Indian J Anim Health (2021), 60(2): 160-171
DOI: https://doi.org/10.36062/ijah.2021.07321

Review Article

A brief review on seroprevalence of bovine leptospirosis in India

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Abstract

Leptospirosis is a zoonotic, infectious bacterial disease that affects both animals and humans and has worldwide in distribution. The causative agent is a corkscrew-shaped spirochaete bacteria of the genus *Leptospira*. An infected animal spreads the bacteria by contaminating the area with uterine discharges, urine and materials from aborted foetuses. Bovine leptospirosis is linked with various symptoms such as stillbirth, infertility, birth of debilitated and weak calves, reduced production and milk yield. Carrier animals frequently discharge the organism in their urine, making them a source of infection for other species. The connection between the host and the agent is influenced by risk variables such as animal risk factors as well as environmental and managerial factors. Sero-epidemiological studies in leptospirosis in the bovine population of India suggest the upsurge in circulating antibodies against *Leptospira* and the emergence of a new serovar. The present review addresses the transmission pattern, epidemiology, clinical manifestations, different diagnostic tests and control measures of leptospirosis in the Indian context.

Keywords: Bovines, Epidemiology, Leptospirosis, Microscopic agglutination test (MAT)

Introduction

Leptospirosis has been defined as a spirochaetal zoonotic disease, which has recently emerged as a serious global veterinary and public health threat in both developed and developing economies. It affects domestic and several wild animals all over the world and the disease is most prevalent in tropical and subtropical regions. Heavy rainfall can act as a predisposing factor for disease outbreak. Adolf Weil, a German physician, was the first to report the disease in the year 1886. Stillbirth, infertility, the birth of frail calves, decreased milk yield and production are all common side effects of bovine leptospirosis. A spirochete belonging to the genus *Leptospira* causes this infection, a direct zoonotic illness (WHO, 2009). The organism remains latent by persisting in the kidneys and genital region, not manifesting any clinical signs of the disease (Ellis *et al*., 1986). Carrier cows frequently excrete leptospires in their urine, acting as a major source of infection for other cows, dairy farm employees and the general public (Waitkins, 1986).

The disease affects several domesticated animals like cattle, sheep, goats, buffalo, horses and pigs, causing major economic losses to farmers due to reproductive concerns (Srivastava, 2008). Though leptospirosis is an occupational hazard, agricultural workers,

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sewage workers, veterinarians and entire communities living in the tropics may be at risk (Campagnolo *et al*., 2000). The disease has gained much importance as it often remains undiagnosed (Iqbal *et al*., 2011). Despite its severity, this disease is largely ignored in most endemic nations due to a lack of knowledge and awareness about the extent of the problem. Leptospirosis is a re-emerging infectious disease in both animals and people, and it has the potential to spread significantly as a result of expected global warming (Kamath and Joshi, 2003; Yang, 2007). Extreme weather events like cyclones and floods, as well as increased rainfall due to global warming, are likely to be major factors in the rise in leptospirosis incidence (Lau *et al*., 2010).

In India, leptospirosis in cattle was first reported by Adinarayanan *et al*. (1960). Cattle are not only unintentionally infected, but also function as maintenance hosts for certain *Leptospira* serovar strains. In the majority of Indian states, leptospirosis is known to be endemic. In order to create leptospiral infection prevention and control programme, the frequency of leptospiral infection in the animal population must be known. So, this review gives brief information about the seroprevalence, transmission, pathogenesis and the different diagnostic tests available for detection of leptospirosis in Indian cattle population.

Etiology

Leptospira organisms are the members of the Leptospiraceae family and they are ubiquitous in nature. Noguchi (1917) coined the term *"Leptospira"* because of its thin spiral structure. *Leptospires* are 6-20 µm long and 0.1 µm in diameter, aerobic, Gram-negative, fastidious, slow-growing and typical movement that resembles that of a corkscrew (Levett, 2001). Because of their smaller size, leptospires are best visualized under dark-field illumination or phase-contrast microscopy. *Leptospires* thrive in humid conditions such as stagnant water or polluted soil, although they may also survive in dry situations. Temperatures of 50°C (122°F) can kill the bacteria, whereas 70 percent ethanol, 1% sodium hypochlorite, formaldehyde, detergents and acids can inactivate it.

