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Leptospirosis a Neglected Re-emerging Zoonoses in India: An overview

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ABSTRACT

Leptospirosis is a zoonotic infectious disease of worldwide economic importance affecting both humans and animals. It is bacterial disease caused by spirochete of genus *Leptospira*. The symptom ranges from flu-like illness to acute kidney failure, jaundice in humans while it causes abortions, stillbirths, reduced milk production in animals. Rodents, domestic and wild animals act as carrier and excrete live organism is their urine. It is an occupational disease affecting farmers, veterinarians, slaughterhouse workers, etc those who are in direct or indirect contact with the carrier animals. The diagnosis is done by direct and indirect laboratory methods for detection of infectious agent and its antibodies. The disease can be controlled through vaccination of domestic animals, control of rodents, strict and proper environmental hygienic measures.

HIGHLIGHTS

- Leptospirosis an infectious zoonotic bacterial disease caused by *Leptospira* spp.
- The disease is endemic in India and often neglected causing occupational hazard.
- Rodents and domestic animals are reservoir host of pathogen hence proper diagnosis and control is essential.

Keywords: Leptospirosis, serovars, zoonoses, occupational hazard, diagnosis and control

Leptospirosis is rapidly re-emerging public health problem globally affecting the health of livestock and human being. It is most widespread zoonotic disease in the world caused by pathogenic spirochetes *Leptospira*. The disease is most commonly prevalent in humid, tropical and sub-tropical climates of South East Asian countries having high rain fall, humidity, presence of marshy land and paddy grown area (Vijayachari *et al.,* 2008; Favero *et al.,* 2017). Leptospirosis, a communicable infectious disease, is caused by more than 250 leptospiral serovars known to infect more than 160 species of mammals (Bhure *et al.,* 2012). It affects cattle, buffalo, sheep, goat, horse, swine, dog etc resulting in huge economic losses to the agriculture sector and potential threat to the associated farming community. Despite of its worldwide severity, the disease is neglected in most of the endemic countries because of lack of information and awareness about the extent of the problem. The disease has been identified as a neglected tropical disease by the World Health Organization and thus requiring further research in its epidemiology and global disease burden. Leptospirosis poses an increasing one health problem worldwide, as evidenced by increasing incidence rates and multiple outbreaks throughout the world (Wasinski and Dutkiewicz, 2013). The disease causes a wide spectrum of clinical manifestations in animals and is responsible for economical losses due to its reproductive impacts like mastitis, repeat breeding, abortion, infertility, early embryonic death, stillbirth, birth of weak calves and decreased milk production in bovines (Vinetz, 2001).

Historical Background

In 1886, the disease was first described by Adolf Weil. Inada and Ito first identified *Leptospira* as the causative

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organism in 1908 (Inada and Ito, 1908). In India, Taylor and Goyal (1931) first described the disease in Andaman Islands as Andaman haemorrhagic fever in humans. From 20th century onwards, leptospirosis is found to be endemic in India and many outbreaks of the disease have been encountered in Tamil Nadu, West Bengal, Gujarat, Kerala, Orissa, Maharashtra, Karnataka and Andaman and Nicobar Islands (Varma *et al.,* 2001). Majority of the disease incidences are occurring during October-November correlating with the monsoon season. In India the disease has attained significant concern in recent years as the incidences are being increased among various livestock species. Leptospirosis in cattle was first reported by Adinarayan *et al.* (1960). Since then several reports emerged confirming the prevalence of leptospirosis in humans and bovines from different states of India (Srivastava *et al.,* 1991; Sivaseelan *et al.,* 2003; Patel *et al.,* 2014; Jain *et al.,* 2019).

Causative Organism

Leptospirosis is a worldwide zoonosis, caused by bacteria *Leptospira interrogans*. *Leptospira* is long corkscrew-shaped organism, too thin to be visible under the microscope. The aetiological agent is a spirochete measuring 0.1 to 0.2 µm in diameter and having 6-25 µm length with tightly set coils, highly motile by rotating and bending, obligate aerobes. Usually one or both ends of the organism are bent or hooked. Two axial periplasmic flagella are located in the periplasmic space. Because of their narrow diameter, dark-field illumination or phase contrast microscopy is required to visualize the leptospires. They do not stain easily with aniline dyes. *Leptospira* are aerobic, susceptible to heat, dry environment, acids and basics disinfectants but can resist alkali pH up to pH 7.8 (Levett, 2001; Prajapati *et al.,* 2018). Silver impregnation staining, immunoperoxidase staining or immunofluorescence is done to observe *Leptospira*. The bacteria being aerobic in nature can be cultured in media enriched with vitamins like B1 and B12, ammonium salts and long-chain fatty acids at 28-30°C. The most commonly used media for culture of *Leptospira* is Ellinghausen-McCullough-Johnson-Harris (EMJH) medium.

