

Review Article

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## A Review on Bovine Leptospirosis with Special Reference to Seroprevalence in India

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### ABSTRACT

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Leptospirosis, a spirochaetal zoonotic disease, has been recognized as an important emerging infectious disease. *Leptospira* spp in bovine associated with infertility, abortion and reduced milk production. Recent seroepidemiology of leptospirosis in bovine population by various workers in India suggest the upsurge in circulating antibodies against leptospira and emergence of new serovar. Present review addresses the issues in the epidemiology with special reference to India, clinical manifestations, diagnosis and management of leptospirosis in bovines.

### Introduction

Leptospirosis has been recognized as an (re-)emerging global public health problem occurs in cattle, buffaloes, pig, sheep, goat, dog, horse, etc. Disease is zoonotic in nature, communicable from man to animals and *vice versa* (Adugna, 2016).

Disease in bovine usually occur in a milder form and responsible for direct or indirect loss in term of abortion and loss of milk

production. In India leptospirosis in cattle was first reported by Adinarayan *et al.*, (1960). Since then several reports emerged confirming the prevalence of leptospirosis in bovines from different states of India (Balamurugan *et al.*, 2018; Balamurugan *et al.*, 2016; Mitra *et al.*, 2015; Patel *et al.*, 2014; Blessy *et al.*, 2017; Patel *et al.*, 2017). Extreme weather events such as cyclones and floods, increased rainfall associated with global warming are considered as the factors for the increased incidence of leptospirosis (Lau *et al.*, 2010).

## Organism

Leptospirosis is caused by the pathogenic spirochetes of the genus *Leptospira* (Adugna, 2016). Organism is usually about 6-25 µm long and 0.1 to 0.2 µm in diameter, highly motile, obligate aerobes. Two axial periplasmic flagella are located in the periplasmic space (Levett, 2001). Term 'Leptospira' was first proposed by Noguchi (1917) based on its thin spiral shape. Organism give optimum growth at a temperature of 28 to 30 °C and at pH range 6.8 to 7.4 and usually survived in a moist environment of soils, mud, swamps, streams and rivers. Antigenic structure of bacterium consists of somatic antigens, surface antigen, outer membrane lipopolysaccharides, and flagellar antigens. The outer membrane lipopolysaccharide is the major antigen is a potent immunogen and responsible for serovar specificity. As per phenotypic classification there are two species namely, the *L. interrogans* (pathogenic) and the *L. biflexa* (non-pathogenic). Pathogenic *Leptospira* spp. classified into 28 serogroups and over 300 serovars based on LPS antigen (Saito *et al.*, 2013). While the genetic homology in DNA hybridization have been identified 20 species of *Leptospira* spp. which includes 9 pathogenic, 5 Intermediate and 6 saprophytic species (Smythe *et al.*, 2013). The host-adapted serovars of serogroup Sejroe, mainly serovar Hardjo affects bovines (Mughini-Gras *et al.*, 2014).

## Epidemiology of leptospirosis

Leptospirosis is worldwide distribution and responsible for one of the major cause of reproductive failure in bovine. Disease is more predominate in tropical countries having high rain fall and humidity and, presence of marshy land and paddy grown area (Favero *et al.*, 2017). The urine of infected animals or healthy carriers, aborted fetus and uterine

discharges which may contaminate soil, pasture, drinking water and feed is the main source of infection (Adugna, 2016). Leptospiral organisms enter into the body most often through cutaneous or mucosal abrasions. Oral transmission may occur when animals are feeding on contaminated pasture or feedstuffs, or drinking contaminated water. The semen of an infected bull may transmit the disease during natural or artificial insemination (Kiktenko, 1976). Warm and humid climate, slightly alkaline soil, presence of organic matter, existence of animals reservoir hosts like rodents, foxes, jacked, raccoon, wild cat etc mainly influence the disease incidence (Radostits *et al.*, 2009). Leptospire persistently present in proximal renal tubules after recovery and excrete intermittently in the environment for months to years (Adugna, 2016). Even organism can persistent in the mammary gland and excrete in milk after recovery (Thiermann, 1982). Hardjo serovar, is mainly associated with cattle however, serovars Pomona, Icterohaemorrhagiae Hebdomadis, Australis, Bankiang and Grippotyphosa can also be associated to bovine leptospirosis (Balamurugan *et al.*, 2018). Infection in cattle can be divided into two groups of strain; strain adapted to bovine and strain adapted to other domestic and wild animal responsible for incidental infection. Animals are infected through direct contact with animals, animal tissues, body fluids (especially urine), or through indirect contact with contaminated environment (Favero *et al.*, 2017). Exotic pure breeds are more susceptible than indigenous pure breeds and cross breeds (Balakrishnan *et al.*, 2011). In India leptospirosis has been reported from various states by different workers.

