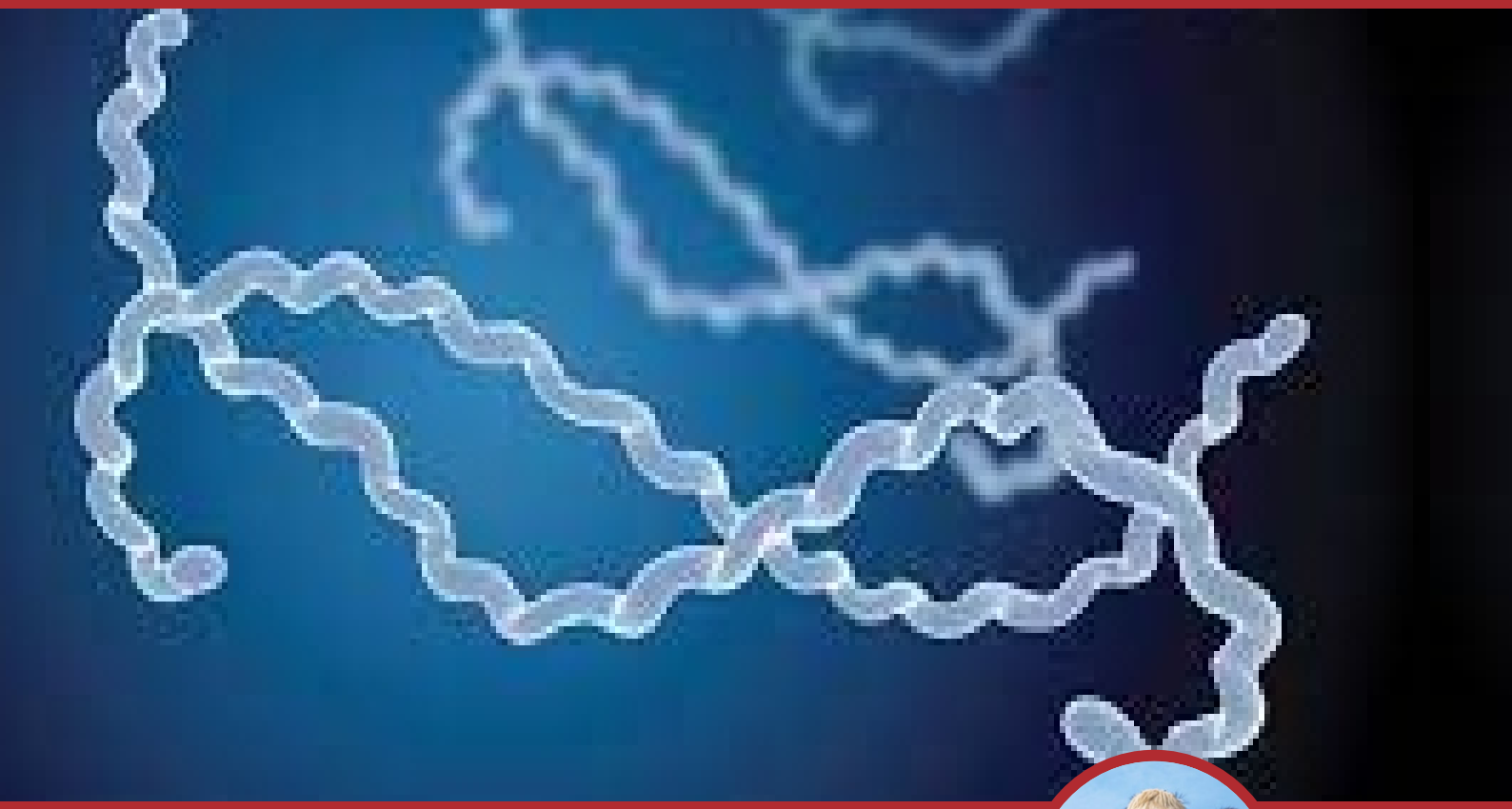
POLICY PAPER



Leptospirosis in Animals

- A Neglected Threat

Prevention, Control & Management

NATIONAL ACADEMY OF VETERINARY SCIENCES (INDIA)

&

GURU ANGAD DEV VETERINARY AND ANIMAL SCIENCES UNIVERSITY

LUDHIANA, PUNJAB

PREFACE

The bacterial illness leptospirosis can affect both humans and animals and, is caused by the spirochete of genus *Leptospira.* It is one of the most prevalent zoonotic causes of high morbidity and mortality across the globe – estimated 1.03 million cases among humans each year. Similarly, cases are reported in high number among animals from different parts of the world. Leptospirosis has the potential to become an epidemic, particularly following periods of intense rain or flooding.

Since the early 20th century, leptospirosis has been recognized as being an endemic disease in India, and outbreaks have grown during the last three decades. The coastal Indian states viz. Gujarat, Karnataka, Andhra Pradesh, Tamil Nadu, and Kerala, and the Andaman Islands have high prevalence rates of leptospirosis.

Vague symptoms, scarcity of quick point-of-care diagnostic tests and a low clinical suspicion among doctors, make early detection of leptospirosis difficult. Treatment options in terminal cases of disease are also limited. Clinical misdiagnosis is common, particularly among animals in tropical areas. Therefore, it is necessary to improve control and management practices for leptospirosis.

The present policy paper summarizes the national and global status of the disease, associated risk factors, transmission, currently available diagnostic methods and reference laboratories working on leptospirosis in the country. Further, it presents a comprehensive outline on prevention, control and management strategies along with recommendations for the policy makers and relevant stakeholders, which will help to tackle this menace at early stages of infection. The authors sincerely hope that this publication will help and guide the policy makers, health workers and other stakeholders to effectively address this problem.

Satparkash Singh
Yashpal Singh Malik

FOREWORD

I am extremely delighted that National Academy of Veterinary Sciences (I) in collaboration with Guru Angad Dev Veterinary and Animal Sciences University has come up with a very important policy paper on “Leptospirosis in Animals-A Neglected Threat: Prevention, Control & Management”. The paper highlights the epidemiology of the disease both nationally and globally along with its associated risk factors, diagnostic techniques and necessary control measures against the disease along with recommendations that can be implemented for its effective management. I express my gratitude towards all those individuals who have generously contributed in completion of such an important publication in the field of veterinary and medical sciences. The readily forthcoming collaboration from GADVASU has been very appreciable in the whole process. I am confident enough that the paper will put light on various aspects of the leptospirosis that will guide the policy makers and associated stakeholders directly or indirectly to make strategies for effective control of this disease in the country.

DVR Prakasha Rao
President
NAVS (India)

FOREWORD

Leptospirosis has long been considered as an important public health concern. It is the need of the hour to be aware about various aspects of this important yet neglected zoonotic disease so that preventive measures can be adopted in a timely manner. It is my pleasure to present this policy paper "Leptospirosis in Animals-A Neglected Threat: Prevention, Control & Management" as an official document published by National Academy of Veterinary Sciences (India), in collaboration with Guru Angad Dev Veterinary and Animal Sciences University. The paper highlights the updated information regarding disease's prompt management, preventive measures and control strategies. It also covers various important aspects of leptospirosis like risk factors, transmission, diagnosis, etc., which will heighten the awareness of the disease in public domain. In addition, various recommendations compiled in this document for prevention, control and management of leptospirosis are quite relevant to different stakeholders. I sincerely compliment the efforts of team from GADVASU and also thank the NAVS (India) for showing their trust in GADVASU for collaboration to bring out this important paper. I am extremely hopeful this document will be helpful to the stakeholders and policy makers to better understand and control this disease in an effective manner.

