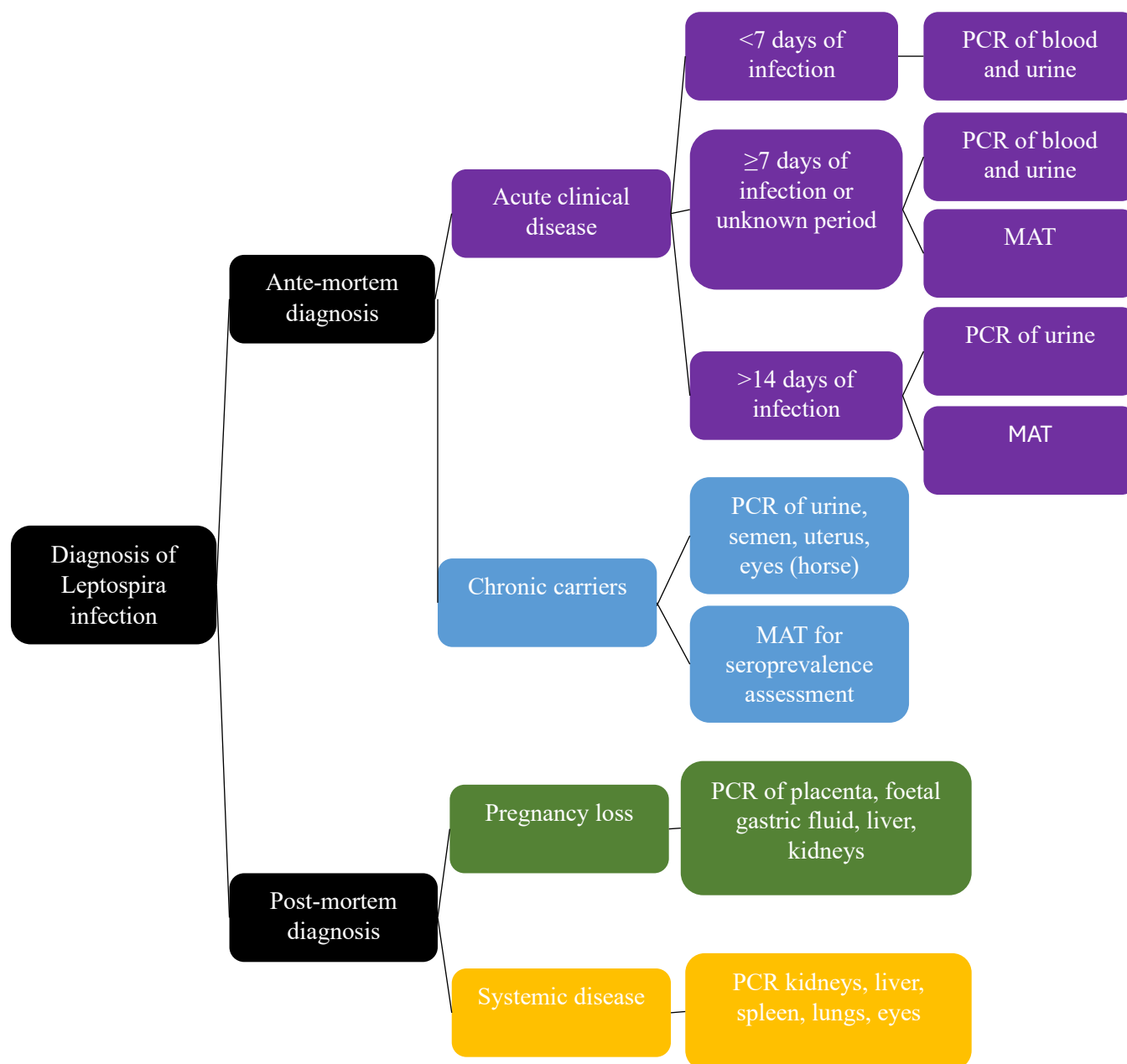


Fig 2. Recommendations for targeted animal testing for leptospira infection [21].

