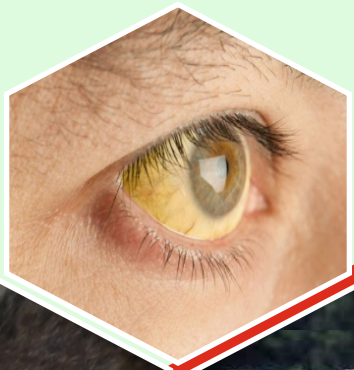
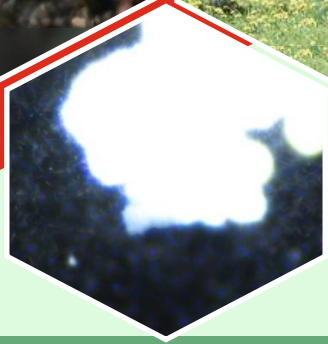
MONOGRAPH

LEPTOSPIROSIS

(An Emerging Zoonosis)







Satparkash Singh, Yashpal Singh Malik

College of Animal Biotechnology
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

MONOGRAPH

LEPTOSPIROSIS
(An Emerging Zoonosis)

IP Innovative Publication Pvt. Ltd.

MONOGRAPH

LEPTOSPIROSIS
(An Emerging Zoonosis)

Satparkash Singh

Yashpal Singh Malik

College of Animal Biotechnology
Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana

IP Innovative Publication Pvt. Ltd.

IP Innovative Publication Pvt. Ltd.
A-2, Gulab Bagh, Nawada, Uttam Nagar, New Delhi - 110059, India.
Ph: +91-11-61364114, 61364115
E-mail: info@ipinnovative.com
Web: www.ipinnovative.com

Leptospirosis (An Emerging Zoonosis)

ISBN : 978-93-91208-72-1

Edition : First, 2022

Foreword

The increasing number of interactions between humans and animals has made it necessary to be aware about various zoonotic diseases so that preventive measures can be taken in timely manner. One of such diseases is leptospirosis which is a zoonosis of worldwide immense importance. It is caused by spirochaetes belonging to the genus Leptospira. It has been identified as one of the emerging infectious diseases, which has been an under-recognized problem with major health impact in developing countries.

I appreciate the efforts of the authors and congratulate the College of Animal Biotechnology to come up with a monograph on this important zoonotic disease. As this monograph covers various important aspects of leptospirosis like diagnosis, pathogenesis, transmission and control apart from its basic introduction, it will be immensely helpful to the students, researchers, academicians, policy makers and other people associated to understand this disease.

I am pleased to present this monograph for the benefit of all concerned.

Inderjeet Singh
(Vice-Chancellor)

Preface

It is evident that livestock diseases play both direct as well as indirect impact on animal and human health, as it not only affects the livelihood of farmers but also induces catastrophic effects on their income. Moreover, the indirect consequences of such diseases on human health are a major concern as more than two third of emerging infectious diseases in humans are zoonotic in nature and involve transmission of disease from an animal to a human host.

One of the most notable zoonotic infections is leptospirosis, which is commonly known as Weil's disease, field fever, mud fever, etc. The lack of knowledge on this disease globally is a major problem. Thus, an effort to reconstitute the current knowledge on it is need of the hour. It is caused by a spirochete Leptospira and is a reemerging, zoonotic and waterborne disease worldwide. Due to negligence, rapid and unplanned urbanization, poor sanitation conditions in most of the developing countries and lack of assessment of current epidemiologic situation, leptospirosis has emerged as a leading cause of severe illness.

This monograph discusses the leptospirosis from all angles pertaining to its risk factors, causative agent, pathogenesis, clinical manifestations and diagnostic techniques with few illustrations from our lab. We hope that this monograph will enrich knowledge of the researchers and students in the field of leptospirosis.