Inderjeet Singh
Vice Chancellor
GADVASU

FOREWORD

I am pleased to present this policy paper "Leptospirosis in Animals-A Neglected Threat: Prevention, Control & Management" as an official document published by National Academy of Veterinary Sciences (India), in collaboration with Guru Angad Dev Veterinary and Animal Sciences University. Leptospirosis has not only been found in India but also recognized as a re-emerging global public health problem due to the increased incidence in both developing and developed countries. This policy paper has meticulously explained about the disease's epidemiology including the national and international status, major risk and transmission factors and guidelines for the diagnosis apart from various methods and recommendations for its effective control and management to reduce the mortality and morbidity rate. I sincerely compliment the joint efforts of all the concerned contributors for the needful compilation and editing of this important policy paper which is need of the hour.

Praveen Malik
Commissioner Animal Husbandry
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CITATION	**:**	**NAVS 2022, Leptospirosis in Animals-A Neglected Threat. Policy Paper No. 8, National Academy of Veterinary Sciences (I), New Delhi, pages 1-28, ISBN: 9789394490413**

Published on behalf of
NATIONAL ACADEMY OF VETERINARY SCIENCES (INDIA)
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PREAMBLE

Leptospirosis, a lethal illness occurring all over the world, is a re-emerging infectious public health problem that has been getting increased attention of the researchers, policy makers and livestock owners as the cases are rising globally. It is a zoonotic disease caused by bacterium *Leptospira*–a pathogenic helical spirochete, is one of the most common zoonotic diseases, causing an estimated 58,900 fatalities annually. It spreads very fast, especially after periods of heavy rain.

Although disease is prevailing worldwide, it is more predominant in countries with humid subtropical and tropical climates. In India, leptospirosis outbreaks are most frequently reported from the coastal states and the Andaman Islands. The number of outbreaks has been rising in the recent past, may be due to availability of more diagnostic facilities and increased interest of researchers in this pathogen. Several risk factors predispose animals and humans to this disease, but the fundamental cause is the lack of knowledge and awareness about the illness in developing nations. Raising awareness can be a proactive measure to tackle this problem. Relevance of a guiding document encompassing upto date critical knowledge in all aspects of leptospirosis was immensely realized.

National Academy of Veterinary Sciences (India) in collaboration with Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, has attempted to come up with a policy paper in light of the importance of leptospirosis for public health on the basis of discussions with decision-makers, public health researchers, and prominent veterinarians. The range of topics covered in this document include leptospirosis and its causes, epidemiology, national and international status, risk factors, mode of transmission, diagnosis, prevention, and recommendations for the control of the disease.

CONTENTS

1. Introduction

Zoonotic diseases impact animal and human health both directly as well as indirectly. Their indirect consequences on human health are becoming a significant concern as more than two-thirds of emerging infectious diseases noticed in humans are zoonotic and involve in the transmission of infection from an animal to a human host.

Leptospirosis is a widely spread zoonotic infection caused by a pathogenic bacterium of genus *Leptospira* (Pappas et al., 2008). The oldest account of this disease is associated with paddy workers in China, thus naming it Rice-harvest fever (Faine, 1994). Leptospirosis, considered an occupational health hazard, is predominately observed in farm workers, abattoir personnel, veterinary doctors, pet traders, hunters, and other occupations such as rodent control program workers, involving frequent animal contact directly or indirectly (Hartskeerl et al., 2011; Musso and La Scola, 2013).

The disease is a global public health concern due to its rising occurrence in developed and developing nations (Vijayachari et al., 2008). The regions of the world reporting the maximum outbreaks are Latin America and the Caribbean (35.8%), followed by Southern Asia (12.9%) and North America (10.7%) (Munoz-Zanzi et al., 2020). In India, leptospirosis has been an endemic disease in the southern regions, with positivity of more than 25% compared to Northern India's 8.3%, and cases peaking from June to October, the monsoon months in India (Kamath et al., 2014). The Andaman Islands have the highest incidence of leptospirosis in India, with estimated 50–65 cases per 100,000 people yearly.

Members of the genus *Leptospira* are also termed leptospires. These are thin, spiral-shaped, and motile due to the presence of endo-flagella (Haake and Mastunaga, 2010). Though they are Gram-negative in nature, the unstained cells are visualized by dark-field or phase-contrast microscopy instead of bright-field microscopy due to their small diameter (Zuerner, 2010). The traditional serological classification based on agglutinating lipopolysaccharide antigens classifies *Leptospira* into 20 serogroups and over 300 serovars (Picardeau, 2013). The genetic, 16sRNA gene-based classification of *Leptospiraceae* members, leptospires are classified into three groups; (i) pathogenic, (ii) intermediate, and (iii) non-pathogenic (Levett, 2015). The main

pathogenic *Leptospira* species are *L. interrogans, L. kirschneri, L. weilii, L. borgpetersenii, L. santarosai, L. noguchii* and *L. alexanderi* (Ahmed et al., 2006). However, serological classification is usually more in practice.

The pathogen's capacity and adaptability to the changing environment are essential for its survival and potential to cause illness (Reis et al., 2008; Lau et al., 2010; Batchelor et al., 2012). Most mammals are carriers of bacteria and shed the organism from the proximal tubules of the kidney in urine, even post-recovery (Haake & Levett, 2015). After excretion, the bacteria can survive for weeks or more in water and moist soil (Trueba et al., 2004). The incubation period of leptospirosis is variable but usually 7-10 days. Major virulence factors of the leptospires are not very clear, but the outer membrane proteins are perceived to be essential for pathogenesis. The lipopolysaccharide (LPS) of the leptospires has low endotoxic potential than other Gram-negative bacteria. The major attributing factor for virulence and invasive nature is corkscrew-like motility of leptospires, which permits easy movement of the bacteria through connective tissue barriers.

The animal species commonly affected by leptospires include dogs, horses, cattle, sheep, and pigs (Khurana et al., 2016). Preference of *Leptospira* serovars to infect different host species was reported (Singh & Malik, 2022). The vulnerability to *Leptospira* infection varies among animals; for example, extensive range of leptospires can infect horses, while cats are rarely infected. The severity of the disease depends on the infecting serovar and the animal species being affected. However, chronic leptospirosis is the one that causes most of the clinical manifestations and, thus economic losses, especially in large animals.

Among farm animals, acute leptospirosis is seen in calves with fever, icterus, anemia, hemoglobinuria, and death in some instances (Radostits et al., 2007). Severe complications such as renal and liver failure, pulmonary haemorrhage, meningitis, and myocarditis have also been documented (Bharti et al., 2003). In adult cattle, infection is usually subclinical, however signs of overt infection like abortion, stillbirth, premature birth reduced milk production (Dhaliwal et al., 1996) or poor growth are also reported. Abortions and stillbirths have also been seen in pigs (Radostits et al., 2007). In dogs, symptoms like hepatic failure, jaundice, renal failure or cardiovascular failure are also associated with this disease and can prove fatal (Evangelista & Coburn, 2010). Incidences of extreme canine leptospirosis are often

coupled with accidental exposure to serovars, like Pomona, Gryppotyphosa or Icterohaemorrhagiae (Ellis, 2015). Humans are only incidental (also known as accidental) hosts for *Leptospira* species.

The situation of leptospiral infection in developing countries represents a significant challenge because animals and humans often live in close proximity. Misdiagnosis of leptospirosis is common because it mimics symptoms generally observed in dengue, hepatitis, or other diseases. This misdiagnosis usually contributes to under-diagnosis and hence under-reporting. Other factors are lack of awareness and neglect by the health system and policymakers. However, over the past few years, this disease is increasingly being considered from a One-Health perspective. Some of the latest diagnostic techniques, like qPCR, lateral flow assays, and latex agglutination test, paved the way for increased reporting of this zoonosis. Although effective vaccines are available commercially in India in canines, similar vaccines are still lacking in farm animals. Vaccination of farm animals with commercial preparations like Spirovac® L5, Bovi-Shield GOLD FP® 5 L5 HB is, however, done in countries like the United States, United Kingdom, and Ireland.