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Microscopy. Direct microscopic observation is used to detect leptospire in biological fluids, to verify culture growth, etc. Darkfield microscopy is most commonly used, although immunostaining is useful in certain cases. Under darkfield and phase-contrast microscopy, leptospire appear as thin, bright, actively motile rods moving with a characteristic rapid rotational and jerky motility. Approximately 10 leptospire/ml are required for a single cell to be visible in the field of view under darkfield microscopy. However, the positive rate of darkfield microscopy falls from 100% to 90.9% when the duration of infection exceeds one week. A further limitation of this technique is that even experienced specialists can easily produce both false-positive and false-negative diagnostic results.

Direct polymerase chain reaction (PCR). PCR is used both to detect and identify leptospire and for rapid diagnosis of leptospirosis [22, 23]. Clinical materials used include blood, urine, cerebrospinal fluid, pieces of parenchymal organs, soil, water and sediment contaminated with leptospirosis [24, 25, 26]. The most specific DNA regions for leptospire are genes encoding the outer membrane lipoprotein LipL32 and the 16S rRNA of pathogenic genospecies of the pathogen [23, 27, 28]. At present, conventional PCR with electrophoretic detection is being superseded by modifications with fluorescent registration of DNA accumulation, the advantage of which lies in high sensitivity and specific detection of reaction products in real time with quantitative assessment. However, conventional PCR with electrophoretic detection remains necessary for amplifying DNA fragments for subsequent detailed investigation: cloning and nucleotide sequencing [28]. PCR analysis is characterized by high specificity, sensitivity and high diagnostic efficacy in the first week of illness (from day 1 onwards), even against a background of antibiotic therapy [29]. Accordingly, it may be recommended as a method of early rapid diagnosis of leptospirosis [25]. The diagnostic efficacy of laboratory investigations for leptospirosis can be significantly increased through the parallel use of the serological test (MAT) and PCR, regardless of the phase of the disease [25, 30]. The World Health Organization currently recommends the following molecular-genetic methods for characterising



leptospire [31, 32]:

- Arbitrarily primed PCR (AP-PCR) or randomly amplified polymorphic DNA (RAPD) fingerprinting;
- Restriction endonuclease analysis (REA);
- Restriction fragment length polymorphism (RFLP) analysis;
- Amplified fragment length polymorphism (AFLP) analysis;
- Pulsed-field gel electrophoresis (PFGE);
- DNA sequencing analysis.

Multilocus sequence typing is currently used to determine species affiliation and intraspecies typing of *Leptospira* spp. [28, 30, 33]. Its principle lies in molecular analysis of a DNA fragment that has been previously selected, cloned and tested by simple methods. The sequencing process produces a formal description of the primary structure of a linear macromolecule as a sequence of monomers in text format. Sequences of gene regions, whole genes, total mRNA and even complete microbial genomes obtained as a result are compared with data stored in the NCBI and *Leptospira*.mlst.net databases [28, 30, 33]. In everyday practice, a promising technique for the rapid and reliable identification of microorganisms of the genus *Leptospira* — MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight) mass spectrometry — can also be used [33]. This method is also highly sensitive, rapid and has low consumable costs. With respect to leptospirosis diagnostics specifically, the method is not yet fully developed, primarily because leptospire grow only on specialised liquid culture media and culture isolation is a time-consuming process requiring considerable effort.

Advantages of PCR diagnostics:

- Testing takes only a few hours, unlike lengthy classical microbiological methods;
- High specificity of 95–100%, as the DNA fragment under investigation is unique to each particular microorganism;
- High sensitivity — the pathogen can be detected even if represented by a single cell in the sample;



- The pathogen can be identified both qualitatively and quantitatively — particularly important when isolating conditionally pathogenic microorganisms that do not cause disease in small numbers;

- Ability to determine the pathogen genotype — essential for rational treatment and prognosis of possible complications.

Limitations:

- Possibility of both false-positive and false-negative results if sample collection rules are not followed or errors are made during testing;

- Significant cost of the investigation.

Enzyme-Linked Immunosorbent Assay (ELISA). This test is based on the detection of IgM antibodies, which appear in the blood approximately one day earlier than those used in the MAT. A weak correlation between MAT and ELISA results on individual serum samples is often observed. The MAT remains the reference standard; IgM antibodies become detectable within the first week of disease, allowing diagnosis to be confirmed and treatment initiated, although the test is likely to be most effective despite the fact that antibody levels are usually low or absent in the early stages of infection [34].