Leptospira organisms thrive optimally at a temperature of 28 to 30°C and at pH range 6.8 to 7.4, and their generation time varies between 7 to 12 hours, yielding 6 to 8 x 10^9 cells/mL. The antigenic structure of the bacteria is made up of somatic antigens, surface antigens, outer membrane lipopolysaccharides and flagellar antigens.The main antigen and a powerful immunogen, outer membrane lipopolysaccharide, is responsible for serovar specificity and is the target of antibody and complement-mediated bactericidal action. Lipopolysaccharide (LPS) present in the outer membrane constitutes the major antigen for *Leptospira* which is serovar specific. Other antigenic components of Leptospira are flagellar antigen, lipoprotein, porin (OmpL1) etc. B and T-cell mediated defence mechanism against the invading organism get activated following its entry into the host system, leading to humoral and cell-mediated immune responses against the organism (Rao *et al*., 2003).

The taxonomy of *Leptospira* is complex. Previously, serological reactions were used to distinguish leptospires, and two species were identified namely the *L. interrogans* (pathogenic) and *L. biflexa* (saprophytic or non-pathogenic). Antigenically linked serogroups are frequently formed from serovars. Pathogenic *Leptospira* spp. are broadly categorized into 300 serovars and 28 serogroups, based on the LPS antigen (Saito *et al*., 2013). The genus *Leptospira* has recently been divided into 35 species, each of which belongs to one of three phylogenetic clusters linked to bacterial pathogenicity (Vincent *et al*., 2019). Nine pathogenic, five intermediate, and six saprophytic species of *Leptospira* spp. have been discovered to show genetic similarities in DNA hybridization. Every serovar is usually

suited to a single mammalian host, although certain serovars can be adapted to several hosts, and one host can contain multiple serovars. Bovines primarily get affected by serovars of the serogroup Sejroe (Mughini-Gras *et al.*, 2014).

Reservoirs and carriers

Puddles, rivers, sewers, ponds, moist soil and agricultural fields are the places where *Leptospira* organisms can be found (Karpagam and Ganesh, 2020). Pathogenic *Leptospira* biofilms have been identified in water bodies, aiding in its survivability (Barragan *et al.*, 2017). The major hosts identified are mice, rats and moles, but cows, sheep, deer, hedgehogs, skunks, rabbits, raccoons, pigs and opossums can also be associated with the disease (Ellis, 2015). Infected animals may excrete leptospires on a regular or irregular basis for months, years, or even their whole lives. Rodents and domestic animals like cattle, pigs, and dogs play an important role as reservoir hosts. Farmers, fisherman, garbage collectors and sewage workers are among those at risk of getting leptospirosis since they are the inadvertent or dead-end hosts of Leptospira (Soo *et al.*, 2020).

Transmission

The transmission pattern of *Leptospira* can be either direct or indirect. Direct transmission occurs when occupational groups or animals are exposed to contaminated products of birth, infected tissues of abortion etc. Besides this, sexual contact or by suckling milk from infected mothers, inadequate husbandry practices like frequent use of calving pens in an unhygienic manner, entry of new animals directly in the herds may act as a route for transmitting the disease directly (Hashimoto *et al.*, 2015).

Animals have been found to contain leptospires in their genital tracts and can transmit them transplacentally (Ellis *et al.*, 1986). Leptospires are thought to enter the host body through intact skin, mucosal membranes and mouth. Animal excreta are the main source of leptospires, which are then expelled into the environment via urine. Humans and animals get it most commonly by contact with contaminated soil or water, and in rare cases, food contaminated by the urine of infected reservoir animals, mainly rats (Faine *et al.*, 1999). Though leptospirosis is thought to be a foodborne but the actual percentage of cases that can be attributed to food is complicated (Unz, 2009). Foodborne leptospirosis was the sole outbreak of leptospirosis described in Greece in the literature (involving drinking water at a cafe) (Levett, 2001). When the environment is immediately contaminated with urine of infected/carrier animals, it can act as a source of infection and the transmission pattern is termed as indirect.