Satparkash Singh

Yashpal Singh Malik

Contents

Preface ..... *vii*

1. Introduction 1
2. Etiology 2
3. Transmission 3
4. Pathogenesis and Pathology 4
5. Risk Factors 5
6. Diagnosis 6
7. Epidemiology: International and Indian Status 16
8. Treatment 19
9. Prevention and Control 19
10. Referral Laboratories 19
11. References 20

1. Introduction

Leptospirosis is one of the fastest re-emerging infectious and neglected disease which is caused by bacteria belonging to genus *Leptospira*. The cases of Leptospirosis are higher in tropical region than in temperate region because leptospires can survive longer in warm and humid conditions. Countries with higher chances of interaction between human population and infected animals have greater probability of infection in humans. The host range of *Leptospira* is diverse because of its capability to infect and survive in different hosts. It occurs in vast variety of wild, domestic and marine animals. Rodents, cattle, pigs and dogs act as animal reservoirs. Urine of infected animals like cattle, goat, horse, pigs serve as a main source of infection. Infection can also spread by coming in contact with infected water and soil. Since it affects many domestic animals as well as wildlife and also humans, leptospirosis is classified as a zoonosis (Lim, 2011). Human leptospirosis is primarily characterized by acute clinical features known as Weil's disease, with intense signs as fever, icterus, renal insufficiency and mortality (O'Toole et al., 2015). In bovine, as well as in other ruminants, the acute and severe form of leptospirosis is uncommon and frequently associated with sporadic outbreaks in calves caused by incidental strains (Loureiro & Lilenbaum, 2020). Indeed, the subclinical and silent presentation of animal leptospirosis is very often neglected (Adler & de la Peña Moctezuma, 2010). In most of the infected domestic animals, bacterial shedding is intermittent and not very intense; besides, they present low antibody titres (Nally et al., 2018), making difficult both direct and indirect diagnosis.

In urban areas of developing countries, various factors such as overcrowded slums, inadequate drainage and sanitation facilities for man and animals and people walking bare foot contribute to the spread of the illness. Also, a contaminated environment due to presence of infected or carrier animals like stray dogs, cattle, pigs, domestic rats, bandicoots, poor conditions of slaughter houses enhance the risk of getting infection. It is estimated that approximately 500,000 high-risk

cases occur globally with a 30% fatality rate per annum (Tilahun et al., 2013). However, under-estimating the global impact of this disease has halted its worldwide surveillance and control. Hartskeerl et al. (2011) emphasized that leptospirosis is significantly underestimated due to the lack of notification and epidemiological efforts in various countries.

2. Etiology

Leptospira species are spirochetes belonging to the division-*Gracillicutes*, class-*Scotobacteria*, order-*Spirochaetales* and family-*Leptospiraceae*. They are obligate aerobes, highly motile having features from both gram positive and gram-negative bacteria with 0.1–0.15μm thickness and 6–20 μm length (Johnson & Beck, 2018). The genome length ranges from 3.9–4.6 Mb. This variation is large in comparison to the other spirochetes and this helps *Leptospira* spp. to survive in diverse environmental conditions and infect various hosts. Under an electron microscope the cell has a cylindrical body with two axial filaments. An external sheath envelops the axis and protoplasmic cylinder. The axial filament periodically contracts, causing rotation and leading to its movement. There are two flagellae present inside the cell, hence termed as endoflagella. The flagella of leptospires consists of three major parts: the filament, flexible hook and the basal complex. The hook filament junction connects the filaments with basal complex. The viscosity of the media greatly influences swimming manner of leptospires. Screw-like motion, progressive movement, and circular motion are possible movements in leptospires. The extremes of *L. interrogans* form a question-mark shape, hence the name is given.

Initially, leptospires were classified as infectious species including *L. interrogans* and saprophytic species including *L. biflexa*. This classification was further divided into specific serovars by using the cross-agglutination adsorption test (CAAT). The serological classification is based on the surface epitopes present on the lipopolysaccharide membrane and antigenically related serovars are arranged into

