Network for wildlife health surveillance in Europe

Diagnosis Card

Leptospirosis

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Etiology

Caused by non-spore-forming obligate aerobic Gram-negative spirochetes of the genus *Leptospira*. Can form biofilms, including *in vivo* (e.g., in renal tubules of *Rattus norvegicus*). The highly immunogenic lipopolysaccharide structure on the outer-membrane is the basis for the serological classification of over 300 serovars, further grouped in 30 serogroups according to cross-agglutination patterns. Initially classified into two groups, pathogenic (*L. interrogans sensu lato*) and saprophytic, non-pathogenic (*L. biflexa sensu lato*). The uptake of molecular methods has greatly modified the classification of this genus, currently divided into two clades: pathogenic (P) and saprophytic (S), which are further divided into four subclades (P1, P2, S1, S2) and 74 genomospecies. The approved nomenclature is *Genus species* Serovar_name, for instance, *Leptospira interrogans* serovar Australis. Strains classified in one given serovar or serogroup can be very distant genetically, and conversely, closely genetically related strains can belong to different serovars (e.g. one indel on LPS-biosynthesis-related gene differentiates *L. interrogans* serovars Copenhageni and Icterohaemorrhagiae).

Affected species (wildlife, domestic animals, humans)

All mammals including humans are considered susceptible species, and the bacteria have also been occasionally isolated from other—often under-sampled—vertebrates (fish, birds, reptiles, amphibians). Broad range of clinical signs, ranging from asymptomatic or subclinical to multiple organ failure and death. Although mice or rats have often been referred to as reservoir species because of asymptomatic chronic renal carriage, a reservoir should be defined at the ecosystem level, including the environment. Species within a given ecosystem should be considered part (or not) of the maintenance host community for a given strain regardless of their clinical manifestations. Cases of seronegative and asymptomatic renal carriers are described in several species including dog, rodents and livestock.

Epidemiological characteristics and disease course

Leptospirosis occurs worldwide with a higher prevalence in tropical and subtropical areas where moist and warm conditions improve the environmental survival of the bacteria. A neglected tropical disease with an important but often overlooked toll for humans and the agricultural sector, with the potential to be greatly impacted by climate change. Risk factors for human leptospirosis are contact with urine of infected animals, and outdoor work or recreational activities, especially around water.

Clinical signs

Clinical signs of acute leptospirosis may include jaundice, haemoglobinuria, meningitis, acute renal failure.
Clinical signs of chronic *Leptospira* infection may include stillbirth, abortion, infertility, chronic renal failure or hepatitis, uveitis.

Gross lesions

Gross lesions may include icterus, enlarged and pale kidneys, congestion and haemorrhages in various tissues.

Histological lesions

Renal lesions may include interstitial nephritis, lymphoplasmacytic inflammation, glomerulonephritis, interstitial fibrosis, protein casts, tubular dilation/atrophy

Differential diagnosis

Other diseases where acute milk drop, hepato-renal failure or reproductive wastage (abortion, reduced litter size, stillbirth and infertility) may occur, e.g., brucellosis, *Neospora*, Q fever, BVD, chlamydiosis, toxoplamosis, Q fever, etc.

Criteria for diagnosis

Exposure to *Leptospira* can be assessed by serology. A four-fold rise in Microscopic Agglutination Test (MAT) antibody titres in paired acute and convalescent serum samples is diagnostic but seronegative chronic carriers are frequent. *Leptospira* can be isolated and/or detected from several internal organs and body fluids of colonized species. The presence of the bacteria should be put in perspective with the presence/absence of consistent lesions/clinical manifestations to distinguish cases of acute leptospirosis and chronic *Leptospira* infection. In the former, leptospires can be found in several internal organs (liver, lungs, brain, kidneys) and body fluids (blood, milk, peritoneal, cerebrospinal, thoracic fluids), while chronic carriers harbour leptospires in their urine, kidneys, or reproductive tissues.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

The choice of sample(s) and test(s) will depend on the purpose (diagnosing an acute case of leptospirosis or investigating chronic ± asymptomatic carriage at the population level).

Samples:

Urine samples should be tested within 3 days (ideally 1 day) of storage at 4°C, blood within 14 days at 4°C, and both urine and blood samples within 28 days of storage at -20°C (storage duration impacting PCR sensitivity), with limited freeze-thaw cycles as each results in a log-fold decrease in lower limit of detection. Separated serum that has been stored ≤ 10° C may be tested up to 1 month post collection.