Although the microscopic agglutination test (MAT) is considered the gold standard in leptospirosis diagnostics, its use as a routine diagnostic test in clinical laboratories is limited. The test is complex and labour-intensive for routine use. Numerous studies have shown that Pan Bio ELISA is more sensitive than MAT for case detection in the early stages of acute disease [35]. IgM antibodies begin to appear within the first week of illness, although antibody levels are low or undetectable in the early stages. Leptospirosis can be diagnosed on the basis of IgM antibodies using Pan Bio ELISA on a single serum sample collected during the acute phase of illness. To confirm results, a sample taken two weeks after recovery is required. A limitation of using a single serum sample for IgM antibody detection is the absence of antibodies in the early stages of infection or their persistence. IgM antibodies in leptospirosis persist for a prolonged period with varying rates of decline [36]. A single serum sample taken during an acute febrile illness with leptospirosis symptoms constitutes only presumptive evidence of infection and therefore



requires confirmation by additional investigations [24].

In summary, leptospirosis is a zoonanthroponosis that is dangerous to both animals and humans, causing significant social and economic losses and, when timely and qualified assistance is not provided, becoming a fatal disease. Rapid definitive diagnosis is therefore of critical importance. Particular attention should be paid to the fact that timely laboratory diagnostics using modern molecular-genetic investigations must be an integral part of contemporary laboratory diagnosis of leptospirosis.

Specifics of diagnostics in raw materials of animal origin. Diagnosis of leptospirosis in foodstuffs is aimed at detecting the *Leptospira* pathogen. The process is complex, as the bacteria die rapidly in meat at room temperature due to lactic acid formation. The principal focus is on investigation of raw materials from slaughtered animals (meat, milk, offal) to prevent infection from reaching the consumer.

Leptospire are highly sensitive to an acidic environment. In muscle tissue after slaughter, due to lactic acid fermentation (meat maturation), they die within a few hours. However, they survive longer in fresh meat, lymph nodes, blood and kidneys.

In fresh milk from sick cows, leptospire can remain viable for up to several days. Before investigation, milk is centrifuged to isolate the sediment where bacteria concentrate.

Immunochemical staining methods. Detection of leptospire is also possible using various immunochemical staining methods, such as immunofluorescence and various immunohistochemical techniques. Such methods are very useful for diagnosing infection in pathological material unsuitable for culturing, or when a rapid diagnosis is required. Since the success of these methods depends on the number of organisms present in samples, they are less suited to diagnosing chronic carrier status, in which organism numbers may be very low or confined to certain organs. Leptospire stain poorly with aniline dyes, and silver staining methods lack adequate sensitivity and specificity, although they serve as a useful adjunct to histopathological diagnostic methods [20].



CLINICAL MANIFESTATIONS AND BIOCHEMICAL BLOOD CHANGES IN LEPTOSPIROSIS

Incubation period for leptospirosis lasts from 2 to 12 days, although some serogroups cause a longer period, such as *L. icterohaemorrhagiae* — up to 20 days. From the moment leptospires enter the body, they multiply rapidly in the blood and migrate to various tissues where they persist and replicate. Target organs include the kidneys, liver, spleen, central nervous system, conjunctiva and reproductive system [15]. The onset of disease (usually acute) is associated with massive entry of leptospires and their toxins into the blood (microscopy of blood reveals dozens of leptospires in the field of view). In adult dogs, the haemorrhagic form of disease is more commonly observed. It is characterised by weakness of the hind limbs, refusal of feed and depression. The severity of disease and the extent of organ damage depend not only on the serotype of the pathogen but also on the reactivity of the host organism. Leptospires, their toxins and metabolic products cause pronounced intoxication, which is particularly rapid in the first 2-3 days from the onset of clinical signs. In addition, leptospires produce haemolysin, which leads to the destruction (haemolysis) of red blood cells [37]. Symptoms of renal insufficiency and liver damage appear approximately 7 days after infection, and the animal may die. Antibody titres rise, ensuring elimination of circulating leptospires (8-10 days after infection). Persistence of leptospires is only possible in the kidneys, as this organ retains the lowest antibody concentration. Leptospiruria begins 15 days after infection. The liver and kidneys are the primary target organs; the spleen, as frequently noted in the literature, is a site of pathogen replication. Damage to the central nervous system or eyes may lead to the development of encephalitis or uveitis [38]. Pathognomonic symptoms, as reported in numerous retrospective studies, include: lethargy, anorexia and vomiting. Fever is observed in only up to 52% of cases [39]. Changes in biochemical parameters primarily include increases in serum urea and creatinine concentrations (detected in 94–100% of cases). Hyperphosphataemia is frequently observed. Increases in serum hepatic markers (alanine aminotransferase, alkaline phosphatase, total bilirubin) are noted in 33–65% of cases

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according to the literature. Haematological disorders manifest as neutrophilia, neutropenia and subsequent development of thrombocytopenia [1].