Intensive surveillance and accurate diagnosis can achieve a good information of the disease's epidemiology, including the reservoir animals, circulating serovars, seasonal patterns, and occupations at risk. Therefore, efforts are needed to improve its diagnostic facilities, spread more awareness and proper attention towards this important disease to control its spread in animals and humans.

2. Epidemiology

Leptospires prefer humid and warm climate with alkaline soil. The prevalence of the disease is positively correlated with the presence of animals, which are carriers or reservoirs such as rodents, raccoons, foxes, or other wild species, and even infected or carrier domestic animals (Radostits et al., 2009). Leptospires can even persist in renal tubules of kidneys of recovered animals and may be excreted intermittently in the environment for months to years (Adugna, 2016). Even the organism is reported to persist in the mammary gland and discharged in milk after the recovery of animals (Thiermann, 1982).

Populations dwelling in developing countries with inadequate sanitary conditions have a high chance of getting infected through contaminated water or soil.

In India, leptospirosis poses a significant problem in the low-lying areas, which are highly populated and face excessive water-logging or floods during monsoon. It is one of the significant zoonotic threats, and human outbreaks have been reported after floods (Akhilanand, 2016).

The disease is studied mainly in humans, especially in coastal areas of the country, where leptospirosis is predominant. In India, leptospirosis accounts for about twelve percent of acute febrile cases reaching hospitals (Sehgal et al., 2003). In July 2015, the Brihanmumbai Municipal Corporation (BMC) reported 15 fatalities in Mumbai due to leptospirosis within ten days, while in Chennai 1,204 cases of leptospirosis were reported after a massive flood (Akhilanand, 2016). In September 2018, an outbreak of human leptospirosis after floods in Kerala claimed approximately 70 deaths (James, 2018).

The epidemiology of leptospirosis in animals is not well established due to limited studies in various parts of the world. Since the isolation of the leptospires is difficult, a thorough understanding of the serovars in a population has been limited. Infecting serovars in the animals vary geographically depending on the exposure to the reservoir domestic or wild hosts. The animals which are non-maintenance hosts, also known as accidental hosts, may get the infection by incidental contact with urine or soil containing leptospires from the carrier or infected animals. For instance, serovar Hardjo is predominantly found in cattle population, however Australis, Gryppotyphosa, and Pomona may also be present in leptospirosis of bovines. Therefore, understanding the relationship between different hosts and *Leptospira* serovars in a specific ecosystem is very important (Cilia et al., 2020).

3. Global and National Status

Leptospirosis is classified as a neglected zoonotic disease with a colossal impact on the economy as well as animal and human health. The worldwide and Indian status of leptospirosis is described as follows:

3.1 Global Burden

Based on global estimates collected on incidences of leptospirosis, the International Leptospirosis Society (ILS) reported 0.35 to 0.5 million cases of leptospirosis annually (Ahmed et al., 2012). According to another report, about 60,000 deaths occur because of leptospirosis annually (Costa et al., 2015). The

country with the maximum occurrence of leptospirosis worldwide is Sri Lanka, with over 700 deaths per annum (more than twice that for dengue fever) and an estimated annual incidence for hospital admission of 52.1 patients/100,000 population (Warnasekara et al., 2019). Studies have been conducted worldwide to check the seroprevalence of *Leptospira* species in animals and humans. The tropical and sub-tropical countries of Latin America, the Caribbean, Southeast Asia, and Oceana are major foci of leptospirosis.

In reports from Latin America and the Caribbean, the annual incidence of leptospirosis in Costa Rica, Uruguay, Cuba, and Brazil was 67.2, 25, 24.7 and 12.8 cases per 100,000 population, respectively (Pappas et al., 2008). In the same report, the number of cases per 100,000 in Barbados and Jamaica was 100 and 78, respectively. In another study, Pratt et al. (2017) reported high occurrence (73.2%) of leptospirosis in dogs on the Caribbean Island of Saint Kitts. Similarly, in 2018, a study conducted in Brazilian wildlife using serological and molecular diagnostics on free-ranging animals from 16 species (the majority of the animals included were opossums and coatis) revealed infection in 11% of cases using microscopic agglutination test (MAT) and 5.5% of cases in polymerase chain reaction (PCR) (Fornazari et al., 2018).

In Southeast Asian countries, the disease is hyper-endemic in Sri Lanka, especially in the provinces of north-central and south. In the Sabaragamuwa, a southern province of Sri Lanka, the yearly occurrence is more than 140/million (Ministry of Health, Sri Lanka, 2007). Cosson et al.(2014), using real-time PCR, found the prevalence of four major species of *Leptospira* in rodent samples collected from different localities of Southeast Asia (Thailand, Lao PDR and Cambodia) with 56% positive samples for *L. borgpetersenii*, 36% for *L. interrogans*, 3% for *L. kirschneri* and 2% for *L. weilli*.

From Oceana, a cross-section survey in New Caledonia was carried out on wild and domestic animals in which samples collected from cattle, deer, cats, dogs and horses showed 40-80% sero-positivity suggesting a high prevalence of different serogroups of pathogenic *Leptospira* species (Roqueplo et al., 2013). In Africa, Desa et al. (2021) reported 57 dairy farm samples in and around Jimma Town, Southwestern Ethiopia to be positive out of total 77. Hammond et al. (2022) in the US Virgin Islands, examined 140 rodent samples, out of which 49 samples were positive

by dark-field microscopy, 60 by culture, 61 by qPCR and 63 by fluorescent antibody testing.

3.2 National status

The states that are endemic for leptospirosis in India include Kerala, Tamil Nadu, Gujarat, Maharashtra, Karnataka and Islands of Andaman and Nicobar. Sporadic cases of leptospirosis have also been reported from other states like Andhra Pradesh, Odisha, and Assam. By the late 1990s, the Regional Medical Research Centre (Indian Council of Medical Research), Port Blair established facilities for serological characterization of leptospiral isolates and it was designated as the National Leptospirosis Reference Centre in 1999, and as the WHO Collaborating Centre for Leptospirosis in 2004. During the 12th Five-Year Plan, Government of India launched a program for the prevention and control of leptospirosis in endemic states, including Tamil Nadu, Maharashtra, Gujarat, Karnataka, Kerala as well as the Union Territory Islands of Andaman and Nicobar. National Centre for Disease Control (NCDC), New Delhi, is the Nodal agency for this program.

Disease has been reported by various research groups across India; some of which are described here. In India, leptospires were identified for the first time in 1931, from the Islands of Andaman and Nicobar (Taylor & Goyle, 1931). Since then, the disease has been reported in different animal species and humans, and studies have been made across India to establish the prevalence of leptospirosis. Among studies involving humans, five instances of jaundice were discovered to have serological evidence of *L.* Icterohaemorrhagiae and Canicola in 1960 (Dalal, 1960). In 1995, patients in Puducherry with fever and jaundice had a seroprevalence rate of 12% for leptospirosis (Prabhakar et al., 1995). Similar results were compiled by Shekatkar et al. (2010) with 40 (36%) blood samples out of total 110 suspected samples found to be positive by MAT, however, 37% positivity was seen by IgM ELISA. A 10-year study showed 26.90% (391/1453) seropositivity by IgM ELISA in New Delhi, India. A cross-sectional study in humans in Puducherry, India was performed to determine the seroprevalence of leptospirosis in patients with acute febrile disease in which out of total 597 samples, 18.25% were seropositive for anti-leptospira-specific immunoglobulin (IgM) antibodies by using IgM ELISA (Moinuddin et al., 2020).