Whole blood: the leptospiremic phase is brief and usually within 5-7 days depending on the animal species and should be considered in the early stage of the clinical manifestations. Heparin is the preferred choice for culturing but can interfere with molecular assays, for which EDTA tubes are best.

Urine: leptospiruria appears after the delay needed for kidney colonization, usually after 5 days of clinical manifestations (DNA can be detected earlier by PCR), and can last in apparently healthy carriers of *Leptospira*.

Kidneys: Leptospires preferentially accumulate in the cortex or medulla, the choice of the area sampled can therefore impact the concentration of *Leptospira* and subsequent detection and measurement. For species with voluminous kidneys, sampling more than one lobule increases the detection rate.

Microscopy: Warthin-Starry (WS) staining or Fluorescent Antibody Test (FAT) can be used to visualize leptospires in tissues (or fluids). WS also stains other spirochetes and *Helicobacter pylori*. Useful in conjunction with hematoxylin-eosin staining to confirm lesions are due to *Leptospira*, however low sensitivity.

Serology: although immunochemical tests (e.g. ELISA and complement fixation tests) have been developed for leptospirosis diagnosis, finding an appropriate species-specific antigen can be complicated with wildlife, and non-serogroup-specific results are of limited epidemiological interest. MAT should be preferred, and the panel of serovars tested representative of all endemic serogroups and serovars known to be harboured elsewhere by the target species. The positivity threshold for assessing exposure at population level can be set lower than for diagnosing clinical leptospirosis (e.g., 48 or 100), or titres analysed as ordinal data. Serotyping remains important to assess circulating serogroups and appropriateness of vaccines in use. Especially true in chronic infections, MAT titres are not a reliable indicator of the infecting serovar(s), with important variations in cross-reactivity patterns across species or titre magnitudes between laboratories. MAT is serogroup-specific and only cross agglutination absorption tests (CAAT) provide serovar-specific results.

Culture: Leptospires are notoriously difficult and slow to grow. Newer media (e.g., HAN) or supplementation of usual growth media with cocktails of antibiotics (e.g., EMJH + STAFF (sulfamethoxazole, trimethoprim, amphotericin B, fosfomycin, and 5-fluorouracil)) or with 5-fluoracil only improve isolation rates for fastidious strains and/or contaminated samples (e.g. urine, soil) but can impact culture sensitivity and careful choice of media, clean sample collection and use of transport media are key. Cultures should be examined by dark-field microscopy every 1–2 weeks for at least 16 weeks (preferentially 26 weeks) before being deemed negative. Despite the difficulty of isolation, culture is a

choice method for the subsequent identification and classification of circulating strains (MLST, cgMLST, serotype), with WGS recently becoming the new gold standard for *Leptospira* taxonomy.

Nucleic acid detection: several PCR assays have been developed, some targeting all *Leptospira* (e.g., *gyrB, rss, secY* genes), others only pathogenic *Leptospira* (e.g., *ligA* gene). Although *lipL32* PCRs are the most commonly used in animal studies, they target the P1 clade only, and like most PCRs assays, they were developed for human diagnosis and often lack proper validation for animal samples.

Typing: Serotyping using MAT is serogroup-specific and should not be used to determine the infecting serovar(s). Single locus typing (eg *lfb1*, *glmU, secY*) can provide information on the infecting species. Three MLST schemes (PubMLST) and a core genome cgMLST (Pasteur BIGSdb) exist and can be achieved directly from samples. Other typing methods like VNTR or MST are less widely used and types difficult to compare with MLST types.

EWDA proposed harmonized protocol (for harmonization at large scale)

The aim of the protocol being surveillance of *Leptospira* circulating in wild populations, it targets chronic carriage and exposure to *Leptospira* rather than the individual diagnosis of acute leptospirosis.

PCR assays on kidneys (and/or alternatively urine if live animals) should be preferred for their sensitivity, with appropriate validation for the matrix used and species targeted.

The presence/absence of *Leptospira* spp. without typing is of little value and PCR screening should be complemented by typing by MLST, using culture-dependent or independent methods.

Exposure to *Leptospira* may also be assessed using MAT and can be useful to compare serological profiles for a given species with historical data or with data from different locations, but researchers must be aware of its limitations and should interpret MAT results with the utmost care.

Laboratories that can be contacted for diagnostic support

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