Differential diagnosis:

1. Diseases in which a sharp drop in milk yield may occur, such as acute viral infections and sudden absence of drinking water;
2. Diseases presenting with renal or hepatic insufficiency;
3. Diseases characterised by perinatal mortality — abortions, reduced litter size, stillbirths and infertility, such as brucellosis, Neospora, Q fever and bovine viral diarrhoea, chlamydiosis and toxoplasmosis in sheep, Q fever in goats, etc.

REDUCING THE RISK OF LEPTOSPIROSIS INFECTION IN HUMANS AND ANIMALS

Leptospirosis poses challenges at the human-animal interface and requires robust public health management mechanisms for effective intervention. Intervention measures are aimed at preventing bacterial exposure in humans and reducing disease severity through various public health strategies and management mechanisms. Effective public health management of leptospirosis includes risk communication, epidemiological surveillance, policy development and community engagement [28, 37, 38]. Risk communication is a fundamental aspect of public health management of leptospirosis, aimed at educating the public about disease risks, symptoms and preventive measures. Effective communication strategies include dissemination of information through various media channels, community outreach programmes and public health campaigns. The World Health Organization (WHO), for example, emphasises the need for clear and accessible information to raise awareness of leptospirosis, particularly in high-risk regions [27, 38, 39]. It has been noted that the use of rodents as a central element in vector control messaging is important given their visual impact; however, such framing may potentially limit broader preventive actions by oversimplifying the task to merely killing rats [41].

Surveillance and early diagnosis are the most important components of public health management for leptospirosis control at the human-animal interface. Surveillance systems are designed to monitor morbidity, detect outbreaks and track trends over time [44]. Effective surveillance systems integrate data from clinical cases, laboratory reports and environmental monitoring to provide a comprehensive picture of leptospirosis dynamics [45]. Early diagnosis programmes are aimed at prompt case detection to initiate treatment and prevent further transmission. Public health management frameworks often support these programmes through funding of diagnostic tools, training of healthcare workers and establishment of reporting mechanisms. Policy development is another important aspect of public health management for leptospirosis control. Policies should address various elements of disease management, including vaccination strategies, access to healthcare and emergency response plans. For example, the development of vaccination programmes for high-risk groups, such as those living in flood-prone areas or working in occupations involving animals, is carried out on the basis of policies ensuring vaccine availability and distribution [18].

Control measures for leptospirosis at the human-animal interface are largely focused on effective treatment and on the development and distribution of vaccines. Timely and accurate diagnosis is critical for initiating appropriate antibiotic therapy, which is the cornerstone of leptospirosis treatment. Antibiotics commonly used include doxycycline, penicillin and ceftriaxone, which are effective in reducing disease severity and duration when administered early [42]. In severe cases, supportive inpatient therapy may be required to address complications such as renal insufficiency, liver damage or severe pulmonary syndrome [41].

Vaccination is the most important preventive strategy, particularly for high-risk groups such as agricultural workers, veterinarians and residents of endemic regions. Vaccines against leptospirosis for humans are currently available in some countries, but their use is often limited by variable efficacy, short duration of immunity and the need to develop serovar-specific formulations [43, 44]. The wide diversity of serovars across different geographical regions limits the cross-protection of vaccines. One identified



challenge is the optimisation of the antigenic components used in recombinant and DNA vaccines to ensure broad and lasting immunity. However, advances in vaccine research are focused on developing more effective broad-spectrum vaccines providing long-lasting immunity against multiple *Leptospira* serovars [18].