Epidemiology

The leptospires are worldwide in distribution. The disease has been given various names depending on the occupational groups involved and the nature of the disease, such as Harvest fever, Mud fever, Cane cutter's disease, seven-day fever, Ricefield workers disease, Weil's disease and Infectious jaundice etc. The *Leptospira* infection has been documented to occur in more than 150 mammalian species. Several nations, including India and other South-East Asian countries, England, China and Europe, suffer with Weil's disease, which is one of the most severe forms of the condition (Sehgal, 1998).

Rodents were the first to be identified as leptospire carriers and sources of infection in humans (Dhanze *et al.*, 2013). Tropical regions with heavy rainfall and humidity along with temperate zones with higher precipitation rates are more susceptible to the disease (Fávero *et al.*, 2017; Patel *et al.*, 2017). The prevalence of the disease basically increases during the month of July to September because flood water may ease in transmitting the *Leptospira* organism to both animals and humans when mixed with urine or aborted materials of infected animals (Pawar *et al.*, 2018). During the flood seasons, cattle, when exposed to a wet environment, become more susceptible of

getting contracted with infected rodents directly or indirectly, subsequently leading to leptospiral infection.When humans and animals are exposed to damp environments for extended periods of time during natural disasters like cyclones and floods, leptospirosis is becoming more widely recognised as a possible outcome.

The urban areas are generally more polluted due to uneven garbage dumping sites, poorly constructed sewage systems and industrial waste, which favour the growth of *Leptospira* organisms and increases the rodent populations, thus acting as a carrier for *Leptospira* infection (Dhanze *et al.*, 2013). Inadequate husbandry practices such as wet floor system, use of unclean water, e.g. stagnant water, improper drainage system, reduced floor space and absence of biosecurity measures, can also lead to infection.The disease occurrence is influenced by the presence of reservoir hosts such as rats, foxes, rabbits, raccoon and wild cats, warm and humid climate, alkaline soil and organic waste (Radostits *et al.*, 2009).

Infection in cattle can be with serovars Hardjobovis, Pomona and Grippotyphosa. Infection with Icterohaemorrhagiae, Bratislava, Hebdomadis, Autumnalis, Australis, Sejroe, Canicola and Batavia can also be seen (Balamurugan *et al.*, 2018). Cattle are the only reservoir of *Leptospira* Hardjo and serve as its maintenance host. Balakrishnan *et al.* (2011) reported that exotic purebreds and crossbreeds are more susceptible to disease than native purebreds and crossbreeds. But in many studies in India, the higher seropositivity found in cross-breed cattle are due to poor disease resistance (Nagarajan, 2005; Pandian *et al.*, 2015). The odds of acquiring leptospiral infection through artificial breeding techniques are higher when compared to breeding by natural service; the semen of an infected bull may increase the chances of getting an infection. Gender associated stress factors like lactation, pregnancy and parturition etc, in the female population, may increase the danger of infection (Sharma *et al.*, 2003; Agarwal *et al.*, 2005; Shafighi *et al.*, 2010).

The larger herds with high animal density present greater probabilities of infection by the organism (Oliveira *et al.*, 2010). The disease is most prevalent in higher age groups like 5-7 years or above. Once the animal recovers from leptospiral infection, it may serve as a reservoir host or maintenance host. The pathogen has an affinity for the reproductive organs and reaches its predilection site, i.e., urinary tubules and from time-to-time releases slowly during urine voiding. Due to the repeated exposure to the infection, the antibodies may remain for a longer duration of time in the animals (Kocabiyik and Cetin, 2004; Balakrishnan *et al.*, 2011; Patel *et al.*, 2014; Pandian *et al.*, 2015). It is a major cause of miscarriage, stillbirth, infertility, reduced milk yield, and mortality in cattle (Bharti *et al.*, 2003; Patel *et al.*, 2014). The most common serovars prevalent in India are Autumnalis, Grippotyphosa, Pyrogenes, Australis, Canicola, Javanica, Ballum, Sejroe, Louisiana and Pomona (Victoriano *et al.*, 2009). In India, the pervasiveness of leptospira infection in cattle has been documented from different parts of the country by various workers, which has been shown in Table 1.

Pathogenesis

Despite scientific efforts, the pathophysiology of leptospirosis remains a mystery (Karpagam and Ganesh, 2020). Although the animal body has many ways to combat the germs, *Leptospira* is well suited to the inflammatory condition it causes.

