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Molecular detection of Leptospira interrogans DNA

Background

Pathogenic *Leptospira* are Gram-negative, spirochaete bacteria with over 200 serotypes, and are the causative agent of Leptospirosis. Leptospirosis is the most widespread global zoonosis across the world[1], with highest estimates of morbidity and mortality in parts of sub-Saharan Africa, parts of Latin America, and in the Caribbean. Although mechanisms of virulence and host survival tactics used by *Leptospira* are currently unknown, human disease is generally contracted through contact with the urine or faeces of infected host animals, including livestock, dogs, cats and rodents[2]. This contact typically occurs through broken or abraded skin and mucosal surfaces; however, it may occasionally occur through the inhalation of droplets of infected urine or contaminated water. Those at higher risk of contracting Leptospirosis include farm or sewage workers and individuals who undertake recreational water sports activities. Additionally, the disease is emerging in travellers to/from tropical regions, such as South East Asia, where rice paddy field workers are at regular occupational risk.

Signs and Symptoms

The spectrum of symptoms of Leptospirosis infection is extremely broad. Patients can present with generalised and mild symptoms or more severe forms of disease such as Weil's[3]. Leptospirosis is a biphasic disease with infection initially presenting as flu-like symptoms within the first week (septicaemic phase), followed by the immune phase, in which more severe forms of disease may ensue. It is during this immune phase that most complications with infection occur. The infection has an initial 7 to 12 day incubation phase, followed by leptospiraemia with pyrexia, myalgia and rigors for 4 to 7 days. This is then followed by the immune phase, characterised by antibody production and the presence of *Leptospira* in urine, with approximately 10% of patients going on to develop more severe forms of disease such as vasculitis, aseptic meningitis, pulmonary haemorrhage and Weil's disease; with the latter characterised by jaundice and acute renal failure. *Leptospira* that are typically excreted in the urine during the convalescent phase can alternatively be localised within tissues. Subclinical infection typically occurs in those in high-risk occupations. Overall, mortality is up to 10%, increasing to 40% in those with severe liver/kidney impairment[4].

Diagnosis

Diagnosis can be difficult, due to the non-specific symptoms that can mimic the clinical manifestations of conventional flu[5], hence it is frequently misdiagnosed. Abnormal liver and kidney function are typical of Leptospirosis, with increased levels of creatine kinase also suggesting infection. *Leptospira* can be identified from the blood and cerebrospinal fluid (CSF) during the immune phase, approximately two weeks after infection, and from the blood specifically less than 48 hours post onset of jaundice. Urine is also an appropriate sample type, as the bacteria can be shed in the urine.

Serology tests can also be performed, which involve performing microscopic agglutination tests (MAT) with live *Leptospira*. Tests may also be performed with killed bacteria, although this has a lowered sensitivity. These tests can be relatively specific to serovars; however, a large number of antigens must be tested, and cross reactions can occur. Alternatively, polymerase chain reaction (PCR) tests offer a sensitive and more timely method for the detection of *Leptospira,* capable of detecting the bacteria in the first week of infection e.g. in whole blood samples. Early diagnosis is beneficial as samples can be sent when patients are exhibiting non-specific symptoms in the initial phases of infection, allowing exact treatments to promptly commence. In addition, PCR can also be used after antibiotic treatment has commenced, and positive results are confirmatory for *Leptospira* infection.

Our Assay

At Micropathology Ltd., a nested PCR assay targeting the *L. interrogans* specific repetitive element is used for the detection of *L. interrogans* DNA, using standard block thermocyclers with subsequent visualisation using agarose gel electrophoresis or melt curve analysis using LightCycler 480s. UKAS accredited sample types for this assay include EDTA whole blood, CSF and urine.

Other sample types may be tested, and are reported alongside a caveat to state that the assay is not UKAS accredited for testing alternate sample types. Turnaround times are stated in the laboratory user handbook with results usually available in practice much sooner than the given timeframe. Where there is a delay, we are usually confirming a result and addressing clinical data given with the specimen.

References

1. Adler, B. and de la Pena Moctezuma. 2011. Leptospira and leptospirosis. Veterinary Microbiology, vol. 140, no. 3-4, pp. 287-296.
2. Gomes-Solecki, M., Santecchia, I. and Werts, C. 2017. Animal models of Leptospirosis: of mice and hamsters. Frontiers in Immunology, vol. 8, no. 58.
3. Levett, P. 2001. Leptospirosis. Clinical Microbiology Reviews, vol. 14, no. 2, pp. 296-326.
4. Török, Estée, et al., 2017. “Chapter 7: Bacteria.” Oxford Handbook of Infectious Diseases and Microbiology, Oxford University Press, Oxford, UK, pp. 340–342.
5. Yaakob, Y., Rodrigues, K. and John, D. 2015. Leptospirosis: recent incidents and available diagnostics - a review. The Medical journal of Malaysia, vol. 70, no. 6, pp. 351-355.