PREVENTION OF LEPTOSPIROSIS INFECTION IN HUMANS:

- Swimming should take place only in designated areas; leptospirosis is most commonly acquired in enclosed stagnant water bodies, especially shallow ones;
- Do not swim in water bodies and rivers if there are wounds, scratches or areas of inflammation on the skin or mucous membranes;
- Do not walk barefoot in marshy areas;
- Do not drink unboiled water from open water bodies or unchecked sources;
- Thoroughly wash and scald with boiling water any fruit and vegetables;
- Do not consume vegetables and fruit (especially those stored in cellars and basements) showing signs of damage by animals;
- Protect drinking water sources and food from rodent access;
- Do not consume meat from animals sick with leptospirosis;
- Observe personal hygiene rules when caring for livestock — wash hands regularly, use protective equipment (work clothing, rubber boots, gloves);
- Wash hands with soap before eating or preparing food;
- Carry out regular rodent control in residential premises and animal housing facilities (traps, snares, mousetraps, toxic substances);

CONTROL MEASURES

1. Upon confirmation of a leptospirosis diagnosis, local self-government bodies and local state executive authorities, on the submission of the chief veterinary medicine officer

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of the district, city or urban district, issue a decision declaring the holding (or its autonomous part) or the locality as unfavourable, shall introduce quarantine restrictions for animals and shall approve an action plan for the control of the disease. Simultaneously, the chief veterinary medicine officer of the district, city or urban district notify the regional state veterinary medicine authority and the chief medical officer of the district sanitary-epidemiological station.

2. Veterinary medicine specialists carry out clinical examination of animals with temperature measurement in those suspected of disease. Sick animals and those suspected of disease are isolated and treated with hyperimmune serum in accordance with the instruction for use, and with antibiotics (streptomycin sulphate — intramuscularly at 25,000 units/kg body weight at 12-hour intervals for 4–5 days; for pigs, additionally ditetracycline intramuscularly at 30,000 units/kg body weight twice at 48–72-hour intervals; kanamycin is administered intramuscularly at 15,000 units/kg body weight three times daily at 8-hour intervals for 4–5 days).

3. Under quarantine restriction conditions, the following are to be prohibited:

- removal (transport) of animals from the unfavourable holding and use of sick animals for reproduction, or sale of animals to members of the public;
- regrouping of animals without the permission of the chief veterinarian of the holding;
- removal of animals not vaccinated against leptospirosis;
- grazing and watering of unvaccinated animals at sites where leptospirosis-affected animals have grazed;
- use of meat and slaughter products from sick or suspected animals for food or feed purposes without appropriate decontamination;
- use of milk from sick animals for commercial purposes without boiling. Milk from clinically healthy animals whose blood serum gives a positive MAT without rising titres is used without restriction.

4. Breeding animals infected with leptospires (positive MAT or leptospires in urine) are isolated, semen collection is suspended and they are treated with leptospirocidal



preparations. After 10–12 days, treatment efficacy is monitored by microscopy of urine. If leptospires are detected in urine, the treatment course is repeated and efficacy re-checked. Semen stocks collected from carrier breeding animals within 6 months before the diagnosis was established are destroyed.

5. Feed that sick leptospirosis animals had access to is fed to leptospirosis-vaccinated livestock.

6. Breeding animals at breeding enterprises (stations, artificial insemination centres) located in natural leptospirosis foci are vaccinated against leptospirosis.

7. Repeated blood serum testing by MAT and urine microscopy of all breeding animals at the previously unfavourable enterprise (station) are conducted after three months, and upon obtaining negative results, every 6 months thereafter.

8. Slaughter of sick animals is carried out at a sanitary slaughterhouse, or in its absence, at the slaughter facility of a meat-processing plant at the end of a shift after all carcasses and offal have been removed from the hall. The premises and equipment of the facility are disinfected after slaughter.

9. In service dog breeding kennels, clinically sick animals and those suspected of disease are isolated and treated with hyperimmune serum and streptomycin sulphate. Streptomycin sulphate is administered to sick dogs once daily for 4–5 days at 25,000 units/kg body weight.

Clinically healthy dogs of all age groups are vaccinated against leptospirosis. Sale of dogs from a leptospirosis-unfavourable kennel is prohibited.

10. After isolating sick animals and those suspected of leptospirosis, mechanical cleaning and disinfection of premises and enclosures are carried out using a 1–2% formalin solution, a 2% sodium hydroxide solution, or a clarified bleach solution containing 3% active chlorine.