serogroups. The pathogenic group includes pathogenic strains that are responsible for human and animal infection and the saprophytes or saprophytic group includes nonpathogenic strains. Pathogenic spirochetes of the genus *Leptospira* belong primarily in the P1 subclade of the new classification (Vincent et al., 2019). Serologically, there are more than 300 distinct leptospiral serovars recognized and these are arranged in 30 serogroups. There are more than 300 serovars, arranged into 30 serogroups. Intermediate leptospires are the biochemical intermediates of saprophytic and pathogenic leptospires. The other notable intermediate leptospires include spps *broomi, licerasiae, wolffii* and *fainei*. Pathogenic leptospires need temperatures between 20–35°C and resemble that of Gram-negative bacteria (Benacer et al., 2013). Compared to saprophytic leptospires, the leptospires from this group have significance to public health. Common examples of pathogenic leptospires are *L. interrogans, L. borgpetersenii, L. weilii, L. kirschneri, L. noguchii* and *L. santarosai* (Dos Santos et al., 2017) can cause severe disease leading to high morbidity and mortality rates.

Leptospires are catalase and oxidase positive and most commonly cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium containing rabbit serum or enrichment at pH 6.8–7.4. Most suitable growth temperature ranges from 28˚–30˚C and subculture is done in every 7–10 days. The addition of selective agents like 5-fluorouracil or antibiotics such as polymyxin B, and neomycin are used to prevent contamination

3. Transmission

Transmission of leptospirosis is either by direct contact with infected animal or by indirect contact with soil or water contaminated with urine of infected animals (Haake & Levett, 2015) Rodents are major reservoir host for leptospires. A cut or abrasion on skin and exposure to water infected with urine of infected animals can lead to infection. Human to human transmission is rarely reported (WHO, 2003). Leptospires may

get settled in the kidneys and be shed in the urine for a long period ranging from a few weeks to many months. Transmission of leptospirosis is explained in Figure 1.

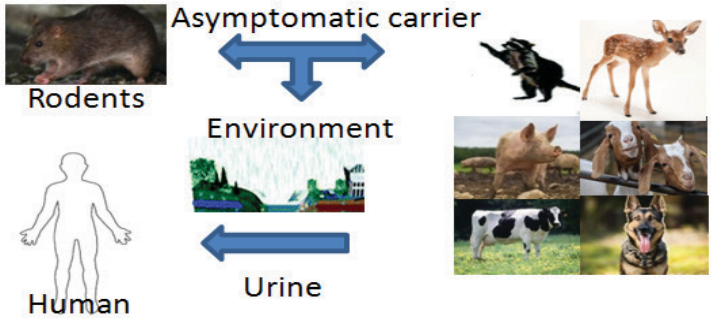


Figure 1. Transmission of leptospirosis: *Infected or carrier shedders contaminate the environment including water and soil which act as source of infection to other healthy animals as well as human beings*

4. Pathogenesis and Pathology

Incubation period usually lasts for 7–10 days but it can vary from 2–30 days. Initial phase is bacteremia phase in which symptoms like fever, headache, muscle pain, nausea and jaundice appear. The next phase is immune phase where anti-leptospiral antibodies are developed in the body but bacteria reside in renal tubules and is shed in urine of infected animals. Cell-mediated immunity is not important in case of leptospirosis but may be responsible for some of the late manifestation of the disease. Immunity is primarily humoral as the result of B-cell and T-helper cell stimulation. The disease-causing ability of *Leptospira* spp is usually attributed to the outer membrane proteins, such as LipL32. Also, the endotoxic potential of leptospiral LPS is significantly lower as compared to the average Gram-negative LPS (Fraga et al., 2007). Therefore, the proteins of leptospires outer membrane are thought to contribute more to their virulence and pathogenicity. The corkscrew-like motility permits their easy movement through more viscous barriers like