The genus *Leptospira* includes two types of species based mainly on their pathogenicity: pathogenic and saprophytic. *Leptospira interrogans* consist of pathogenic strains while *L. biflexa* consist of non-pathogenic free living saprophytic strains. The saprophytic and pathogenic leptospires are morphologically indistinguishable (Adler and Moctezuma, 2010). The pathogenic leptospires were previously classified as members of the species *Leptospira interrogans*, however the genus *Leptospira* has been reorganized based on DNA hybridization into 20 species including nine pathogenic, five intermediate and six saprophytic species. Most of pathogenic serovars reported worldwide belongs to three species namely *L. interrogans, L. borgpetersenii,* and *L. kirschneri* (Picardaeu, 2013). Recently the genus *Leptospira* is divided into 35 species belonging to three phylogenetic clusters, which supposedly correlate with the bacterial virulence (Vincent *et al.,* 2019). Despite the advances in molecular taxonomy, the subdivision of the genus *Leptospira* in to serogroups and serovars remains widely used. Each and every serovar has its own distribution area and its own host maintenance species. Presently there are nearly 300 pathogenic *Leptospira* serovars based on their antigenic relatedness which cannot be differentiated on the basis of morphology. Usually, every serovar is adapted to a specific mammalian host like insectivores, rodents, pigs, dogs and cattle. Each serovars can also be adapted to several hosts, while one host might harbor several different serovars. Hardjo infection is the only exception where the serovar Hardjo is maintained specifically by cattle and sheep and there are no known wildlife hosts to this serovar (Hartskeerl *et al.,* 2011).

Mode of Transmission

Rodents, cattle, pigs, dogs, cats and wild animals are considered as common reservoirs of leptospires. The organism cannot be eradicated since rodents and insectivores are major natural reservoirs. Leptospirosis is an occupational disease affecting farmers, veterinarians, slaughterhouse workers, rodent catchers, pet traders and sewer workers. Agricultural workers are considered as the main occupational risk groups, who during their daily activities are exposed to contaminated wet soil and water. Individuals working directly with animals like veterinarians, farmers, cowherds, abattoir workers, etc. can acquire the infection through contact with infected urine, infected carcasses, aborted fetuses or parts of placenta and during milking, and after animal bites. In the tropical climatic zone, the highest morbidity is noted in

environmental conditions which are most favourable for survival of leptospires like extreme weather conditions, cyclones and floods with increasing intensity and frequency, increased rainfall associated with global warming may potentially leads to an increase in the disease incidence and leptospirosis outbreaks (Wasinski and Dutkiewicz, 2013).

Pathogenesis and Clinical Symptoms

Pathogenic leptospires live in the kidneys and genital tract of their natural hosts. A wide range of mammalian species are carriers of pathogenic leptospires including farm and pet animals, semi-domestic and feral as important source of infection. Humans are considered as dead end hosts of leptospires. Cattle and wild rodents are the main host which excretes leptospires in their urine but may not display symptoms of active infection thus acting as potential source of infection to humans and other animals. Urine of healthy carriers or infected animals, aborted fetus and uterine discharges which may contaminate feed, drinking water, soil and pasture which in turn act as main source of infection. Leptospires excreted in urine can survive for many months depending up on favorable environmental conditions. Pathogenic strains of *Leptospira* enter through skin abrasions and cuts, through mucous membranes of nose, eyes, mouth and genital tracts of domestic animals and humans (Sunder *et al.,* 2018).