Leptospires can enter an animal's body by a variety of channels, including abraded skin, feet and legs, mucous membranes and the mouth. The most common points of entrance for cattle are field ponds and marshy areas. After penetrating the skin, the organisms grow quickly in the bloodstream. *Leptospira* spreads quickly through the bloodstream to all organs (Picardeau, 2017). They primarily damage the liver and produce bile leakage into the bloodstream, leading in high bilirubin levels and jaundice. Toxins

Table 1. Bovine leptospirosis seroprevalence as reported from various parts of India

Place / States	Sample size	Test used	Sero-prevalence	Serovars	References
Assam and Bihar	680	ELISA	Assam (1.2%) and Bihar (4.5%)	*Leptospira* Hardjo	Leahy *et al*., 2021
Districts of lower Brahmaputra valley, Assam	380	IgG ELISA and MAT	17.89% and 11.58%	Autumnalis (6.05%), followed by Ballum (2.63%), Bataviae (1.31%), Ichterohaemorrhagie (0.7%), Javanica (0.5%) and Sejroe (0.2%)	Kader (2019)
Meghalaya	276	Double sandwich ELISA	8.33%	*Leptospira* Hardjo	Milton *et al*., 2019
Andhra Pradesh	106	MAT	70.8%	Visakhapatnam (71.43%), Chittoor (70.83%), Guntur (70.45%), Kurnool (69.86%), Godavari (67.78%), Srikakulam (65.79%) and Prakasam (61.97%) districts	Alamuri *et al*., 2019
Gujarat, Haryana, Punjab, Maharashtra, Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Chhattisgarh, Sikkim and Uttarakhand	373 associated with a reproductive disorder	MAT	70.51%	Hardjo (27.76%), Pyrogenes (18.63%), Canicola and Javanica (17.49%), Hebdomadis (17.11%), Shermani and Panama (16.73%), Djasiman (16.35%), Tarassovi, Grippotyphosa and Pomona (15.97%), Icterohaemorrhagiae(15.59%), Copenhageni (14.83%), Australis (13.69%), Kaup and Hurstbridge (10.65%), Bankinang(10.27%) and Bataviae (9.51%)	Balamurugan *et al*., 2018
Gujarat, India	398	I-ELISA	5.77 %	Hardjo(5.77%)	Patel *et al*., 2017
Pondicherry (Southern India)	250	ELISA & MAT	91 (36.4 %) and 62 (24.8%)	Hardjo (41.94%),Grippotyphosa(24.19%), Pomona (16.13%), Icterohaemorrhagiae (11.29 %) and Canicola(6.45 %)	Rajan *et al*., 2017
South Andaman	427	MAT	42.15%	*L. hebdomadis* (14.1%), L. icterohaemorrhagiae (12.9%), *L. lai* like (11.9%), *L. australis* (10.8%), *L. grippotyphosa* (5.6%), *L. pomona* (4.9%), *L. hardjo* (4.7%), *L. canicola* (3.5%), *L. pyrogenes* (2.8%) and *L.autumnalis* (0.5%) respectively	Sunder, 2014
South Andaman	108	MAT	69.44%	Automatic (53.70%) followed by Sejroe (28.70%) and Hardjo (22.22%).	Mitra *et al*., 2015
South Gujarat Region	676	MAT and Real-time PCR	29% and 12%	*L. Ballum* (19%), *L. Autumnalis* (18%), *L.Icterohaemorrhagea* and *L.Hardjo* (8%), *L. Pomona* (7%), *L. Hebdomadis* (7%), *L.Canicola* (7%), *L. Australis*(5%), *L. Pyrogen* (4%) and *L. Batavia* (4%)	Panwala and Mulla, 2015
Bihar	450	DAS-ELISA	9.11%	*L. Hardjo*	Pandian *et al*., 2015
Odisha and West Bengal	350	MAT and rLipL32 ELISA	50.85% and 56%	Icterohaemorrhagiae (67.98%), Hebdomadis (33.14%), Grippotyphosa (29.21%), Hardjo (25.84%), Australis (13.48%) and Pomona (3.37%)	Behera *et al*., 2014