host connective tissues, adding to their highly invasive nature (Charon et al., 1992). According to Levett et al. (2006), leptospires can induce chronic renal diseases in the animal carriers; therefore, the urine of infected animals often contains huge numbers of the leptospires. These organisms gather at the convoluted tubules of kidney, colonizing and multiplying rapidly, whereas few are released via urination into the environment (Kurilung et al., 2017). In human beings, bacteria in the blood can sometimes invade even further to distant tissues leading to Weil's disease, an extremely severe form of the disease (Asensio-Sánchez et al., 2018). In cattle, infection with serovar Hardjo is less severe as cattle are considered to be maintenance host for this serovar where as other strains cause more illness in cattle. Infection in dairy cattle causes agalactia, abortion, still birth, reduced fertility, mortality in calves etc. It is most often caused by adapted strains from the Sejroe serogroup, and is associated to early embryonic losses and consequent oestrus repetition, very probably due to uterine inflammation and/or direct invasion of the embryo by leptospires (Mori et al., 2017; Libonati et al., 2018). Similar to other ruminants, abortion is the most important clinical consequence of leptospiral infection in caprine (Dehkordi & Taghizadeh, 2012). Pyrexia, reproductive problems/disorders, abortions, jaundice are common symptoms in cattle, sheep, goats and swine. In human beings, clinical signs and symptoms in leptospirosis are similar to other diseases like dengue, chikungunya, influenza etc.

5. Risk Factors

In humans, the risk factors associated with leptospirosis are occupational groups mainly farmers, abattoir workers, veterinarians, rice field workers and animal handlers or recreational activities like swimming and hunting. For animals. the important risk factors include shared grazing with common water resources, purchase or introduction of new 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, etc.

6. Diagnosis

The diagnosis of leptospirosis is challenging and cumbersome due to various reasons like need of sophisticated lab and well-trained laboratory personnel, clinical similarity with other infections like hepatitis, dengue, scrub typhus, etc. and lack of standard testing technique to detect infection at all stages. General strategy for the diagnosis of leptospirosis is given in Figure 2.

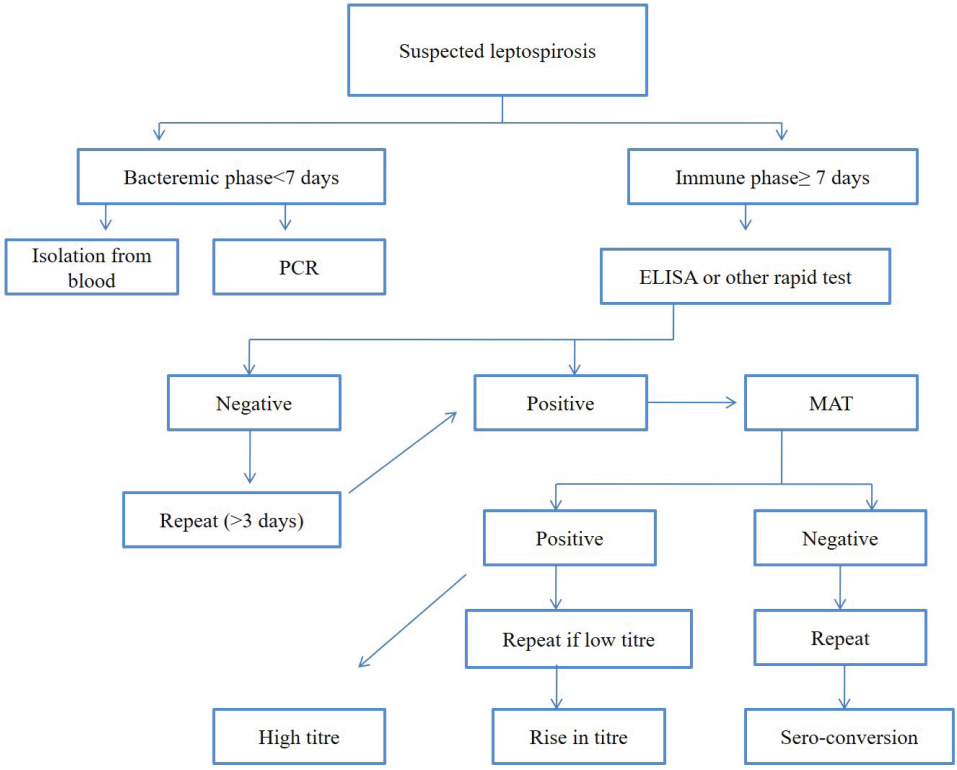


Figure 2. Strategy for the diagnosis of leptospirosis

There are 2 criteria for diagnosis: Presumptive diagnosis and Confirmatory diagnosis.

