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REVIEW

Leptospirosis: a re-emerging infection

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Abstract

Leptospirosis is a re-emerging zoonotic infection. In developing countries large outbreaks have occurred in urban slums and following floods. Individuals from developed nations are also now more frequently exposed to the infection as a result of international travel and greater participation in certain outdoor recreational activities. Leptospirosis remains a diagnostic challenge since it often presents as a non-specific febrile event and laboratory diagnosis is still currently inadequate. Rapid tests may not be sufficiently sensitive in early disease and culture facilities are not widely available. A severe pulmonary haemorrhagic form of the infection is increasingly being encountered in many countries including Malaysia. The control of leptospirosis is largely dependent on general hygienic measures and rodent control. An effective human vaccine is still not available. There remains much that is unknown about this disease and there is scope and opportunity for good quality research.

Keywords: leptospirosis, re-emerging infection, zoonosis, Weil.

INTRODUCTION

Leptospirosis is a zoonotic infection that occurs globally. It is the most widespread zoonosis in the world. Weil first described its clinical features in 1886 but it was not until 1915 when Inada and Ido successfully isolated *Leptospira*, the aetiological agent. Leptospirosis is a re-emerging infection because of the occurrence of recent large outbreaks. Although it is largely a disease of the developing world, individuals from the developed countries are also exposed through global travel, military expeditions and participation in certain outdoor recreational activities.[1]

Aetiological agent

Leptospirosis is caused by *Leptospira interrogans*, a spirochaete. The organism is 6 – 20 mm long, hooked at one or both ends and is motile. The organism is an aerobic and slow growing bacterium. *Leptospira interrogans* is subtyped into 24 serogroups and over 200 serovars based on the outer envelope lipopolysaccharide (LPS). A genotypic classification has been introduced but is currently not clinically useful. There is a lack of correlation between the serological and genotypic classifications.

Serological classification

The leptospiral serovars are defined by agglutination after cross absorption with homologous antigen. Related serovars are grouped into serogroups. Serovars and serogroups have no taxonomic standing but are epidemiologically useful. The commonly encountered serogroups include *icterohaemorrhagiae, hebdomanis, autumnalis, pyrogenes, grippotyphosa, canicola, australis, pomona and javanica*. There is some evidence that certain serovars like *icterohaemorrhagica* may be associated with more severe disease.[2]

Molecular typing

Subserovar differences may be detected by molecular typing methods, Such typing is useful for epidemiological investigations. The molecular methods used include digestion of DNA using restriction enzymes (REA), pulse field gel electrophoresis (PFGE), ribotyping, restriction fragment length polymorphism (RFLP) and sequencing of amplicons.

Genotypic classification

Genotypic classification is based on DNA hybridisation studies. Twenty genomospecies have been identified with 9 genomospecies in

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the pathogenic clade. The 20th (*Leptospira kmetyi*) was recently isolated from soil in Johor, Malaysia.[3] There is no correlation between genomospecies and serovars.

Whole genome sequencing

The complete genomes of *L interrogans, L borgpetersenii* and *L biflexa* have been sequenced. The leptospiral genome comprises of 2 circular chromosomes and show extensive differences from other spirochaetes like *Treponema pallidum* and *Borrelia burgdorferi*. Genes related to motility, chemotaxis, adhesion and invasion of cells have been identified. Its broad array of genes reflects the ability of the organism to adapt to diverse environmental stimuli. [4,5]

Disease incidence

Leptospirosis occurs in many countries but the incidence varies considerably and marked under-reporting is common. Generally there is a higher incidence in tropical countries. Males are more affected than females. The aetiological agent is also encountered in a wide variety of wild and domestic animals but rodents, cattle, dogs and pigs appear to be the predominant animal hosts in many countries. Different serogroups predominate in different countries but *icterohaemorrhagiae* is encountered in the majority of countries.

In the Asia-Pacific region, countries with high incidence rates (> 10 per 100,000 population) are Bangladesh, Cambodia, Fiji, French Polynesia, the Andaman and Nicobar Islands in India, Laos, Nepal, New Caledonia, Sri Lanka, Thailand and Vietnam. Countries like China, mainland India, Malaysia, New Zealand, the Philippines and Mongolia have moderately high incidence rates (1 – 10 per 100,000 population) while low incidence rates (< 1 per 100,000 population) are seen in countries like Australia, Hong Kong, Japan, South Korea and Taiwan.[6] In the developing countries of the Asia Pacific region, leptospirosis is largely a water-borne disease and climate change may further aggravate the extent of the problem.