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Place / States	Sample size	Test used	Sero-prevalence	Serovars	References
South Gujarat	398	MAT	12.81%	Pomona (28.89%), Hardjo (15.56%), Canicola (12.22%), Patoc (5.56%), Icterohemorrhagiae (5.56%), Hebdomadis (5.56%), Pyogenes (4.44%), Bellum (4.44%), Bataviae (4.44%), Autumnalis/Bankinang (3.33%), Australis (2.22%), Hurstbridge (2.22%), Javanica (2.22%), Grippotyphosa (1.11%), Shermani (1.11%) and Kaup (1.11%)	Patel *et al*., 2014
Andhra Pradesh	1499	MAT	19.1%	Autumnalis (19.29%), Canicola (9.12%),Grippotyphosa (26.31%), Hebdomedis (4.21%), Hardjo (12.98%), Ictero (4.91%) and Javanic (12.63%)	Rani Prameela *et al*., 2013
Odisha	120	MAT	42.5%	*L. Australis* (50.9%) and *L. Hardjo* (23.5%)	Balamurugan *et al*., 2013
Gujarat state	544	MAT	34.74%	Hardjo (47.69%), Hebdomadis (36.31%), Ballum (10.15%), Australis (5.54%) and Pomona (0.31%)	Balakrishnan *et al*., 2011
Andaman and Nicobar Islands	124	MAT	40.32%	Grippotyphosa (31.6%), Australis (15.8%), whereas in Nicobar district Australis(58.3%) and Sejroe (33.3%)	Sharma *et al*., 2003

produced by *Leptospira* causes either renal failure or interstitial nephritis (Chin *et al.*, 2019). Leptospires may be able to avoid phagocytes in the bloodstream by causing macrophage apoptosis. Generally, the incubation period of leptospirosis varies from 3 to 7 days. In acute form of leptospirosis, after penetrating the skin or mucosa, the organisms grow in the liver and travel to the peripheral circulation, where they can be isolated for several days until the fever goes away. Serum antibodies start to emerge at this point, and organisms can be identified in the urine.

Despite the numerous *Leptospira* serovars and host species, the main phases in the disease development are the same in all host/serovar combinations. Months to years after initial infection, Leptospira organisms are shed in the vaginal and urinary secretions of chronically infected animals, and these animals serve as an important reservoir of infection, with the potential to transmit the infection to other incidental hosts or reservoir hosts.

Clinical symptoms and signs

The clinical manifestations of leptospirosis vary with the pathogenicity of the infecting serovars, host susceptibility, age and physiological condition of the animal. The majority of leptospiral infections are asymptomatic, particularly in non-lactating and non-pregnant animals, which can be detected at slaughter by interstitial nephritic lesions.

Accidental hosts are most usually connected with acute or subacute leptospirosis, while subacute infection is less severe (Adugna, 2016). In calves infected with accidental serovars, notably serovar Pomona, severe acute illness occurs seldom. Clinical signs include a high fever ranging from 103°F to 107°F, hemolytic anaemia, haemoglobinuria, jaundice, lung congestion, meningitis and death. Other body systems may be impacted, leading to clinical issues such as uveitis, pancreatitis, haemorrhage, muscle pain or respiratory disease. When bovines are

infected with host-adapted serovars, clinical symptoms are frequently minor or non-existent. Clinical symptoms might range from mild to severe in infections caused by non-host-adapted serovars. In lactating cows, milk production has significantly diminished, and the discharge is reddish in colour or contains blood clots, with the udder limp and floppy (Radostits *et al*., 2009). Abortion owing to a systemic response in adult cattle can occur during the acute stage of the disease. Infection with *Leptospira* serovars Pomona and Hardjo causes mastitis and a drop in milk output in nursing animals (Thiermann and Garrett, 1983).

Most typically associated with serovars Hardjo and Pomona, the chronic form of infections and clinical indications of the disease causes foetal infection in pregnant cows, resulting in abortion, stillbirth, or the birth of premature and weak infected calves. In persistent infections, the host-adapted serovars can cause chronic interstitial nephritis, impaired milk output and poor development (Adugna, 2016).