## Clinical features

Clinical manifestation of leptospirosis depends on type of serovar, infectious dose, and

virulence of serovar. It also depends on age, health and immunological status of the host. In bovines, clinical signs are usually mild or inapparent when infected by host-adapted serovars. However, for infections with non-host-adapted serovars, clinical symptoms may range from mild to severe (Lilenbaum and Martins, 2014).

### **Acute form**

Acute leptospirosis in these animals is infrequent and has been associated mostly to incidental serovars such as Pomona, Grippotyphosa, and Icterohaemorrhagiae. Calves up to one month of age are generally affected with this form. The disease is characterized by high fever (40.5°C to 41.5°C), septicaemia, anorexia, petechiae on mucosa, acute haemolytic anaemia, haemoglobinuria, jaundice, and decrease milk production in adult (Radostits *et al.*, 2009).

Ulceration on the cutaneous and mucous surfaces, subcutaneous oedema, gastroenteritis and cherry red coloured urine are also common finding. *Leptospira interrogans* serovar Hardjo and Pomona infection in lactating animals result in mastitis with sudden reduction in milk production (Thiermann, 1982). Abortion, stillbirth, premature birth of weak calf, congenital infection, may occur in pregnant animals (Radostits *et al.*, 2009).

### **Subacute form**

In subacute form animals show clinical expressions as acute but for lesser severity (Adugna, 2016).

### **Chronic form**

Chronic infections usually involves infection by host-adapted serovars, which can lead to chronic interstitial nephritis, poor reproductive performance, abortion, stillbirth, weak

offspring, reduced milk production, and poor growth (Adugna, 2016).

### **Diagnosis**

Diagnosis is usually based on the history of disease and laboratory confirmations. Standard tests, such as culture isolation of the organism from clinical specimens and the microscopic agglutination test (MAT) are generally employed for diagnosis of infection in most of laboratory due to high specificity. Robust and easy to use diagnostic tests are still lacking for diagnosis of leptospirosis in both humans and animals.

### **Dark field microscopy**

Leptospira organism can be visualized and identified in the clinical sample by the use of dark field microscope after silver staining or Wrthin-Starry staining. Immunostaining which requires a primary antibody specific for the serovar can be used to increase the specificity of procedure (Suman and Khalid, 2014)

### **Isolation and identification of organism**

Leptospira organisms could be isolated from body fluids, mainly urine, tissue from dead animals such as kidney, liver, lungs, brain ((Suman and Khalid, 2014). In suspected abortion cases the agent can be isolated from abortion materials or tissue samples from a freshly aborted fetus kidney, liver and lungs (Monte *et al.*, 2015). Presence of organism in internal organ of fetus indicates maternal infection. But due to the low level of recovery, it is not used as a routine diagnostic method. Liquid and semisolid Ellinghausen-McCullough-Johnson-Harris (EMJH) media commonly employed for isolation purposes that are enriched with 1.0% rabbit serum and bovine serum albumin and supplemented with 5-fluorouracil to prevent saprophytic leptospira growth.

### Seroprevalence of bovine leptospirosis in India

States	Sero prevalence	Test used	Sample size	Serovars	References
Odisha	42.5%	Microscopic agglutination test (MAT)	120	L. Australis (50.9%); L. Hardjo (23.5%)	Balamurugan <i>et al.</i> , 2013
Nine districts of Bihar	9.11%	DAS-ELISA	450	L. Hardjo	Pandian <i>et al.</i> , 2015
Four districts of South Gujarat	5.77 %	I-ELISA kit	398	L. Hardjo	Patel <i>et al.</i> , 2017
Pondicherry	36.4 % and 24.8 %	I-ELISA and MAT respectively	250	L. Hardajo (49%); L. Grippotyphosa (24.19%); Pomona (16%)	Blessy <i>et al.</i> , 2017
South Gujarat districts	12.81%	MAT	398	Pomona (28.89%)	Patel <i>et al.</i> , 2014
South Andaman	69.44%	MAT	108	Automnalis (53.70%) Sejroe(28.70%) and Hardjo (22.22%)	Mitra <i>et al.</i> , 2015
Kokan region Maharashtra	41.04 %	MAT	575	Australis (23.61%) Hardjo (19.44%), Hebdomadis (16.67%), Bankinang (15.28%), Icterohaemorrhagiae (14.58%)	Balamurugan <i>et al.</i> , 2016
Maharashtra, Gujarat, Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Punjab, Haryana, Chhattisgarh, Sikkim and Uttarakhand	70.51%	MAT	373	Hardjo (27.76%), Pyrogenes (18.63%), Canicola and Javanica (17.49%), Hebdomadis (17.11%), Shermani and Panama (16.73%), Djasiman (16.35%)	Balamurugan <i>et al.</i> , 2018

Antimicrobial agents should also be added to inhibit the growth of contaminants, but allow the multiplication of *Leptospira* spp. *Leptospira* grow beneath the surface of the semisolid medium and form a discrete zone, known as a Dinger's ring.