11. A holding (farm, herd) is considered free of leptospirosis after veterinary measures have been carried out and in the absence of sick animals and leptospira carriers.

Before lifting quarantine restrictions, 1–2 months after measures have been implemented, at least 50 blood serum samples from young animals are tested by MAT (no



positive reactions should be present) and at least 100 urine samples from every 1,000 adult animals, but not fewer than 100 animals, with no leptospira carriers present among them. The microagglutination reaction in adult animals may remain positive. Repeated testing for leptospirosis in previously unfavourable holdings is carried out 6 months after the lifting of quarantine restrictions [45].

CONCLUSIONS AND OUTLOOK

1. The “animal – water – soil – human” transmission chain is confirmed in the study area. Test results from water and soil samples collected in the Danube Delta area indicate environmental contamination with leptospires that substantially exceeds the boundaries of clinical manifestations in animals alone and is consistent with the “iceberg phenomenon” model.

2. Molecular-genetic diagnostic methods are the priority. Analysis showed that real-time PCR combined with ELISA provides the highest diagnostic efficacy at all stages of disease. These methods form the foundation of the Protocol as the recommended approach for unified cross-border screening and monitoring.

3. The absence of a harmonised approach between Ukraine and Romania is a critical gap. Before the launch of the SMS-RO-UA project, epidemiological data collection and analysis by both countries were conducted without the necessary coordination, making timely assessment of cross-border risks impossible. The Joint TOR and the developed Protocols represent a first step towards closing this gap.

4. Stray animals and wildlife remain the key reservoir of leptospira carriers. In the context of inadequate management of stray animal populations, this risk factor is of particular significance and requires systemic management decisions.

5. The seasonal pattern of morbidity reinforces the need for preventive measures. The peak of human infection falls in June–September, coinciding with the active tourist

and fishing season in the Danube Delta. Scheduled preventive campaigns should be launched no later than April–May each year.

Implementation of the One Health principle in the cross-border dimension is the central strategic objective for further development of the leptospirosis monitoring system. The specific prospects below take into account EU priorities.

Integration into the EU joint epidemiological surveillance system. The results of the SMS-RO-UA project lay the foundation for connecting the laboratories of PP2 (DSVSA-Tulcea) and PP3 (OSAU) to the EU data sharing system, enabling real-time tracking of cross-border outbreaks and timely response.

Development of a GIS platform for predictive monitoring. The GIS platform developed under the project has potential for further expansion through the integration of climate data, animal migration data and results from new field studies. Predictive modelling of leptospirosis outbreaks using artificial intelligence algorithms is consistent with the EU Veterinary Big Data Strategy 2022–2027.

Harmonisation of Ukrainian national legislation with EU standards. In the context of Ukraine's candidate status for EU membership (June 2022), the approach to joint collection of standardised and harmonised data established in this Protocol is a practical instrument for aligning Ukrainian veterinary legislation with Regulation (EU) 2016/429 ("Animal Health Law"). The methodological approaches developed herein are recommended as a reference basis for developing national regulatory instruments.

Development of broad-spectrum vaccination strategies. One of the key challenges identified in the study is the limited cross-protection of available vaccines against different *Leptospira* serovars. In line with the priorities of the EU Farm to Fork Strategy and WOAHA recommendations, further research should focus on identifying the serovars circulating in the cross-border region in order to develop regionally adapted multivalent vaccines.

Incorporation of results into educational programmes. The materials of this Protocol and the Practical Guide for the Assessment of Emerging Diseases are recommended for integration into the curricula of the Faculty of Veterinary Medicine at



OSAU and partner educational institutions, in line with the objectives of the Erasmus+ Programme and human capital development priorities under the EU Strategy for the Danube Region (EUSDR).

Community engagement and public awareness. The project results highlight the need for systematic public information campaigns, primarily targeting fishermen, farmers and pet owners in the border zone.

Establishing a cross-border laboratory surveillance network. The SMS-RO-UA project lays the foundation for establishing a sustainable cross-border network between laboratories and potential new participants from Moldova and Bulgaria. Such a network aligns with the priorities of the EU's macroregional strategies and could be supported under future Interreg or Horizon Europe programmes.

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