1. Presumptive diagnosis
    i. IgM based serological tests is positive.
    ii. Microscopic Agglutination Test (MAT) is positive.
    iii. Visualization of leptospires by Microscopy.
2. Confirmatory diagnosis
    i. Isolation of leptospires from clinical specimen or positive by PCR assay.
    ii. Four-fold or higher rise in the titre between paired serum samples.

6.1 Samples for Diagnosis

For diagnosis of leptospirosis, urine, cerebrospinal fluid, infected tissues, body fluids are preferred. At the initial stage of infection (1–7 days), leptospires can be found in the blood and in later stages when antibodies are produced in the body, urine can be used to diagnose the presence of leptospires and blood samples can be used to check antibodies. Details of different samples utilized for diagnosis is as follows:

6.1.1 Blood

Isolation of leptospires from blood sample can be done when blood is collected within 10 days of infection. After this period antibodies can be detected from serum sample. Blood should be collected by adding heparin or any other suitable anticoagulating agent. One or two drops of blood is inoculated into 10 ml of semi-solid EMJH media with 5-fluorouracil and incubated at 29°–30°C.

For antibodies detection, blood should be collected and serum should be isolated properly without lysis and contamination and stored at **4°C. The procedure** for separation of serum from blood is as:

1. Collect 5 ml of blood and let it clot at room temperature.
2. Without disturbing the clot collect the serum using pasture pipette.
3. If necessary, centrifuge the serum at 1000 × g for 5 minutes to remove cells.
4. Supernatant is transferred to sterile vial and then stored at -20°C.

6.1.2 Cerebrospinal Fluid (CSF)

CSF should be collected preferably in the first 10 days of infection. Leptospires can be visualized by dark field microscopy. Culture isolation is done by inoculating 0.5 ml CSF in 5 ml of semi-solid EMJH media and incubation for at least 2–3 months.

6.1.3 Urine

Midstream urine should be collected in sterile container and transported immediately as leptospires dies quickly in urine. The survival period can be increased by making urine neutral. For isolation the samples should be proceeded immediately by adding one to two drops in semi-solid EMJH medium. Some researchers use dilutions of urine (1:10, 1:100 or 1:1000 in PBS) for inoculation in EMJH media. Pellet from centrifuged urine sample is also used for PCR. Processing of urine sample is as described in Figure 3.

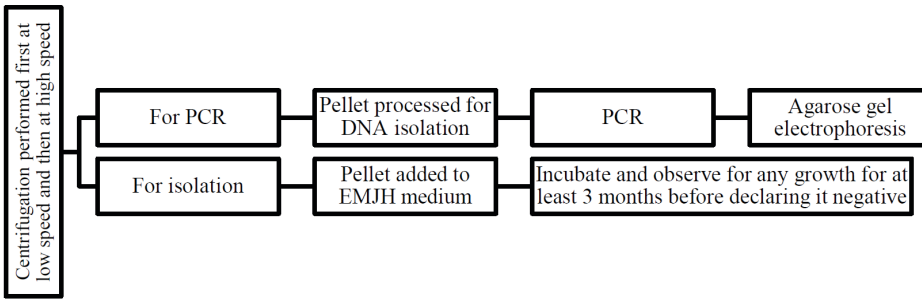


Figure 3. Processing of urine sample for diagnosis of leptospirosis

6.1.4 Post-mortem Samples

Post mortem tissue samples (lung, kidney, liver, heart blood etc) should be collected aseptically as soon as possible after death and transported at 4°C. It should be inoculated in EMJH for isolation of leptospires. Samples may be triturated in PBS and supernatant is inoculated in EMJH and kept at 29˚C for cultural isolation.

6.2 Diagnosis Methods

There are many techniques available for diagnosis of leptospirosis including direct methods like microscopic detection, culture isolation from clinical samples and indirect methods like molecular and serological methods. The molecular tools appear are proposed as the most significant methods for the diagnosis of the silent form of leptospirosis in domestic animals.

Different methods of diagnosis of leptospirosis are given in Figure 4.

