Epidemiological Status of Leptospirosis in India

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Abstract

A corkscrew-shaped spirochaete named \textit{Leptospira interrogans} causes the infectious disease called leptospirosis. Leptospirosis, a growing public health concern worldwide, from subclinical infections to potentially lethal pulmonary hemorrhage. The disease caused by the pathogenic Leptospira, poses a threat to both humans and animals, and its transmission occurs through contact with infected animals, contaminated water, and soil. The prevalence of leptospirosis is influenced by various factors, including climate, urban development, and animal-rearing practices. It can manifest with severe symptoms in humans, making early diagnosis crucial. Diagnostic methods like microscopic agglutination test (MAT) and enzyme-linked immunoassay (ELISA) are widely used for the screening of leptospira infection. Molecular technique like PCR and qPCR offering higher sensitivity and rapidity. This paper meta-analyses the incidence of leptospirosis in various animals based on the literature published from 2005 to 2023 and provides prevalence of the disease in various animal including humans. Results suggest the significance prevalence of the disease in humans and various animal species, namely buffalo, rodents, and dogs. Coastal regions in India were particularly vulnerable to the disease. Efforts to control leptospirosis include surveillance programs and public health initiatives. Understanding the epidemiology and prevalence of leptospirosis, as highlighted in this paper, is essential for implementing effective preventive measures. Finally, a continued research, diagnostic advancements, public awareness campaigns and addressing research gaps in epidemiology of the disease are critical in mitigating the impact on human and animal health. This review provides important data for public health authorities, veterinarians, scientists and for the public, in general.

Keywords: Leptospirosis, Risk Factor, Prevalence, Diagnosis, Zoonotic Disease

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Citation: Choudhary S, Choudhary RK, Kumar M, Singh S, Malik YS. Epidemiological status of Leptospirosis in India. J Pure Appl Microbiol. 2023;17(4):1968-1977. doi: 10.22207/JPAM.17.4.44

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INTRODUCTION

Leptospirosis is becoming a significant concern for public health, causing subclinical infections to lethal pulmonary hemorrhage. The disease is widely distributed and occurs both in developed and developing countries in suitable habitat where the conditions are suitable for the growth of causative organisms. Incidences of the presence of pathogenic leptospires have been reported in a variety of domestic or pet animals, such as dogs, cattle, horses, rats, and pigs and also in several wildlife animals including coyotes, sea lions, raccoons, opossums, bats, white-tailed deer and surprisingly in frogs and caimans. Rats, canines, buffaloes, and cattle are the hosts for leptospirosis.

Humans or animals are infected accidentally by direct or indirect contact with leptospira infected animals or contaminated water or urine. Infection is also possible by other methods, like handling clinically infected animal tissues, inhalation of droplets of urine, and consuming contaminated food and water. Rodents, especially the common rat (Rattus norvegicus) is the most common host. Certain serovars are linked with particular animal hosts e.g., Icterohemorrhagiae with rats, canicola in dogs, and Hardjo in cattle. In humans, the early stages of leptospirosis are characterized by high fever, headaches, jaundice, and mucosal hemorrhages.

In humans, the early stages of leptospirosis are characterized by high fever, headaches, jaundice, and mucosal hemorrhages. Other essential factors responsible for the rise in cases are natural calamities like heavy rainfall, unhygienic cattle-rearing practices, and unplanned development of cities. Pathogenic strains of leptospires can survive in soil and water, indicating their survivability outside the host. For the survival of the leptospires, moisture is an essential key factor in the environment. Recent estimates show the annual incidence of leptospirosis worldwide is around one million cases and contributes to around 60,000 deaths. MAT and ELISA are widely used tests, of which the microscopic agglutination test (MAT) is considered the most authentic.

For the clinical detection of leptospirosis, molecular diagnostic methods are more popular as they are more sensitive and rapid than other methods, such as culture. Also, these techniques are less time-consuming and are more accurate than the conventional serological test, making the detection of organisms right in the samples in a short time. Among molecular biology techniques, real-time polymerase chain reaction (qPCR) is advantageous. It is more sensitive in diagnosing various infectious diseases compared to conventional PCR assays.