In the light of a leptospirosis outbreak in cattle, forty cows were screened in a community close to Chennai and leptospires-specific antibodies were discovered in

68% of the animals (Ratnam et al., 1983). According to a serosurvey, approximately 40% of the cows and 26% of the bulls in the Islands of Andaman and Nicobar were seropositive (Sharma et al., 2003). Investigations were carried out to ascertain the cause of abortions during six months to term in Nili-Ravi buffaloes in Nabha subdivision of Punjab, which revealed 13 out of 28 sera samples (65.00%) positive for *L.* serovar Hardjo antibodies in ELISA. This was claimed to be the first report of this infection in Nili-Ravi buffaloes (Rana et al., 2011). Sharma et al. (2014) studied seroprevalence in 184 cattle and 202 goats slaughtered in an abattoir in Andaman and found 37% positivity in cattle and 29% in goats. To observe the distribution and seroprevalence of leptospirosis amongst buffaloes and cattle having a history of reproductive complications, 373 bovine samples from endemic states were tested with MAT and seropositivity of 70.51% was observed (Balamurugan et al., 2018). In Andhra Pradesh, more than 400 samples were analyzed for antibodies by MAT in which overall seropositivity for both cattle & buffaloes was about 68.8% (Alamuri et al., 2019). In Chhattisgarh, serum samples from more than 350 bovines were analyzed using ELISA in which overall seropositivity was ~60% (Jain and Kumar, 2020).

In companion animals also, studies have been conducted to evaluate the role of dogs in disease spread to humans. Studies on 58 canine and 40 equine sera from different parts of West Bengal were conducted using MAT, which showed that leptospiral antibody titers were present in 20 canine and 12 equines. *L.* serovar Pomona was the more prevalent serovar in equine while *L. interrogans* serovar Icterohaemorrhagiae was more predominant in canine (Debnath et al., 2005). In a study at IVRI, Izatnagar, India, a total of 807 serum samples from dogs of 17 breeds were examined by microscopic agglutination test and recombinant leptospiral lipoprotein 32 (rLipL32) based ELISA for the presence of anti-leptospiral antibodies, in which an overall seroprevalence of 7.07% was observed by MAT, whereas 10.29% seropositivity was observed in ELISA (Kumar et al., 2009). Ambily et al. (2013) studied the seroprevalence of canine leptospirosis in Kerala using MAT performed on 205 serum samples, out of which 146 (71.12%) were seropositive. The highest positivity was against *L. interrogans* serovar Autumnalis, whereas least cases were positive with serovar Patoc. Immune response dynamics in about 100 dogs after administration of a commercial vaccine was studied by MAT and LAT based on genus-specific recombinant protein antigens of *Leptospira*, viz. serovars Canicola,

Pomona, Grippotyphosa and Icterohaemorrhagiae. Of the four serovars used in the vaccine, Pomona was the most immunogenic, followed by Icterohaemorrhagiae, Canicola and Grippotyphosa. Surprisingly, only 69.60% of the dogs showed post-vaccination titers against either of the four serovars, which warrants the increased efficacy and quality control of vaccines (Chandra et al., 2013). In a recent study at Tirupati, Andhra Pradesh, about 50 samples each of serum and urine were collected from clinically suspected dogs with hepatic and renal failure. Out of 50 urine samples screened, 18 were found positive on dark field microscopy, whereas, out of 50 serum samples subjected to MAT revealed seropositivity of 36%. The highest seropositivity (55.5%) was reported against *L. interrogans* serovar Canicola (Prameela et al., 2018). Desai et al. (2020) found the prevalence of canine leptospirosis in south Gujarat by analyzing 46 serum and 33 urine samples from suspected dogs using dot-ELISA test and dark field microscopy (DFM). A total of 17 (36.95%) serum samples were found positive by dot-ELISA whereas 13 (39.39%) urine samples were confirmed by DFM.

Meta-analysis of the published data on animal leptospirosis from India between 2005-2021, suggested the highest prevalence of leptospirosis in water buffalo (45.75%), followed by rodents (40%), dogs (26%), cow (24.26%), goat (19.42%), and pigs (15.8%) (our unpublished work). Further, the meta-analysis indicated that state-wise prevalence was highest in Andhra Pradesh (64.71%), followed by Kerala (60.5%), Chhattisgarh (54.7%), and Odisha (52.26%), while the lowest prevalence of the disease was found in Assam (0.9%) and Haryana (1.4).

In 2018, a proposal was sanctioned from DBT to develop a dedicated laboratory at GADVASU for diagnosis of leptospirosis, an essential yet under-recognized zoonosis. More than 1,000 serum samples mainly from dogs, bovine, horses and humans were tested with MAT, which revealed approximately 10% positivity overall.

4. Transmission and Risk Factors

Agro-climatic conditions such as excessive rainfall, high level of water table, and clay soil favor endemicity of this disease (Shivakumar, 2008). Transmission of leptospirosis occurs either by direct contact with an animal which is infected or through indirect contact with water or soil contaminated with urine of infected animals (Haake & Levett, 2015). Rodents are known to be the major reservoir host for leptospires. An abrasion or cut on the skin followed by exposure to matter

contaminated with infected animal's urine can lead to infection. Human-to-human transmission of leptospirosis is rarely reported (WHO, 2018). Organisms may get lodged in the kidneys and be shed in the urine for an extended period ranging from a few weeks to years.

The exposure of humans & domestic animals to other infected or carrier domestic or wild animals increases the risk of transmission. Leptospirosis presents a significant problem, especially in areas with heavy monsoons and excessive water logging along with high temperature and humidity. A schematic representation of the transmission pattern of leptospirosis in animals and humans is described in Figure1.

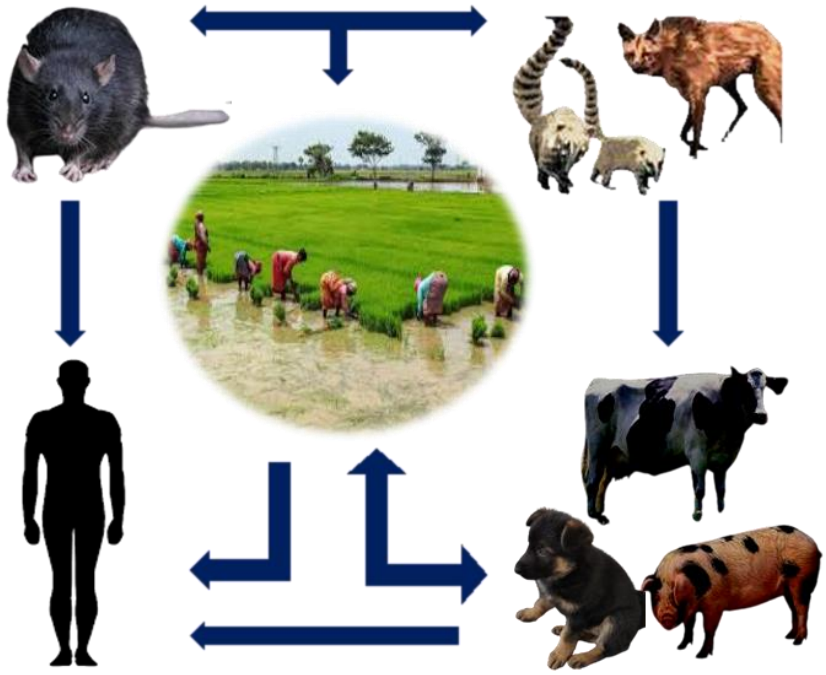

Figure 1: Transmission of leptospirosis: *Rodents, wild reservoirs, and infected or carrier animals contaminate the environment, including water and soil, which serve as a source of infection to other healthy animals as well as human beings.*

For humans, there are several occupations that are considered high-risk for leptospirosis, like sewer workers, slaughterhouse staff, veterinarians and animal caretakers, rodent control workers, dairy farmers and military personnel, etc. Indirect exposure is also responsible for illness during occupations like farmers working in irrigated rice fields, sugarcane or banana farms, septic tank cleaners and freshwater fishermen. Rice / Paddy work is an important risk factor in Sri Lanka, Thailand, India, Indonesia, Iran, the Philippines, Tanzania, and Korea; the disease has hence been termed 'Rice field fever'.