Diagnosis

The history of the disease, vaccination, and laboratory tests are commonly used to diagnose leptospirosis. The most prevalent method for diagnosing leptospirosis in animals is to use serologic assays. The diagnostic techniques for leptospirosis such as the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA) are used to detect antibodies against the pathogen. The organism can be detected in tissues or bodily fluids by culture isolation. During a period of evident fever, organisms can be identified from the blood of cattle. Leptospirosis can also be diagnosed by detecting the antigen and isolating the bacterium (Levett, 2001). Most reliable and simple diagnostic tests for leptospirosis in both humans and animals are still lacking to use.

Microscopic agglutination tests (MAT): According to OIE (2013), serological MAT is the gold standard for diagnosing leptospirosis. The most common serological test for diagnosis of leptospirosis is the MAT using live antigen and it can measures both IgM and IgG antibodies. For maximum sensitivity, it should use antigens representative of all known serogroups in the region where the animals are located and, preferably, strains representing all known serogroups. But the specificity of the MAT is quite good because antibodies against other bacteria normally do not cross-react with *Leptospira* to a substantial level. MAT is quite helpful in determining the acute or severity of an infection. When compared to a control culture diluted 1/2 in phosphate buffered saline, the endpoint is defined as the serum dilution that results in 50% agglutination and 50% free cells. A titre of 1/100 is considered as positive, however due to the MAT's high specificity, lesser titres can be interpreted as indication of earlier *Leptospira* exposure. The MAT has the drawbacks of being time-consuming and requiring a live culture, both of which offer a risk of human infection. Because the titres after vaccination and those after spontaneous infection may be of equal magnitude, the test is unable to distinguish between them. Cross-reactivity of antibodies is also a problem (da Silva Pinto *et al*., 2016).

Enzyme-linked immunosorbent assays: ELISA detecting anti-leptospiral antibodies have been created employing a variety of antigen preparations, assay procedures, and assay platforms, such as plate tests and dipstick tests. By employing antigen from whole-cell *Leptospira* or outer membrane protein, ELISA, has been developed to circumvent the difficulty found in MAT (OIE, 2013). Validation issues are a key stumbling block in evaluating most ELISAs. Almost all have been tested against the MAT (using MAT titres of 1/100 or above), which is a flawed test with a sensitivity of less than 50% in some chronic infections. The ELISA test is very sensitive and can quantify the amounts of IgM and IgG antibodies in serum (Budihal and Perwez, 2014). The use

of dead antigen and the capacity to assess distinct immunoglobulin classes are two of the test's advantages. ELISA can detect recent leptospires infection in cattle based on the detection of IgM. MAT is less sensitive and selective than ELISA-IgG (O "Keefe, 2002; Patel *et al*., 2017).

Isolation and identification of *Leptospira*: Isolation of the bacteria is considered as the most specific methods of confirming the presence of *Leptospira* in tissues and urines if the proper pH is maintained. The Ellinghausen-McCullough-Johnson-Harris (EMJH) medium is most widely used for *Leptospira* cultivation. Leptospiral culture can be carried out in a semisolid or liquid (0.1–0.2% agar) medium containing BSA and either Tween 80 or a combination of both Tween 80 and Tween 40. In semisolid culture media, adding 0.4–5% rabbit serum improves the odds of isolating fastidious leptospiral serovars. Samples should be incubated at 29±1°C for at least 16 weeks and preferably for 26 weeks. The amount of time taken to discover a positive culture depends on the number of organisms and the type of leptospiral serovar in the sample.

Immunochemical staining technique: Leptospires can also be detected in blood, tissues or urine sediment using a range of immunochemical staining techniques such as immune-histochemical and immunofluorescence techniques for a quick diagnosis (Barnett *et al*., 1999). Immunohistochemistry can be used to detect leptospires in formalin-fixed tissue, although its sensitivity varies due to the small number of organisms present in various tissues.

Dark-field microscopy (DFM): DFM is a cost-effective and quick technique, however it is not regarded sensitive because it requires at least 10 leptospires per millilitre to be seen. Direct microscopic inspection of clinical specimens can reveal leptospires. DFM of body fluids such as blood, urine, CSF, and dialysate fluid, as well as tissues from carcasses or abortion products, can be used to quickly determine the presence of leptospires. This method has advantages in terms of early detection but it has low sensitivity and specificity, and offers no information on the infecting serovar, despite its utility in settings where laboratory resources are restricted. A variety of staining approaches, including immunofluorescence staining, have been utilised to boost the sensitivity of direct microscopic inspection of leptospires in veterinary specimens. The *Leptospira* organism can be recognised and identified in the clinical sample using a dark field microscope after silver staining or Within-Starry staining. Immunostaining, which requires the use of a primary antibody specific for the serovar, can be employed to boost the procedure's specificity (Budihal and Perwez, 2014).