Etiology

The causative agent of leptospirosis is the spirochaete, which belongs to the order Leptospirales, family Leptospiraceae, and genus Leptospira containing 82 species. Based on their pathogenicity, the genus is divided into pathogenic and non-pathogenic strains. The differentiation of various serovars is due to the difference in agglutination reaction in MAT. Furthermore, 60 serovars for *L. biflexa* and above 300 serovars for *L. interrogans* have been reported.

Leptospires are aerobic and motile, and they can sustain alkaline pH but cannot survive in the hot and dry environment. The bacteria are Gram-negative. In addition to Gram staining, silver impregnation staining and immunofluorescence are used, followed by visualization using a dark field microscope. Ellinghausen-McCullough-Johnson-Harris (EMJH) medium is the most popular medium for the culture of *Leptospira*.

As the pathogenic and non-pathogenic strains cannot be differentiated based on their morphology, a few tests, like testing in vivo pathogenicity and detection of outer membrane proteins, are done. Outer membrane proteins and other virulence factors have been characterized well in the past.

Epidemiology

Leptospirosis outbreaks have been reported globally. Human infections are rampant in tropical regions with high rainfall, where the human population gets exposed to water contaminated with the urine of infected animals. People who live in underdeveloped nations with poor sanitation have a significant risk of contracting an infection through polluted water or soil. Leptospirosis is a serious issue in low-lying, densely inhabited parts of India that experience...
frequent flooding or water logging during the monsoon season. Farmers, sewage workers, butchers, and other occupational risk groups are most vulnerable to getting the illness. Swimming, sailing, and other water sports are among the leisure activities that are thought to increase the risk of contracting leptospirosis.

A recent meta-analysis in humans found that exposure to rain was a significant risk factor for leptospirosis and that the majority of cases were reported from the coastal zone and among farmers. 97% of patients had fever, and 35% of them had conjunctival suffusion (red conjunctiva). In 34% and 35% of the patients, the liver and kidney, respectively, were affected. The combined mortality from multiple studies was estimated to be 11%.30

Research gaps in epidemiology

In India, several research gaps exist that hampers understanding of epidemiology of leptospirosis in human and animals. Some of the key research gaps are;

• Zoonotic disease surveillance: Limited surveillance and data collection on zoonotic diseases that are infection of human and animals and vice versa.
• Antimicrobial resistance (AMR): Understanding of transmission dynamics of AMR in leptospirosa in human and animals are limited.
• Emerging infectious disease: Pathway of spillover from animal to human and factors that facilitate the events are poorly understood.
• Data Accessibility: Lack of data sharing facility and transparency hinders collaborative research and policy formulations.
• One health approach: Insufficient research on One Healthy Approach due to insufficient interdisciplinary research programs and efforts.

Addressing these research gaps in the epidemiology of diseases in India is essential for developing evidence-based strategies for disease prevention, control, and mitigation of leptospirosis in human and animals.

Clinical Manifestations

The symptoms of leptospirosis include fever, renal and hepatic insufficiency, pulmonary signs, and reproductive failure across human beings and domestic animals, primarily dogs, cattle, and pigs. Fever, jaundice, vomiting, diarrhea, intravascular disseminated coagulation, uremia followed by renal failure, hemorrhages, and death are possible symptoms of typical canine leptospirosis. Leptospirosis symptoms in cattle and pigs include reproductive failures, such as abortion, fetal mummification, weak new born, and agalactia.31 Humans may experience symptoms ranging from a mild, influenza-like illness to a severe infection with respiratory distress and renal and hepatic failure (Weil’s disease).

Risk factors and transmission

The cases of leptospirosis are higher in the tropical regions than in the temperate parts because leptospires can survive longer in warm and humid conditions. Countries where there are chances of exposure of the human population to infected animals have greater chances of infection in humans. The host range of Leptospira spp is diverse because of its capability to infect and survive in different hosts. It occurs in a wide variety of wild, domestic, and marine animals. Rodents are a primary reservoir host, while other animals like cattle, pigs, and dogs may also act as carriers and sources of infection to other animals or humans.32 Controlling rat infestation on farms and fencing to keep stray dogs out of the farm can be effective prevention strategies.33 In humans and animals, infections can occur by coming in contact with infected water and soil.34,35