Leptospirosis is characterized by a wide range of clinical symptoms in livestock with slight difference between affected species. Clinical signs of acute or sub-acute disease are seen in the leptospiremic phase and it is characterized by septicaemia, anorexia and high fever, depression, petechiation of mucosa, paleness, and acute hemolytic anaemia with hemoglobinuria and jaundice. Clinical signs of chronic leptospirosis in livestock are generally associated with reproductive problems like infertility, stillbirth, abortion, drop in milk production and mastitis. Abortion in animals usually occurs during the last trimester of pregnancy (Adugna, 2016).

Bovine leptosirosis is one of the major causes of reproductive failure. The clinical signs linked with bovine leptospirosis are variable and depend upon the infecting serovar as well as the susceptibility of the individual animals (Adler and de la Pena Moctezuma, 2010). Serovars viz., Hardjo, Pomona, Icterohaemorrhagiae, Australis, Hebdomadis, Bankinang and Grippotyphosa are found mainly associated to bovine leptospirosis. *Leptospira* infection occurs via bacterial exposure through mucous membranes and results in no or very mild acute clinical symptoms. As a result of serovar Hardjo infection, abortions, birth of weak calves or stillbirths may occur, but the symptoms are usually seen only when animal is infected during her first pregnancy. Abortion may occur several weeks after the infection without any noticeable signs of illness. Infertility is also commonly seen in bovine leptospirosis. Persistent infection of the reproductive tract of the male and female cattle may be the most economically important feature of serovar Hardjo infection. The disease has enormous economic impact on the international trade of animals and semen also (Balamurugan *et al.,* 2018).

Small ruminants are considered as accidental hosts, being affected by incidental serovars of *Leptospira*, carried by other domestic and wild animals. The disease occurs rarely in sheep and goats but the symptoms are similar to the bovines with major illness only in young or pregnant animals. In most cases they develop acute septicemia and are found dead. In Hardjo infections, abortion may be the only sign, but milk drop syndrome can also be observed.

Canine leptospirosis is characterized by anorexia, lethargy and vomiting, weight loss, polyuria, diarrhea, abdominal or lumbar pain, musculoskeletal pain and dehydration. The clinical symptoms of equine leptospirosis are similar to those of cattle, with listlessness, low-grade fever, anorexia, anaemia, conjunctival suffusion, petechial hemorrhages on the mucosa, jaundice, and general depression. Renal failure may also occur, especially in foals. In pregnant mares infection may results in abortion, placentitis and stillbirths (Simbizi *et al.,* 2016).

In humans, the clinical manifestations of leptospirosis are flu-like illness, pneumonia, pulmonary hemorrhages, jaundice, acute kidney failure, etc (Karpagam and Ganesh, 2020). The disease is characterized by fever, myalgias, severe headache, chills, nausea and vomiting, diarrhoea, anuria or oliguria, jaundice (yellowing of skin and eyes), conjunctival suffusion, haemorrhages, aseptic meningitis, skin rash, joint pain, cardiac arrhythmia, psychosis and restlessness. Sometime infected persons may not have any symptoms. Illness usually starts suddenly with fever and other symptoms appear after 7-12 days incubation period. The classical form of severe leptospirosis is known as

Weil's disease which is characterized by jaundice, bleeding and kidney failure (Hartskeerl *et al.,* 2011).

Diagnosis

The diagnosis of leptospirosis is difficult in the clinic and the laboratory both because of which the disease is not recognized frequently and therefore severely neglected. In laboratory, diagnosis of leptospirosis is broadly classified into direct diagnosis or confirmation by isolation of causative organism or demonstration of leptospires by dark field microscopy or by PCR amplification of specific segment of leptospiral genome; and indirect diagnosis by detection of antibodies against leptospires using Microscopic agglutination Test (MAT), Enzyme linked immunosorbent assay (ELISA), Lepto dipstick assay and Indirect haemagglutination assay (IHA).

Diagnosis is difficult due to easily contamination of samples with other bacteria, thus hampering isolation while the serological measurements always do not give a positive reaction of an animal's infectious status (Heinemann *et al.,* 2000).