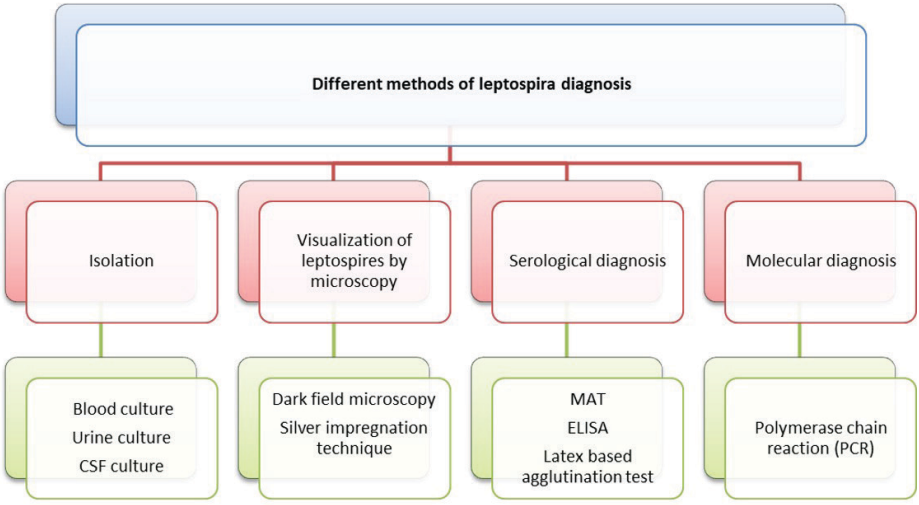


Figure 4. Different methods of diagnosis of leptospirosis

6.2.1 Direct Visualization Methods

6.2.1.1 Dark Field Microscopy

Dark field microscopy is used to detect leptospires from clinical samples. It is successful only when the bacteria are in blood which is the case only in early phase of infection. But it is not recommended as it gives false positive or false negative results. It also requires expertise to examine and its sensitivity decreases as infection progresses.

For examination of blood sample, a 5 ml of blood treated with an anticoagulant is centrifuged at 1000 g for 15 min. About 10 µl of plasma is examined under dark field microscope. Similarly for examination of urine, sample is centrifuged at 3000 g for 10 min. and a drop of the deposit is examined by dark field microscope (Figure 5).

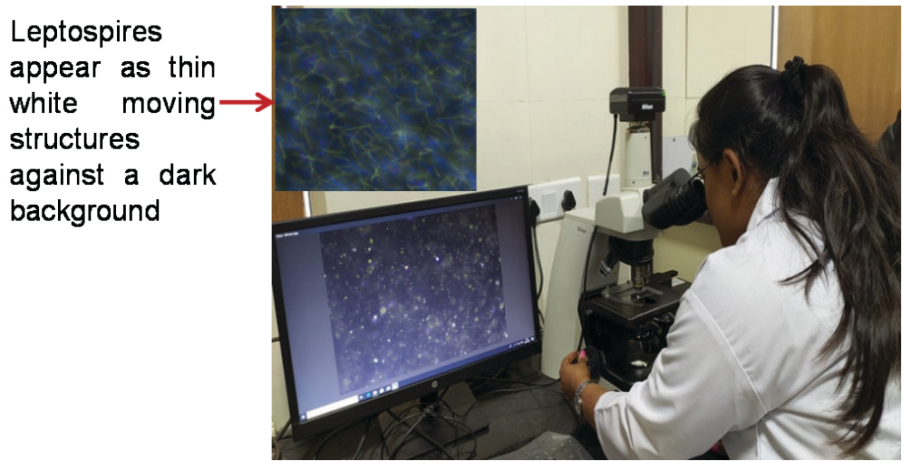


Figure 5. Dark field microscopy: *Leptospires are visualized as thin white motile structures against dark background*