Apart from these occupations, activities like water sports or swimming, kayaking and rafting in contaminated lakes and rivers are also significant risk factors for contracting the disease (Kumar et al., 2012). The risk is likely greater for those participating in these activities in tropical or subtropical climates.

For animals, the important risk factors include shared grazing with common water resources, introduction of newly infected cattle, rodents on the farm, level of hygiene in milking and status of leptospiral vaccination, presence of other animals on the farms like dogs, sheep and goats, horse, pigs, etc. Animals acquire infection mainly from environmental exposure, but venereal transmission is also frequent in some mammalian species.

5. Signs and Symptoms

Leptospirosis may occur in the form of outbreaks, but many infections may pass unnoticed. Introducing an infected animal into an unvaccinated or unexposed herd may result in a severe epidemic. Signs of the disease in different species vary and depend on the infecting serovar.

5.1 Animals

In cattle, infection with serovar Hardjo-bovis may result in a storm of abortion in females within five months of pregnancy, especially where the herd is unvaccinated and not exposed previously. In dairy cattle there may be a sudden drop in milk production. Animals may develop symptoms of mastitis with milk becoming thick and yellow without swelling of the udder. Infection may also result in early embryonic losses due to inflammation of the uterus (Mori et al., 2017). Sheep and goats may also show similar symptoms as that of cattle. In dogs, infection results in various symptoms depending on geographical location, host immunity and serovar involved. In dogs, four manifestations have been recognized: hemorrhagic, icteric, uremic, and reproductive (abortion or weak pups). Some dogs develop renal or hepatic lesions, while others display mild or no symptoms. Typical symptoms in dogs may be seen as pyrexia, diarrhea, hepatic lesions, intra-vascular disseminated coagulation, hemorrhages, uremia due to kidney failure, and death. Other symptoms seen in dogs are polyurea, dehydration, vomiting and anurea, etc. (Kohn et al., 2010).

5.2 Humans

Human beings show symptoms overlapping with diseases like dengue, chikungunya, etc.; cases may be icteric or anicteric. The disease manifestation in humans is inconsistent, ranging from one day to four weeks after infection, and infection can last for months in survivors. Infection ranges from mild, influenza-like symptoms to a severe illness with kidney and liver failure, and even death (the classical form known as Weil's disease).

6. Diagnosis (National Institute of Communicable Diseases, 2006)

The diagnosis of leptospirosis is cumbersome and challenging because of clinical similarity with other infections like hepatitis, dengue, scrub typhus, etc. It requires sophisticated lab and well-trained laboratory personnel. Laboratory support is required because:

- Leptospirosis is difficult to distinguish from several other diseases on clinical grounds, and thus the laboratory methods help to confirm the diagnosis.
- For epidemiological and control strategies namely, to determine the causative serovar, the likely source of infection and the potential reservoir.

Samples like urine, cerebrospinal fluid, infected tissues, and body fluids are preferred for the diagnosis of leptospirosis. During initial stage of disease (up to 7-8 days), leptospires can be found in the blood and in later stages, antibodies produced in the body can be detected by serological tests.

6.1 Samples for diagnosis

The types of samples to be collected for the diagnosis of leptospirosis are:

- **Blood for culture isolation**: Blood sample more than ten days after disease onset is not accepted for culture isolation because leptospires have mostly disappeared from the blood. Therefore, it should be best collected within the first seven days after the commencement of infection. Samples for blood culture should be stored and transported at room temperature.
- **Serum**: Serum is used for detection of antibodies against leptospires. Serology should preferably be done twice at an interval of 7-10 days. The screening of paired sera is essential to identify a rise in titers between the two samples, which will confirm the diagnosis of leptospirosis. It may be noted that a

negative result in the early phase of the infection does not exclude leptospirosis.

- **Urine**: Urine can also be used to diagnose the presence of leptospires after 7-10 days of infection. Midstream urine should be collected in a sterile container and transported immediately as leptospires die quickly in acidic urine. Therefore, it must be inoculated into culture medium within 1-2 h of voiding. The survival period of leptospires can be increased by making the pH of the urine neutral.
- **Post-mortem samples**: Specimens may be collected from the brain, cerebrospinal fluid, lungs, kidney, liver, and heart blood. Samples should be collected as soon as possible after the animal's death and inoculated into culture medium immediately. These samples should be stored and transported at 4^{o}C to prevent tissue autolysis.
- **Cerebrospinal fluid**: It should be collected preferably in the first ten days of infection and processed similarly to blood for bacterial isolation.

6.2 Techniques for diagnosis

Many techniques are available for diagnosing leptospirosis, including direct methods like microscopic detection, culture isolation from clinical samples, and indirect methods targeting antibody detection. Confirmatory diagnosis is when leptospires are isolated from clinical specimens or positive by PCR assay. Further, a four-fold increase in the antibody titer between paired serum samples is also confirmatory.

6.2.1 Microscopy

Leptospires can be visualized by dark field microscopy. This method is used to detect leptospires from clinical samples. It is successful only when the bacteria are in the blood, which is the case only in the early phase of infection. Leptospires are visualized as thin white motile structures against a dark background. Silver impregnation technique is another method in which spirochetes are stained brownish black on a yellowish background and can be visualized by an ordinary bright field microscope (Gangadhar & Rajsekhar 1998). This technique lacks sensitivity and is not a preferred method of diagnosis and is used only for academic purposes in the teaching labs to demonstrate leptospires.

6.2.2 Culture isolation

Leptospires can be isolated from blood and CSF samples during the first week of disease and the second or third week of disease from urine. Samples should be inoculated into Ellinghausen-McCullough-Johnson-Harris (EMJH) or other media and incubated at 29˚C. The organism is slow growing and culture should be declared negative only after 8-12 weeks of incubation. Various types of culture media in use are– EMJH (Ellinghausen-McCullough-Johnson-Harris media), Stuart's medium, Fletcher's medium, Korthof's medium. Growth in the semi-solid medium is very typical in the form of a ring near the surface of the medium known as Dinger's ring due to the organism's aerobic nature (Figure 2).

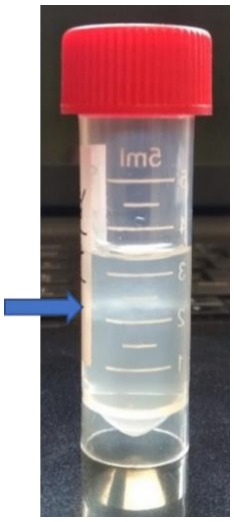

Figure 2: Culture showing characteristic Dinger's ring (*indicated by arrow*)

6.2.3 Microscopic agglutination test (MAT)(Office International des Épizooties, 2021)

MAT is the gold standard test for diagnosing leptospirosis due to its high specificity. The live cultures of various serovars of *Leptospira* are reacted to test serum to detect the presence of antibodies. The 50% agglutination or reduction in culture indicates positive results. For the confirmatory diagnosis, the test requires two samples at weekly intervals. This test is costly as there is a need to maintain different leptospiral serovars. It does not differentiate between recent and previous infection. Moreover, it carries little importance during the early stages of leptospirosis as the antibodies will not be present in the early stages of disease.

6.2.4 Enzyme-linked immunoassay (ELISA)

Commercial kits or antigens derived from specific serovars are available to detect *Leptospira*-specific IgM and IgG in the serum. The Bovine Leptospira ELISA kit (Linnodee) is a sandwich-ELISA kit that detects the antibodies against the LPS epitope of serovar Hardjo in either sera or milk. The Human Leptospira IgM ELISA kit (Panbio) is one of the most commonly used kits for detecting anti-leptospiral IgM antibodies and diagnosing human leptospirosis.