#### Microscopic Agglutination Test (MAT)

Test is the most widely used for diagnosis and epidemiological surveillance in India and worldwide. More confirmative diagnosis requires the use of both acute and convalescent sera, because of the frequent low

or possibly negative MAT titres in animals recently infected with *L. Hardjo*. Cross-reactive antibodies caused by exposure to leptospires of the same serogroup also common.

The MAT has the disadvantages that it is tedious and time consuming and requires the live culture which imposes a risk of human infection. Test is also unable to differentiate between titres after vaccination and those after natural infection, since the titres may be of similar magnitude (Pinto *et al.*, 2016).

### **Enzyme-Linked Immunosorbent Assay (ELISA)**

The ELISA test is highly sensitive, easy to perform and can measure IgM and IgG antibody levels in serum (Suman and Khalid, 2014). Advantages of test include uses of killed antigen and ability to measure different immunoglobulin classes. ELISA based on detection of IgM is able to detect recent infection of leptospire in cattle. ELISA-IgG is more sensitive and specificity than MAT (O'Keefe, 2002; Patel *et al.*, 2017).

### **Direct or Indirect Fluorescent Antibody Test (FAT)**

The FAT is a very useful technique for demonstrating leptospire antigen in tissues from infected animals and aborted fetuses material (Dhaliwal *et al.*, 1996). FAT is particular important to demonstrating the presence of leptospire in autolysed materials (Smith *et al.*, 1966). It can also employ in urine sample or cultured samples for fast and accurate diagnosis (Rajeev *et al.*, 2010).

### **Restriction Endonuclease Analysis (REA) and Pulsed-Field Gel Electrophoresis (PFGE)**

REA can be used to type leptospire that are serologically undistinguished. In this technique, a large number of fragments are generated after restriction with an endonuclease enzyme such as EcoRI, HhaI, or HindIII (Eljalii and Ali, 2006). Recently, pulsed-field gel electrophoresis (PFGE) has also been introduced for identification and an epidemiological study of leptospiral isolates (Galloway and Levett, 2008).

### ***In situ* DNA hybridisation**

It is rapid, specific and sensitive means for diagnosis of leptospirosis (De Brito *et al.*, 2006). Le Febvre, (1987) described a DNA

probe that could identify *L. Hardjobovis* in cattle. DNA hybridisation was more sensitive than either FAT or culture techniques for the diagnosis of bovine leptospirosis (Herrmann, 1993).

### **Genome specific amplification and identification**

The PCR is a rapid, reliable and sensitive test for the diagnosis of leptospirosis. The application of the PCR for the diagnosis of leptospirosis has been reported by different workers based on specific gene targets, most frequently 16S or 23S rRNA genes (Merien *et al.*, 1992) and repetitive elements (Zuerner *et al.*, 1995). A nested PCR with primers derived from the LipL32 sequence has been reported by Nassi *et al.*, (2003). The product obtain after amplification were subjected to REA and the profiles correlated well with phylogenetic relationships between these serovars. The main concern with PCR assay is the false-positive results which are caused by contamination with previously amplified DNA or target DNA.

### **Prevention and control**

Various control measures include that minimize the accidental infection of cattle by carrier and reservoir hosts, and minimize spread of infection within a herd by vaccination or combined vaccination with aim to prevent reproductive losses, renal colonization and urinary shedding of organism (Martins and Lilenbaum, 2017). Cattle should be separated from pigs and sheep, and should not allow for gazing near ponds and marshes. Vaccination of susceptible animals is most economically way to control disease in large organized farm. Inactivated vaccine is available for commercial use in some countries. It requires booster immunization either annually or 6 month interval to raise protective immunity depending upon herd structure and risk of

exposure (Bolin, 2003; Wang and Węgrzyn, 2007). Regular screening of bulls is also important and if they are infected they should not be used naturally or for artificial insemination. Quarantine and screening of newly introduced animals is important before allowing them to enter the herd. Damp areas near the farm should be drained or fenced and use suitable disinfection in farm. Since rats and other wild animals may act as a source of infection, contact between them and farm animals should be controlled by using rat bait and fencing of farm (Martins and Lilenbaum, 2017).

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