Transmission of leptospirosis is either by direct contact with an infected animal or by indirect contact with soil or water contaminated with the urine of infected animals.17 Human-to-human transmission is rarely reported. Infected urine is the source of infection in humans and animals coming in contact. In humans, the risk factors associated with leptospirosis are mainly occupational groups, i.e., farmers, abattoir workers, veterinarians, rice field workers, and animal handlers, or recreational activities like swimming and hunting. Hence, it is also known as paddy field worker disease, mud fever, or sewer worker disease. For animals, the important risk factors include shared grazing with common water resources, purchase or introduction of infected
cattle, rodents in the farm, level of hygiene in milking and status of leptospiral vaccination, presence of other animals in the farms like dogs, sheep and goats, horse, pigs, and others (Figure 1). Leptospirosis can also be transmitted through the semen of infected animals.36

METHODOLOGY

Data source and study selection
The published data on leptospirosis was retrieved from all animals (including dogs, cows, buffalo, pigs, and goats) reported from India using PubMed and Google search engines. Terms like “leptospira”, “leptospirosis” and “dogs” or “cows”, or “buffaloes”, or “pigs” or “goats” were searched. We included data from 2005 to 2023, along with a list of authors, year of publication, geographic location of Indian states, sample type, sample size, methods of detection, and prevalence (any kind, including culture/sero/molecular/others) of Leptospira spp. During the initial screening of titles and abstracts, criteria were determined to include based on their suitability. Inclusion criteria for detail analysis was defined for each species. Otherwise, review articles, metanalysis, and case reports were excluded from this analysis. The full-text documents were assessed, and data were retrieved from the table or the abstract. Publications in other languages were excluded from this study. Microsoft Excel using was used with “Data Analysis” tool functions for the data analysis.

RESULTS

A meta-analysis of published data from India included in this study suggested the highest prevalence (%) of species-wise livestock leptospirosis in India (total data of 2005-2023)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sum of # of samples</th>
<th>Sum of Favorable</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>1176</td>
<td>538</td>
<td>45.75</td>
</tr>
<tr>
<td>Cow</td>
<td>8398</td>
<td>2038</td>
<td>24.26</td>
</tr>
<tr>
<td>Dog</td>
<td>1039</td>
<td>271</td>
<td>26.08</td>
</tr>
<tr>
<td>Goat</td>
<td>2900</td>
<td>563</td>
<td>19.42</td>
</tr>
<tr>
<td>Human</td>
<td>4394</td>
<td>416</td>
<td>9.47</td>
</tr>
<tr>
<td>Pig</td>
<td>177</td>
<td>28</td>
<td>15.80</td>
</tr>
<tr>
<td>Rodents</td>
<td>60</td>
<td>24</td>
<td>40.00</td>
</tr>
</tbody>
</table>

Figure 1. A flow diagram of transmission of Leptospira and organisms in humans and animals showing the zoonotic importance of the disease. Bacteria are shed into the water and soil from the mammalian reservoir host from where humans and animals (cattle, dogs, pigs, horses, and others) pick infection, and further shed organisms in the urine. Pathogen-containing urine further contaminates water and soil. Leptospirosis is considered an occupational health hazard because the farmers, personnel working in the slaughterhouse, veterinarians and other workers coming in close proximity to infected animals are prone to get the disease.
prevalence of leptospirosis in buffalo (45.75%), followed by rodents (40%), dogs (26%), cows (24.26%), goats (19.42%), and pigs (15.8%) (Table). Apart from the analysis of retrieved data, some of the recent reports are mentioned below.

A study was conducted using animal samples from several districts in the south Gujarat region that were tested for leptospirosis. A total of 151 (or around 11%) of the 1406 animal samples of goats, cows, buffalo, and bullocks tested positive. In cows, buffaloes, bullocks, and goats, the most prevalent serovars were Icterohemorrhagiae (22%), Patoc (58%), Hardjo (50%), and Autumonalis (50%), respectively. A recent study was conducted on a total of 300 cow samples from cases of mastitis, miscarriage, and recurrent breeding from both organized and unorganized farms in Jabalpur to check for leptospirosis. According to the findings, leptospirosis seroprevalence among suspected cases was 48.67%, and it was notably higher in the organized sector and with high seropositivity in older cattle.

In another recent study conducted on canines in Gujarat, 45 of 410 sera were found to be positive, showing a seroprevalence of 10.98%, with serovar Pyrogenes being the predominant serotype. According to MAT, ImmunoComb, and PCR, the total prevalence of leptospirosis was 16.59%.