6.2.5 Molecular detection

Polymerase chain reaction (PCR) or real-time qPCR involves amplification of leptospiral specific DNA and thus detects its presence. The test does not necessitate the presence of live bacteria and a diagnosis at early stages can be done since bacterial DNA can be seen even before developing a serological response to bacteria. Many PCR protocols are available for detecting *Leptospira*, but primer sets G1/G2 and B64-I/B64-11(Brown et al., 1995) and primers specific for 16s rRNA (Merien et al.,1992) have been mostly used. However, in recent times, *lipL32* gene-based primers are one of the most commonly used targets for detection of leptospires, with approximately 50% of researchers using this marker. A PCR for the detection of pathogenic *Leptospira* species by amplification of a partial fragment of the major outer membrane lipoprotein *lipL32* gene confined to pathogenic strains only could detect the majority of serovars of *L. interrogans* (Bhure et al., 2009). Another PCR was developed to differentiate pathogenic and saprophytic leptospires based on the *hap1* gene, which is only amplified in the pathogenic *Leptospira* strains (Kumar et al., 2010). In a study on serum and urine samples spiked with a known amount of leptospires to compare the efficiency of conventional and SYBR green-based real-time PCR on the locus LA0322 of *L. interrogans*, the conventional PCR had an average detection limit of 3.2-4.6 x 10^3 organisms. In contrast, real-time PCR was sensitive enough to detect as minimum as 32 to 41 leptospires per ml (Malathi et al., 2010). Similarly, a multiplex PCR developed for detection of *Brucella*, *Leptospira* and bovine herpesvirus-1 (BoHV-1) targeting conserved regions of *BCSP31*, *LipL32* and *gB* genes, respectively which could detect as low as 15 pg of each template. With this PCR, three out of five human urine samples tested were found positive for *Leptospira* and other two were positive for both *Leptospira* and *Brucella* (Bhure et al., 2012). A multiplex PCR was developed to simultaneously detect infectious causes of abortion in bovine (BHV-1,

Brucella spp and *Leptospira* spp) with high sensitivity and specificity. This assay could detect BHV-1 and *Brucella* spp from field samples; however, none of the field samples was positive for *Leptospira* spp. (Sharma et al., 2013). A real-time PCR was also developed to detect pathogenic leptospires based on *lipL32* gene with high specificity and sensitivity (Ahmed et al., 2020).

PCR is efficient but may yield false-positive results in case of contamination with DNA or give false-negative results due to inhibitors, which are sometimes there in the materials being tested.

6.2.6 Lateral flow assay (Leptocheck-Rapid test)

Leptocheck-Rapid test (Zephyr Biomedicals) is a lateral flow immunoassay for detecting specific IgM antibodies from human patients. It uses a broadly reactive genus-specific antigen for the sero-diagnosis of recent infection. It works on the principle of immune-chromatography. A 10 micro litres of serum/ plasma/ whole blood is added into the sample port, followed by adding a few sample buffer drops in the buffer port. After 15 min, if red to deep purple coloured band appears only in the control window, then it is declared negative. If, in addition to the control band, another band appears in the test window, it is considered positive. The control band should appear in positive/negative both to validate the test results.

A presentation of strategies for diagnosing leptospirosis is given in Figure 3.

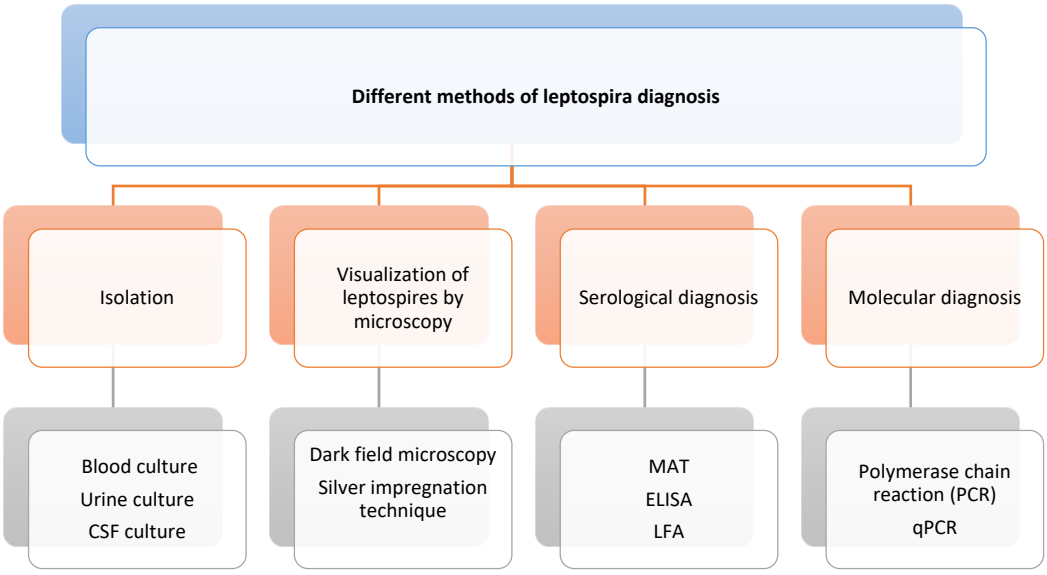

Figure 3: Schematic presentation of strategies for diagnosis of leptospirosis

7. Lab Requirements

To set up a lab for working on leptospires, the general requirements are essentially similar to any diagnostic laboratory in terms of instruments, technical staff and training, safe laboratory practice etc., with some special equipment.

One of the essential instruments, which is must for a laboratory on leptospirosis is dark field microscope. This is because leptospires can be seen only with dark field microscopy as they are poorly stained with normal bacterial staining procedures. Another essential piece of equipment is a refrigerated incubator called BOD incubator to provide the optimum growth temperature from 28^{o}-30^{o}C for leptospires. This temperature will not be achieved with a standard incubator when the ambient temperature is higher, which is very common in tropical and sub-tropical countries such as India.

The laboratory practices of BSL-II need to be followed since leptospires belong to biosafety level 2 pathogens. Apart from general good laboratory practices like use of Personal Protective Equipment (PPE), proper discard and disinfection facilities, laboratory should have a bio-safety cabinet at least Class II type A2 or preferably B2, to handle leptospires.

Three levels of diagnostic capability are commonly found:

- First level: Simple screening methods for anti-leptospira antibodies are available.
- Second level: Moderately complex serological methods are available and cultures are handled.
- Third level: National or international reference laboratories that maintain culture collections, perform typing and monitor the performance of other laboratories.

8. Prevention and Control

Animal leptospirosis has shown a massive impact on animal health, culminating in extensive economic losses and the development of risk situations in the animal-human-ecosystem interface. Despite the extensive research progress made by researchers and efforts of government agencies, there is still a lot to do.

In the 5th Global Leptospirosis Environmental Action Network, International Workshop, Rio de Janeiro, Brazil in 2015, the focus was on health policy to develop a

road map for research on leptospirosis. The attendees included researchers and policy makers from different continents.