LEPTO Dipstick assay: The LEPTO Dipstick assay is a recently developed test for the diagnosis of leptospirosis that detects IgM antibodies using a widely reactive antigen. The test was validated using 867 serum samples from known cases of leptospirosis and controls in the Andaman and Nicobar Islands. The test exhibited a high level of agreement with the usual paired microscopic agglutination assays for diagnosis. In serum samples from known cases of leptospirosis in the Andaman and Nicobar Islands, the effectiveness of this test was compared to that of an IgM ELISA. The assay had a 91.0% positive predictive value, with a sensitivity of 78.7% and specificity of 88.3% (Sehgal *et al*., 1999).

Molecular methods: A number of PCR procedures are available for detecting the organisms. Pathogenic leptospires can be detected in blood, tissue or urine samples using PCR techniques, but the infectious serovar cannot be determined. A molecular method for detecting pathogenic leptospiral infection in bovines had been developed by concurrently targeting the outer membrane proteins LipL32 and LipL21 in a multiplex PCR (Meenambigai *et al*., 2011). The only way to definitively identify the infectious

serovar is to culture blood, urine, or tissue specimens.

Zoonotic risk of leptospirosis

Leptospirosis has become a major infectious illness in human medicine over the last few decades. It is a major zoonotic disease all over the world. People are susceptible to infection with the majority of *Leptospira* pathogenic serovars, but they are only accidental hosts and hence are not important infection reservoirs. Leptospirosis is a serious zoonotic disease that poses a risk to veterinary doctors, veterinary employees, livestock producers, mine workers, fish workers, and dairy workers etc. Contamination with contaminated urine or uterine contents is the most common way for humans to become sick. Although Leptospirae can be found in cow's milk for a few days during a fever's peak, the bacteria do not survive long in milk and are destroyed by pasteurisation. Farm workers who milk cows, are particularly vulnerable to serovars hardjo infections. The major route of infection is through mucosal membrane contact with infectious body fluids (blood in acute cases or urine in chronic infections).

Prevention and control

Understanding the epidemiological characteristics of leptospirosis is an important step in developing treatments to reduce the likelihood of disease transmission. Many stages in the leptospirosis transmission cycle can be targeted by intervention measures. Some of the treatments for reducing the risk of leptospirosis transmission include rodent management, particularly with slow-acting rodenticides, and enhanced environmental hygiene to prevent the likelihood of water, soil, and food contamination. Because rodents, raccoons, opossums, and skunks are abundant in rural and urban areas, it's difficult to prevent contact with free-ranging wildlife and domestic animals that could be *Leptospira* maintenance hosts. Despite the fact that there is little that can be done in wild animals, leptospirosis in domestic animals can be controlled through vaccination with inactivated whole cells or an outer membrane preparation.Vaccination with polyvalent inactivated vaccines is the cornerstone of leptospirosis prophylaxis. Although several vaccines have been demonstrated to greatly reduce renal colonisation and urine shedding, leptospiral vaccines are primarily created and assessed for their ability to prevent clinical indications of disease. Occupational hygiene is crucial for preventing human leptospirosis, which includes wearing waterproof shoes and gloves in sewers, farmers, and other high-risk populations. Mass awareness or public education is of utmost importance. Streptomycin is sometimes added to the semen of bulls kept in artificial insemination centres as a preventive measure.

Conclusion

Leptospirosis is still a major public health issue that primarily affects people in their working areas. The incidence of leptospiral infection in the animal population is crucial for developing leptospiral disease prevention and control programmes. A well-designed multicentric study conducted in several geographic places could provide more information about the epidemiology of leptospirosis.

Conflict of interest: Authors have no conflict of interest in this study.

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Received – 01.07.2021, Accepted – 13.10.2021, Published – 17.11.2021 (Online), 01.12.2021 (Print)
Section Editor: Dr. I. Samanta, Associate Editor