On the basis of our meta-analysis, the prevalence of leptospirosis was found to be...
highest in Andhra Pradesh (64.71%), followed by Kerala (60.5%), Chhatisgarh (54.7%), and Odisha (52.26%). The lowest prevalence of the disease was found in Assam (0.9%) and Haryana (1.4 %) (Figure 2).

The prevalence % of leptospirosis in humans is 9.47% as seen in Table. This disease is most prevalent in coastal areas. The reported incidence of human leptospirosis state-wise in India is described below.

**Kerala**

Leptospirosis is common and endemic in most parts of Kerala. The Autumnalis, Australis and Icterohemorrhagiae, Canicola, Pomona, Shermani are the common serogroups. A study found that leptospirosis is also occupation-related, as it is common in oyster shell catchers (82%). There was an increase of 74 % in the monsoon and a sharp rise in cases in some areas where the disease was not found earlier, such as Kolenchery, a midland, and leptospirosis was rare before 1987. A post-flood outbreak of human leptospirosis in Kerala claimed about 70 lives in 2018.

**Andaman and Nicobar Islands**

Andaman and Nicobar Islands have reported leptospirosis since the early part of the 20th century and are endemic for leptospirosis. There have been reports of outbreaks of Andaman hemorrhagic fever (AHF) since 1988. The disease surveillance system recorded 544 cases and 93 deaths in the Andamans between 2000 and 2004, with 2002 having the highest incidence. Five hundred twenty-four cases of AHF were reported from 1988-97.

**Maharashtra**

Leptospirosis in Maharashtra has been regularly reported Karande et al. and Karande et al. In 2005, because of the large outbreak during monsoon season and floods, 2355 cases

![Figure 3](image.png)  
*Figure 3.* Various approaches to detect leptospires in the blood and urine of humans and animals. PCR is a rapid test for the detection of the nucleic acid of leptospires in blood and urine. In contrast, MAT is the standard gold method for the detection of antibodies in the serum. Isolation and identification of *Leptospira* species are done by the culture of samples (blood, urine, CSF).
and 167 deaths were reported. In Mumbai, during the course of ten days in July 2015, the Mumbai Corporation reported 15 leptospirosis fatalities after massive floods.47

**Gujarat**
The disease is endemic in Gujarat, with the highest number of cases in Valsad, Navsari, and Surat since 1994.48,49 Situations such as heavy rainfall, high water table, and clay soil in south Gujarat favor endemicity for Leptospirosis.42

**Tamil Nadu**
There have been reports of leptospirosis from Chennai since 1980s.50,51 To study leptospirosis, a laboratory at the Madras Medical College was established in 1994.42 In a study, a total of 2035 patient serum samples were tested for the presence of specific IgM antibodies using the Panbio Leptospira IgM ELISA kit to determine the seroprevalence of leptospirosis over a 10-year period from 2011 to 2021 in Chengalpattu district, Tamil Nadu, which revealed a prevalence rate of 9.14%.52

**Karnataka**
In Karnataka, 152 cases and 11 fatalities were reported in 2004 while 224 cases and 19 fatalities were reported in 2005.42 In another study from the state, leptospirosis was shown to be substantially connected with environmental factors, proximity to an open sewer, occupational factors, the existence of skin cuts, direct contact with mud while working, and the presence of rats or rodent habitats, according to a population-based case-control study in the Kodagu district.53

**Odisha**
In Odisha, 142 patients with febrile sickness and hemorrhagic symptoms were assessed during October and November 1999, following the cyclone. Incidences of disease shoot-up during the rainy season. When tested, 28 (19.2%) were found positive for leptospirosis by MAT and 6 were positive by PCR/culture.54

**Other States**
Data from other Indian states is reviewed as follows. The evaluation of acute febrile patients in Uttar Pradesh revealed that 7% had leptospirosis (25/346), and 17 of the 25 patients had jaundice.55 Similarly, in a study of 55 cases of leptospirosis in Hyderabad, 52% had renal failure, and 42% had jaundice. Apart from these, leptospirosis has also been reported in Rajasthan, Uttarakhand, Punjab, and Haryana.

Govt of India launched a pilot project on prevention and control of leptospirosis under the 11th five-year plan, which in the next five-year plan launched a scheme called “Prevention and Control of Leptospirosis.” In 2019, the National Center for Disease Control (NCDC) issued an advisory notice and preparatory checklist to control leptospirosis, especially during the outbreak in affected districts (https://ncdc.gov.in/showfile.php?lid=390).