Leptospirosis in Malaysia

The first case in Malaysia was reported by Fletcher in 1925. The incidence rate in Malaysia is estimated to be between 2 – 5 per 100,000 population. However there has been a significant increase in recent years. The Ministry of Health had reported that the number of confirmed cases had increased from around 263 in 2004 to 1418 in 2009.[7] The male : female ratio is 4:1 with the majority of patients between 20 – 60 years of age. The case fatality rate is estimated to be around 10%. Leptospirosis has also been shown to be a common cause of acute febrile illness in Malaysia.[8]

There is also a high enzootic incidence in the Malaysian domestic animal population.[9] A more recent study revealed that 17% of wild rats at National Service Training Centres in the states of Kelantan and Terengganu had anti-leptospiral antibodies.[10]

Serogroups in Malaysia

Thirty eight serovars have been described in Malaysia and these serovars are divided into 13 serogroups namely *australis, autumnalis, bataviae, canicola, celledoni, grippotyphosa, hebdomanis, icterohaemorrhagiae, javanica, pomona, pyrogenes, sejroe* and *tarassovi*.[11]

Epidemiology

The occurrence of leptospirosis reflects the complex interaction between man, the animal hosts and the environment. Socioeconomic status, occupation, association with animals, recreational activity, climate and rainfall are all linked to the occurrence of leptospirosis. Humans are infected through direct contact with infected animals or exposure to fresh water or soil contaminated by infected animal urine. There is an association between animal host and the offending serovar.

The predominant portal of entry is abraded skin or mucous membranes although water borne transmission has also been reported. Human to human transmission is not considered important. Certain risk factors for exposure are identified. Occupation is an important risk factor and is related to the risk of exposure to animals or contaminated water and soil. Occupations with a high risk include farm workers, rice farmers, fish farmers, miners, sewage workers, rat catchers, soldiers and abbatoir workers. In Thailand an attack rate as high as 41.3% has been reported among pond cleaners.[12]

Outdoor recreational activity has also been associated with leptospirosis. One of the largest outbreaks was reported among participants of the Eco-Challenge that took place in Sabah in 2000.[13] Eighty (42%) out of 189 competitors who were contacted after the event met the case definition of leptospirosis. Twenty nine cases were hospitalised but there were no deaths. Taking doxycycline before or during the race

was protective. Leptospirosis was also reported among athletes competing in triathlons in Illinois and Wisconsin in 1998 as well as other water-related activities like white water rafting.[14]

In environments with poor sanitation, even normal routine activities may expose the population to leptospirosis due to widespread environmental contamination. In urban epidemics, transmission can occur in the household setting of slum settlements.[15] Heavy rain and flooding cause leaching of leptospira from soil into the water. A very large outbreak with over 3000 cases and nearly 250 deaths was reported in the Philippines from 1st October to 5th November in 2009.[16] The outbreak followed the occurrence of severe floods caused by three powerful typhoons that struck the country during that period. The incidence of leptospirosis in Guadeloupe increased fourfold during 2002 – 2004, a period of heavy rainfall associated with the El Nino phenomenon.[17]

Healthy human renal carriage

Humans had often been regarded as accidental or incidental hosts of leptospirosis but a recent study among Amazon villagers in Peru had suggested that healthy human carriage may also occur. This study enrolled 314 healthy subjects; 102 had no clinical or serological evidence of recent infection. Leptospiral DNA was found in the urine of 6 of the 102 (5.9%) who were not newly infected as well as in 7 of the 212 (3 %) with evidence of past infection giving an overall incidence of 4.1%. All the colonised subjects were women.[18]

Clinical Manifestations

The clinical manifestations of leptospirosis are extremely variable.[19] The infection has been misdiagnosed as other infections including dengue, typhoid, hepatitis, influenza, meningoencephalitis, yellow fever and malaria. Asymptomatic infections are common (up to 60-70%) in endemic areas. The majority of patients present as a self-limiting anicteric febrile illness. Because of this non-specific clinical presentation, leptospirosis remains a much underdiagnosed illness.

Only 10% to 15% develop the classical features of Weil's disease with jaundice, haemorrhage and proteinuria. Other clinical manifestations include pulmonary haemorrhage, uveitis, acalculous cholecystitis, myocarditis and pancreatitis. Mortality rates of between 5 – 40% have been reported.