Methods to control leptospirosis in animals are almost similar in animals and humans. Preventive measures in animals control the disease in a particular animal and decrease the risk of zoonosis. Control strategies should be based on epidemiology, host species involved, prevalent serovars, means of spread, precipitating factors in a particular area. Various measures which should be taken to control leptospirosis in animals include

- ***Vaccination***

Vaccination is the most important, convenient and probably most practical way to control leptospirosis in animals. However, there are some limitations like availability, cost and effectiveness of the preparations. Since immunity is considered serovar specific, the vaccine preparation must include the serovars which are prevalent locally. Therefore, if inappropriate antigens are included in the preparation, it will probably not be effective against the prevalent serovars (Ambily et al., 2013). The vaccines are known to prevent disease but do not prevent the shedding of the organism in urine. A few vaccines are commercially available for various animal species, providing limited immunity. Multivalent vaccine preparations have also been sold; however, these are less effective in comparison with monovalent, and their efficacy remains debatable. The vaccination of dogs should be done after birth once the maternal antibodies decline at 8-9 weeks of age, followed by a booster at 12 weeks of age and annual revaccination thereafter. The available vaccines for dogs in different countries effectively protect the animal for a year. Some of the commercial vaccines available are NoviVac DHPPI for dogs (Intervet), Spirovac for cattle (USDA), Farrowsure-plus for cattle and pigs (Pfizer Animal Health, USA) etc. Immunization may be the most cost-effective way to prevent leptospirosis, and its use is critical in leptospirosis control programs. However, only the whole-cell bacterins are accessible commercially, and their effectiveness in preventing leptospiral infection-related reproductive losses is debatable.

- ***Awareness***

Awareness must be created among veterinarians, medical staff, farmers, pet owners, small animal house workers, butchers, and other high-risk occupations about

the measures to prevent the disease. Intensive education and training programs need to be initiated and strengthened. Public awareness campaigns should be carried out. At the very first step, animals should be protected from getting infected. All measures should be followed to avoid direct or indirect contact of animals with urine from infected or carrier animals. Animals should not be allowed access to water or areas where carrier or reservoir host species are potentially prevalent. Field workers should wear rubber shoes as well as gloves to avoid contact directly with contaminated mud and water.

- ***Control of rodents***

Since rodents are the primary reservoir hosts of leptospires, there is a need to undertake selective measures against rodents. Trapping and poisoning programs are required for effective control. These programs are adopting control methods for rodents before the rainy season could make a significant difference.

- ***Maintenance of proper drainage system***

There must be a proper drainage system to avoid waterlogging, which is a potential source of infection once contaminated with infected urine. Urine from animals should be appropriately drained and not dumped into common water bodies like ponds, which are accessible to animals.

- ***Chemoprophylaxis***

During peak seasons and in high-risk areas, doxycycline @ 200 mg once a week may be given to people at high risk according to their occupation.

- ***Reporting of the disease***

An efficient reporting system should be in place, especially in high-risk areas with proper coordination of health and veterinary departments for effective identification and control of outbreaks.

9. Treatment

Renal failure and liver disease are treated with fluid therapy and other supportive measures to maintain normal fluid, electrolyte, and acid-base balance. Antibiotic treatment is indicated whenever leptospirosis is suspected and should be instituted before confirmatory test results are available.

9.1 Treatment in dogs

Renal replacement therapy with intermittent hemodialysis should be considered for dogs that are anuric or oliguric despite appropriate supportive treatment. No experimental studies guide the selection of antibiotic protocols for this species. However, current recommendations are to treat with doxycycline (5 mg/kg, PO, every 12 hours, for two weeks). For dogs difficult to treat with doxycycline, initial therapy with penicillin is appropriate, but a 2-week course of doxycycline should follow this to eliminate the renal carrier phase of infection. Penicillin G (25,000–40,000 U/kg IV q12h) also could be used. Oral administration of ampicillin should be avoided as it is not readily absorbed from the gastrointestinal tract. Doxycycline is the drug of choice for clearance of the bacteria from tissue. Shedding is usually terminated within 24 hours of start of antibiotics, which greatly reduces the chances of infection to humans and other dogs (Goldstein, 2010).

9.2 Treatment in cows

The most commonly prescribed medicine for leptospirosis therapy is dihydrostreptomycin. A single dosage of 25 mg/kg IM is recommended to eradicate the renal carrier status. Successful reductions in the losses associated with leptospiral infection have been obtained through antibiotic therapy to manage infection until generation of immunity by vaccination (Mughini-Gras et al., 2014).

9.3 Treatment in horses

Treatment procedure for horses has primarily been derived from other species, as there is very scanty information available specifically for horses. The antibiotics of preference include penicillin (10,000-15,000 IU/kg) and/or streptomycin (10 mg/kg). However, the use of streptomycin is declining in horses due to significant toxic effects (Newman & Donahue, 2007).

9.4 Treatment in humans

Treatment with effective antibiotics should be started as soon as the onset of disease. Doxycycline 100 mg twice a day for seven days is recommended in adults. Severe cases can be treated with intravenous penicillin. Minor cases can also be treated with amoxicillin or erythromycin. In the case of children, Amoxycillin or ampicillin for seven days should be given in divided doses @30-50 mg/kg/day.

10. Recommendations

Policies for effective control of leptospirosis need to be designed for various stakeholders, e.g., at the level of veterinarians, farmers, animal house workers, government etc.

10.1 For veterinarians and veterinary staff

The literature has confirmed that veterinarians are at far more risk of infectious diseases than normal workers because of their extensive occupational exposure to diseased animals. Proper education should be provided to the veterinary staff as well as the owners of the animal about the potential hazards linked to infectious leptospires and the following strategies should be adopted to reduce the exposure.

- For personal protection of veterinarians, good hand hygiene practice should be followed in handling the animal that is suspected, confirmed or recovering case of leptospirosis.
- Proper disinfection should be done in and around the veterinary hospital where the animals may have urinated/operated/handled.
- Gloves should always be used to clean up the urine.
- Personal Protective Equipment kits should be worn while handling the suspected animals.
- Caution should be exercised in handling animal urine and other body fluids, and they should be considered potential sources of infection until a negative diagnosis has been proven.
- Appropriate hygienic precautions should be taken when examining sick animals.

Suppose a veterinarian encounters a dog suffering from symptoms such as hepatitis, nephritis, increased serum creatinine etc. or cattle with abortion, agalactia, anemia or mastitis or a pig with a history of stillbirth, loss of appetite and abortion. In that case, he should suspect leptospirosis and send the appropriate sample from the diseased animal to the diagnostic laboratory. The sample can be processed for diagnosis by molecular or immunological methods like PCR and MAT, respectively. Moreover, the vet must encourage vaccination of animals. Vaccine preparations for dogs mostly contain *L. interrogans* serovar Icterohaemorrhagiae and *L. interrogans*

serovar Canicola, whereas *L. interrogans* Hardjo and *L. interrogans* serovar Pomona are included in the vaccines for cattle. A booster vaccination is also required every one to two years.

10.2 For farmers/pet owners

Farmers/pet owners must ensure vaccination of their animals with the guidance of a veterinarian to protect the animals from leptospirosis. A veterinarian should be consulted at the earliest for further diagnosis and treatment if any sign or symptom is suspected. Farmers should be encouraged to drain urine from the animal shed into a pit instead of letting it flow and mix with rivers, ponds, etc. Humans or animals that are non-maintenance hosts may become infected incidentally and suffer from illness even multi-organ failure. They are called "incidental" or "accidental" hosts. Therefore, care must be taken by farmers or pet owners to protect themselves and also their animals. Since rodents are a major source of infection to farm animals, rodent-proof buildings should be preferred in the infection-prone areas. Rodents and pests should not have access to the feed storage areas and drinking water. Diseased animals should be kept separated from healthy ones.

10.3 For Slaughterhouse workers/Tannery workers

Slaughterhouses should be built following the proper rules and regulations of the concerned regulatory bodies. Proper disposal of the leftovers needs to be carried out. Routine disinfection of the working areas should be performed. The workers need to wear protective gloves and gumboots since the organism is known to enter through cuts/abrasions on the skin. The owner/in-charge should ensure regular monitoring and routine health checkup of the workers.