Proper diagnosis of leptospirosis is mainly based on laboratory confirmation since its clinical signs are nonspecific and can be usually mistaken with other febrile diseases. The culture of *Leptospira* from body fluids (blood or urine) is the most confirmative test. Direct diagnosis of *Leptospira* infection include fluorescent anti-body testing (FAT), silver staining and immuno-histochemistry, culture of bacteria and PCR from blood, urine and tissue samples (Vado-Solis *et al.,* 2002). Since, leptospires are slow growing and fastidious requiring more than a month for growth of organisms, its isolation is difficult. Therefore, molecular methods using PCR for routine diagnosis are becoming increasingly important. PCR is highly sensitive and specific and may detect up to ten leptospires per milliliter of urine.

Among serological tests, MAT is the most widely used reference and gold standard serological test for diagnosis of leptospirosis (OIE, 2018). The major advantage of MAT is its high specificity. A titre of 1:100 or more indicates infection in seroprevalence studies (Srivastava, 2008). However, MAT is also problematic due to the requirement for live *Leptospira* serovars cultures prevalent in a particular geographical area (Adler and Pen, 2010). Diagnosis of *Leptospira* antibodies by ELISA is serovar-specific and thus limited to regions where the occurrence of the serovars is well defined. This technique can distinguish acute and chronic infections by the detecting their specific immunoglobulin IgM and IgG (Bourhy *et al.,* 2013). A label-based lateral flow dipstick assay was developed for the rapid, easy and visual detection of *Leptospira* using multiplex loop-mediated isothermal amplification (m-LAMP) which can simultaneously detect the target DNA template and a LAMP control in clinical diagnostics thus serving as a point-of-care device (Najian *et al.,* 2016). Further an electrochemical AuNPs based highly sensitive and specific DNA sensor has been developed for diagnosis lipL32 gene which is highly conserved among pathogenic *Leptospira* serovars (Verma *et al.,* 2020).

Prevention and Control

The prevention and control of leptospirosis is difficult in domestic animals and man due to the widely distribution of leptospires in wildlife and reservoir hosts. The infected animals should be immediately isolated and quarantined for at least 14 days and the premises and surroundings should be thoroughly disinfected. Carrier animals shedding the *Leptospira* pathogen in their urine should be segregated or slaughtered. *Leptospira* can be easily controlled by adding antibiotics to the semen. Since, leptospirosis is disease of an occupational hazard, all the persons directly involved with animals and its surrounding should preferably use aprons, gloves, gumboots during their handling. Rodent control using effective rodenticides, proper environmental hygienic measures to avoid the contamination risk of food, water and soil are necessary to further prevent the transmission of leptospirosis (Dhanze *et al.,* 2013). Quarantine and screening of newly introduced animals should be strictly followed. Damp areas near the farm should be drained and suitable disinfectant to be used in farm. Since rats and other wild animals act as infection source, contact between them and farm animals should be controlled by using rat bait and fencing of farm (Martins and Lilenbaum, 2017).

Control of leptospirosis includes measures like identification and treatment of the carriers and infectious source and systematic immunization with commercially available vaccines containing the circulating serovars. Immunization of local reservoirs of the pathogen should be implemented in the areas highly prone to leptospirosis

especially low lying areas, damp and muddy environment and high rice cultivating areas (Routray *et al.,* 2018). Existence of a large number of serovars of *Leptospira* makes it difficult to develop a multispecies universally effective vaccine. Although vaccination is not possible in wild animals, but the vaccination strategy can be applied in domestic animals for control and prevention of leptospirosis. Currently the molecular and cellular studies on leptospirosis vaccines have been focused on whole cell inactivated immunogen, lipopolysaccharides, lipoproteins, outer-membrane proteins and potential virulence factors (Vijayachari *et al.,* 2008).

CONCLUSION

Leptospirosis is an important neglected zoonotic disease of endemic nature in India with considerable impact on veterinary and public health. The key determinants of the incidence and prevalence of the disease are socioeconomic conditions, climatic conditions, reservoir animals, environmental hygiene and occupational associations of human being. The efficacy of disease recognition, treatment and control requires adequate knowledge pertaining to its epidemiology. Reservoir host control measures, animal vaccination and environmental hygiene in conjunction with a strong surveillance, monitoring and networking programmes can significantly reduce the disease. Strict bio-security measures like quarantine and isolation of infected or suspected animals should be implemented for the successful control and eradication of the leptospirosis. An interdisciplinary approach involving medical, veterinary, agricultural and environmental sciences under one health vision needs to be implemented in order to orient knowledge about the detection, prevention and eradication of leptospirosis.

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