**Diagnosis of leptospirosis**
Laboratory support is required for the diagnosis to find the serovar responsible for causing specific infection, a possible source of infection, location, and potential reservoir. The tests performed for leptospirosis are isolated by culture and PCR, while serological tests can be used for the detection of antibodies. For the confirmation of diagnosis, isolation of bacteria is the most definite way. However, blood culture takes much time and is not suitable for an early diagnosis, but it is considered valuable in patients who are at risk of losing life in the first week before developing antibodies. An advanced technique like PCR is promising, and it is sensitive and specific.56,57

The serological microscopic agglutination test (MAT) is the gold standard for the diagnosis. The main advantage of MAT is identifying serovar of epidemiological importance.58 Leptospires are cultured, and an agglutination reaction is carried out in laboratories to detect the antibodies. MAT is laborious and is not an economical method, and needs live bacterial antigen for the test. Because of these factors, MAT is conducted only in particularly designated labs.59 Various approaches to detect leptospirosis are presented in Figure 3.

**Leptospirosis research at IIT Guwahati**
Molecular characterization for a comprehensive understanding of the CRISPR/Cas system of *Leptospira* is being carried out at the Indian Institute of Technology, Guwahati. The long-term goal of the lab is to utilize the outer membrane proteins (OMPs) of the spirochete
to develop a rapid diagnostic assay and vaccine to control the loss incurred by the disease. The overall goal is to elucidate the role of Cas proteins (Cas 6/7/8) in CRISPR adaptive immunity of the spirochetes and develop a CRISPR-Cas, an emerging microbial adaptive immune defense system of microbes (including spirochetes) against the foreign genetic elements that were being transferred horizontally from viruses or plasmid. Recent studies (from the lab of Manish Kumar, the 3rd author) include transcriptional analysis of CRISPR 1-B array of *L. interrogans*, assembly of Cas7 subunit of *Leptospira*, and Cas2 protein of subtype I-C.

**Leptospirosis research at the vet versity**

The Guru Angad Dev Veterinary and Animal Sciences University started working on leptospirosis in 2018, collaborating with IIT Guwahati, a joint collaborative project funded by the Department of Biotechnology- North East Region (DBT-NER).

In 2018, DBT launched a DBT-Canine Research Center and Network, and university is a collaborating institution of this mega project. The overall goal of this mega project was focused on the development of canine diagnostics and vaccines, under which a lab on leptospirosis has been established at the College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University. Under this project, a dedicated Bio-safety lab for diagnosing leptospirosis has been set up for serological diagnosis. Detection of anti-leptospiral antibodies using the Microscopic agglutination test (MAT) and molecular detection by PCR is carried out, in which about 10 percent seropositivity was found in animals using microscopic agglutination test. The samples from various parts of the state are coming for the diagnosis of leptospirosis.

Only a few referral labs maintain the leptospiral serovars because there is no long-term preservation method, and it has to be continuously sub-cultured every week. There is a high risk of contamination and loss of culture. We maintain reference cultures obtained from ICAR-NIVEDI and seven reference serovars that are commonly prevalent in domestic species in India, i.e., Australis, Autumnalis, Canicola, Hardjo, Icterohaemorrhagiae, Pomona, and Pyrogenes are being maintained. These reference cultures are used as antigen panels in the MAT and positive control in the molecular detection of leptospires in samples by PCR.

**CONCLUSION AND FUTURE PROSPECTIVE**

Due to the wide distribution of the disease in domestic and wild animals, prevention and control of leptospirosis are difficult. Control of leptospirosis includes identification of infected and carrier animals, immunization through vaccination, suitable drainage systems, and disinfection of the contaminated source of water. In the future, the adoption of prevention strategies, immunization of susceptible populations using current or next-generation vaccines, and timely reporting of outbreaks could mitigate disease incidences.

**ACKNOWLEDGMENTS**

None.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

**FUNDING**

This study was supported by the Department of Biotechnology, New Delhi, India, (Ref no. No. BT/PR25083/NER/95/1002/2017) awarded to MK & RKC and the establishment of the Leptospira lab under the Canine Research Network Project at GADVASU, Ludhiana, India.

**DATA AVAILABILITY**

All datasets generated or analyzed during this study are included in the manuscript.

**ETHICS STATEMENT**

Not applicable.

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