10.4 For Animal house staff

Rodents are symptomless reservoirs and strong shedders of the bacterium; therefore, animal house workers must take appropriate measures to protect themselves from getting infected by following good management practices. Animal house staff and other workers should always wear protective gear while handling laboratory rodents or animal house waste to minimize the contact with the urine. Skin abrasions or cuts should be covered before handling animals or their waste. Proper disinfection of the animal house areas should be carried out regularly. Leptospires can be inactivated by 1% sodium hypochlorite, 70% ethanol, iodine-based preparations,

quaternary ammonium compounds, hydrogen peroxide, formaldehyde, detergents etc. Disinfectant can be applied either by mopping or spraying by high-pressure sprayers in which the chemical is diluted as per the recommendations. Dead animals should be placed in disposable plastic bags and immediately incinerated upon discovery. Installing an incineration facility for the disposal of pathological and animal wastes should be planned for animal house well in advance during civil and electrical construction. Wild rodents may transmit a wide variety of bacteria, viruses, and parasites, including leptospires to the housed species. Therefore, animal house buildings should be rodent-proof. Wastes should be removed regularly and frequently in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators must comply with regulations of central and local authorities. Waste cans containing animal carcasses, and dangerous waste materials should be packed in leak-proof liners. If wastes are required to be stored before disposal, it should be stored separately from other facilities of storage and storage area should be free of rodents and other animals. Hazardous wastes should be rendered harmless by sterilization, de-contamination, or other suitable means before they are released from the animal facility for disposal. Cages should be carefully washed with detergent and rinsed thoroughly with water to remove all traces of infected urine. Bedding in animal cages or pens should be changed as often as necessary to keep the animals clean and healthy.

10.5 For Government/Administration

The government/administration should take the following concrete steps for the control and prevention of leptospirosis:

- Constitution of the committees for planning and implementing effective control and prevention measures in the community and identifying resources that may increase the scope of community education.
- Establishing rodent control (poisoning, trapping) programs is one of the measures to minimize the chances of infection through infected urine.
- Development of brochures and handbooks about prevention and control of this disease to educate people, community health workers, volunteers, farmers, and veterinarians.

- One health concept should be strengthened and followed to control leptospirosis since it is a major zoonotic disease.
- Funding for research on leptospirosis should be increased and strengthening of already established labs is critical since lack of maintenance and financing of labs may render them non-functional.
- Animal Husbandry departments should ensure vaccination among animals to prevent the spread of the disease to humans.

11. Referral Laboratories in India

- Regional Medical Research Center (ICMR), Port Blair (A&N)
- National Centre for Disease Control, 22-Sham Nath Marg, Delhi
- National Institute of Epidemiology, Chennai
- National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, Karnataka
- Bacteriology & Mycology Division, IVRI, Izatnagar, Uttar Pradesh 243122
- ZRL, Tamil Nadu Veterinary & Animal Science University, Chennai

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ANNEXURE

List of experts participated in the consultation

NAVS officials

1. Dr DVR Prakash Rao, President NAVS, & Chairman and MD, Prakash Food & Feed Mills (P) Ltd, Chennai
2. Dr AC Varshney, Vice-President NAVS & Ex-VC, DUVASU
3. Maj Gen ML Sharma, Secretary NAVS, India (online)
4. Maj Gen Shrikant Sharma, Ex-President NAVS, Ex-Vice-chancellor LUVAS (online)
5. Dr. MP Yadav, Executive Member, NAVS, Ex-Director IVRI (Online)
6. Dr Nem Singh, Executive Member, NAVS & Ex-JD Research, ICAR-IVRI
7. Dr Ravindra Sharma, Executive Member, NAVS & Former Director Research, LUVAS
8. Dr VK Gupta, Executive Member, NAVS, CSK HPKVV, Palampur

DAHD – Govt. of India and States

9. Sh SK Gulati, Ex-Secretary Animal Husbandry, Govt of Haryana
10. Dr Ajit Singh Yadav, Director, CCS National Institute of Animal Health, Baghpat, U.P.
11. Dr OP Chhikara, Former Director General, Dept. of Animal Husbandry, Haryana
12. Dr S Khosla, Former Director, Dept. of Animal Husbandry, Punjab
13. Dr Sireesha G, Deputy Director, Dept. of Animal Husbandry, Andhra Pradesh
14. Dr Anirban Guha, Assistant Commissioner (NADCP), DAHD, GOI, New Delhi
15. Dr Amit Nain, Member, Veterinary Council of India, and Veterinary officer, Fazilka
16. Dr Charanjeet Sarangal, Bacteriologist, NRDDL, Jalandhar, Punjab
17. Dr Anupma Kumari, Punjab Veterinary Vaccine Institute, Punjab

Industry & Farmers' Representatives

18. Dr Manoj, Chief Scientific Officer, Hester Biosciences Ltd., Ahmedabad, Gujarat
19. Dr T Bhattacharya, Brilliant Bio Pharma Pvt. Ltd. Hyderabad
20. Dr. BN Mishra, Brilliant Bio Pharma Pvt. Ltd. Hyderabad (Online)
21. Dr Srinivas Karnati, Indian Immunologicals Ltd, Hyderabad
22. Sh. Daljit Singh, President, Progressive Dairy Farmers Association
23. Sh. Sandeep Singh Randhawa, President, Progressive Livestock Farmers Association
24. Sh.Bikramjeet Singh, Representative, Pig Farmers Association
25. Sh.Kunal Sharma, Representative, Pig Farmers Association
26. Sh.Harkeerat Singh, Representative, Pig Farmers Association

ICAR and other research institutes

27. Dr RK Singh, Ex-Director, ICAR-IVRI, Izatnagar
28. Dr Abhijit Mitra, Director, ICAR-Central Institute for Research on Cattle, Meerut
29. Dr Aniket Sanyal, Director, ICAR-NIHSAD, Bhopal (online)
30. Dr RP Singh, Director, ICAR -Directorate of FMD, Argul, Jatni, Odisha
31. Dr JK Biswal, ICAR-International Center for Foot and Mouth Disease (ICFMD)
32. Dr Sharvan Sehrawat, Biological Sciences, IISER, SAS Nagar
33. Dr NN Barman Professor & Head, Dept. of Veterinary Microbiology, Khanapara
34. Dr NK Kakker, Former Head, Dept. of Vet. Microbiology, LUVAS
35. Dr Naveen Kumar, Principal Scientist, ICAR-NRC- Equines

GADVASU, Ludhiana faculty

36. Dr Inderjeet Singh, Vice-chancellor
37. Dr JPS Gill, Director Research
38. Dr. PS Brar, Director Extension Education
39. Dr Yashpal Singh Malik, Dean, College of Animal Biotechnology
40. Dr MS Oberoi, Former Dean & FAO Expert, College of Veterinary Sciences, Ludhiana
41. Dr RS Sethi, Additional Director Research
42. Dr VK Dumka, Coordinator Research
43. Dr SS Randhawa, Director, Teaching Veterinary Clinical Complex
44. Dr Deepti Narang, Prof & Head, Dept of Vet. Microbiology
45. Dr Paviter Kaur, Dept of Vet. Microbiology
46. Dr Gurpreet Kaur, Dept. of Vet. Microbiology
47. Dr Mousumi Bora, Dept. of Vet. Microbiology
48. Dr BBS Dhaliwal, College of Animal Biotechnology
49. Dr Rattan Choudhary, College of Animal Biotechnology
50. Dr Neeraj Kashyap, College of Animal Biotechnology
51. Dr BV Sunil Kumar, College of Animal Biotechnology
52. Dr Satparkash Singh, College of Animal Biotechnology
53. Dr JS Bedi, Director, Centre for One Health
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55. Dr Rajnish Sharma, Centre for One Health
56. Dr Deepali, Centre for One Health
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ISBN 9789394490413