6.2.1.2 Silver Impregnation Technique

This technique is used to stain leptospires in body fluids and tissues. Technique used is Fontana method and spirochetes are stained brownish black on a yellowish background. This technique lacks sensitivity and is not a preferred method of diagnosis. It is used only for academic purpose in

the teaching labs to demonstrate leptospires. Most common method used is modified Fontana silver staining technique as described by Gangadhar & Rajsekhar (1998). Briefly, smears are fixed by dipping in a fixing agent for two minutes containing 1 ml of Glacial acetic acid and 2 ml of 40% formaldehyde solution made up to 100 ml in distilled water. The slides are removed with the help of a pair of forceps and dried onto a tissue paper. Subsequently, the slides are merged in 100% alcohol for 2 minutes. The under surface of the slides are wiped using tissue paper and the smears are air dried. The slides are dipped in the mordant containing 1 g phenol and 5 g tannic acid made upto 100 ml with distilled water, in a hot water bath pre-heated to 75ºC and kept for 1 minute. The slides are rinsed in distilled water and dipped in ammoniated silver solution, which is pre-heated at 75ºC in a hot water bath and left for 1 minute. The ammoniated silver solution is prepared by dissolving 0.6 g of silver nitrate in 100 ml of distilled water. Sixty ml aliquot of this solution is mixed with few drops of 10% ammonia solution and shaken to obtain a brown precipitate. Further ammonia solution is added till the precipitate dissolved. Finally small quantities of silver nitrate solution are added from the 40 ml aliquot to the above ammoniated solution till a stable precipitate reappeared. After rinsing in distilled water, the slide is air dried and examined under oil immersion objective of a bright field microscope to observe dark brown leptospires against light yellow background.

6.2.2 Isolation

Leptospires can be obtained from blood and CSF samples during the first week of illness, and during the 2nd or 3rd 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. Growth in semi-solid medium is visible as typical Dinger's ring because leptospires tends to gather near the top due to their aerobic nature (Figure 6). Major drawbacks of the isolation method include low sensitivity, slow and time-consuming method. Various types of culture mediain use are:

1. EMJH (Ellinghausen-McCullough-Johnson-Harris media)
2. Stuart's medium
3. Fletcher's medium
4. Korthof's medium

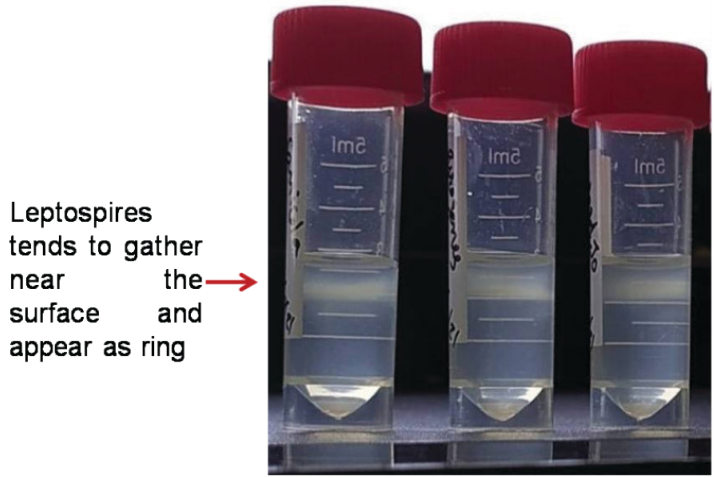


Figure 6. Cultures showing characteristic Dinger's ring. *Leptospires when grown in semi solid medium tends to form a ring or dense area due to their aggregation near the surface***.**

6.2.2.1 Isolation from Blood Sample

1. To 5 ml EMJH medium, 3–4 drops of blood are added.
2. The medium is visualized weekly upto 6–8 weeks for any growth.
3. To avoid contamination, 100–150 µg/ml of 5 FU may be added.
4. About 1% of rabbit serum can be added to medium for fastidious organisms.

6.2.2.2 Isolation from Urine Sample

1. Fresh midstream urine is collected and sample is tested within 2 hours of collection.
2. Urine is centrifuged and pellet is inoculated into culture medium.

3. Urine may be diluted in PBS in various dilutions ranging from 1:10 to 1:1000
4. Few drops of above dilutions are added in 5 ml medium tubes and incubated at 29˚C.
5. Tubes are viewed under dark field microscopy after 7–10 days and regularly thereafter for 6–8 weeks.
6. In case of contamination, filter can also be used and sub cultured into fresh medium.