A clinical study of 559 cases of leptospirosis in Malaysia conducted over three decades ago revealed that the common clinical manifestations were fever (100%), conjunctival injection (54%), jaundice (46%), muscle tenderness (45%), abdominal symptoms (29%), headache (25%), proteinuria (25%), chills & rigors (22%) and hepatomegaly (18%). Splenomegaly (6%), haemorrhagic signs (5%), cough (4%) and loin pain (2%) were less frequently encountered.[20]

In a more recent study based on 1060 cases diagnosed between 2003 – 2005, the most common clinical features were fever (98%), chills (64%), cough (56%) and abdominal pain (43%). A very significant finding which had differed from the earlier study was the number of patients who had severe pulmonary involvement (51%). Pulmonary failure was a major contributory factor in 85% of fatal cases.[21]

Pulmonary Leptospirosis

A severe pulmonary haemorrhagic form of leptospirosis (SPFL) has been increasingly reported in recent years.[22] Pulmonary involvement in leptospirosis has been reported to vary from 20 – 70% of cases. Pulmonary haemorrhage is a prominent finding. Clinicians may fail to recognise this form of leptospirosis as it can present without renal failure or jaundice. It is a cause of serious disease and mortality. In one series of 42 patients who required artificial ventilation, 23 (55%) died.[23]

Ocular Leptospirosis

This is also often an unrecognised condition and may present as a late complication occurring up to 2 years after the acute episode. An acute uveitis occurs in the immune phase of the illness and a hypopyon occurs in up to 12% of patients. Ocular leptospirosis can also be recurrent or chronic and lead to cataract formation and glaucoma.[24]

Laboratory Diagnosis

The laboratory diagnosis of leptospirosis includes darkfield microscopic examination, culture and serological tests. Isolation remains the gold standard and leptospira can be isolated from blood, CSF and urine during the first week of illness. A study from Northeast Thailand showed that the sensitivity of blood culture can be increased using a combination of whole blood and deposit from spun plasma.[25]

However culture facilities are often not routinely available and requires the use of special media eg Fletcher's and EMJH media.

Growth is slow and may take up to 4 months The inoculation of hamsters or guinea pigs is rarely performed today. The serovar of the leptospira may be identified using agglutination tests or PCR.

The majority of clinical cases are confirmed using serological tests. Paired sera have to be obtained since antibodies may not appear early in the disease and seroconversion may take as long as 30 days. The reference standard serological test is the MAT (microagglutination test) which allows for the detection of the infecting serogroup. The panel of antigens in MAT must reflect the prevalent serogroups in the country.

The ELISA test and the dipstick assay are rapid tests that detect Leptospira genus-specific antibodies. In a comparison of serological tests in the United Kingdom, the IgM ELISA (PanBio) had a sensitivity of 90% and specificity of 94% and would appear to be suitable as a screening test.[26] However, in a similar comparison in Malaysia, the PanBio ELISA had a sensitivity of only 54% while the Dipstick had a sensitivity of 83%. The suboptimal performance of the Panbio ELISA test may be due to the blocking effect of high IgG antibodies or the high MAT cut-off titre used in the study.[27] The antigens used in rapid tests may also not cross-react with all serovar antibodies and it is important for these test kits to be evaluated locally before being adopted for routine diagnostic use. PCR assays are now available and are useful for the early detection of the disease. There is probably a need to use a combination of laboratory tests.

Treatment

Penicillin or doxycycline are still the antibiotics commonly used to treat leptospirosis. Surprisingly no comparative trials have been conducted to date. Penicillin has been shown to shorten fever and period of renal dysfunction.[28] Other antibiotics which may be effective include the macrolides, other beta-lactams and aminoglycosides. A recent multicentre, open randomised trial in Thailand involving 69 patients with confirmed leptospirosis showed that a three day course of azithromycin is as effective as a seven day course of doxycycline. Patients on azithromycin had less adverse effects.[29]

Prevention

General hygienic measures including avoidance of contact with potentially contaminated water, wearing protective clothing and footwear and rodent control are the mainstay of prevention. Farm and domestic animals may be vaccinated. Doxycycline 200 mg once weekly has been shown to be effective for chemoprophylaxis although a recent meta-analysis did not find any conclusive proof of benefit.[30,31] Currently there is still no effective vaccine for human use despite decades of research.[32]

Conclusions

Leptospirosis continues to be a major public health challenge in many countries. There is still a lot that is unknown about this disease and there is much scope and opportunity for good quality leptospirosis research in Malaysia. There is a need to increase awareness among physicians of this infection as there is still much under-diagnosis of the condition. Improved laboratory tests should be developed to make confirmation of the infection more rapid and easier. There is definitely also a need for an effective vaccine for humans.

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