6.2.3 Serological Diagnosis

6.2.3.1 Microscopic Agglutination Test (MAT)

MAT is often considered as gold standard test for the diagnosis of leptospirosis. 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 interval. This test is costly as there is 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 the leptospirosis as the antibodies will not be present in early stages of infection. The test is performed as follows (OIE, 2021)

1. For antigen preparation serovars are maintained in the laboratory at 30ºC.
2. 1:50 serum dilutions are made with PBS.
3. Dilutions are added to the 96 well plate.
4. 50 µl of 5–8 days old culture added to each well to make final 1:100 dilution of serum.
5. As control, a mixture of 50 µl of culture and 50 µl of PBS is used.
6. The plate is covered and incubated for 2 hours at 29–30ºC.

7. Results are observed for agglutination or reduction of cells (Figure 7). For this, a drop of mixture from each well is added to a slide and observed under dark field microscope.
8. The results of test sera are compared with control.
9. A 50% agglutination or reduction are considered as positive reaction.

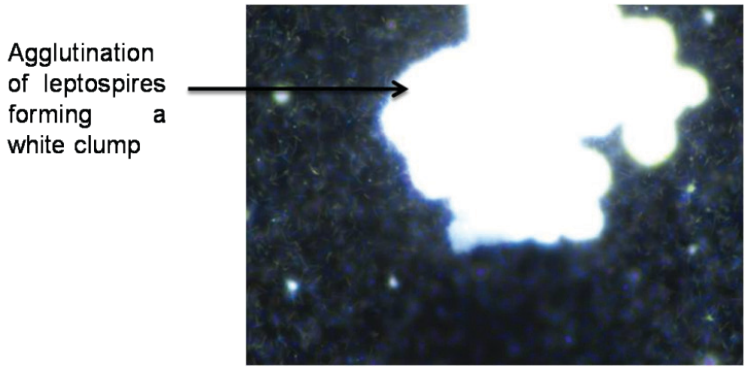


Figure 7. Microscopic agglutination test (MAT). *Leptospires are seen clumped as they are agglutinated by antileptospiral antibodies. A 50% agglutination or reduction in number of free cells as compared to control is considered as positive reaction*

6.2.3.2 ELISA

Leptospira specific IgM and IgG present in the serum can be detected by commercial kits or antigens derived from specific serovars maintained in laboratory. The reaction is recorded ELISA reader using an enzyme labelled anti-IgM or IgG. ELISA obviates the need for live cultures and can be automated. In commercial ELISA kits, antigen is usually used to detect presence of IgM, but IgG can also be detected. Bovine Leptospira ELISA kit (Linnodee) is a sandwich ELISA kit which detects the antibodies against LPS epitope of serovar Hardjo. Human Leptopira IgM ELISA kit (Panbio) is one of the most commonly used kit for detection of anti-leptospiral IgM antibodies and diagnosis of human leptospirosis. ELISAs have been developed using a wide variety of antigen preparations, from

leptospiral sonicates to recombinant lipoproteins such as LipL32, LigA, or the outer membrane porin OmpL1. However, OMPs based ELISA is a genus specific test and specific serovars cannot be determined.

6.2.4 Molecular Detection by Polymerase Chain Reaction

PCR involves amplification of leptospiral specific DNA that confirms its presence. It can help in the diagnosis of leptospirosis in the initial phase of the disease, at the time when bacteria is still present in the blood and antibody titres are yet not at detectable levels. The test does not require the presence of viable organisms and an early diagnosis can be performed, since bacterial DNA can be detected even before the development of a serological response to infection (Waggoner & Pinsky, 2016). There are many PCR protocols available for the detection of *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. Brown et al. (1995) created two sets of primers using genomic DNA libraries of *Leptospira* serovars Icterohaemorrhagiae (strain RGA) and bim (strain 1051). Merien et al. (1992) used *Leptospira interrogans* serovar Canicola *rrs* gene (16S) fragment of 331-bp for detection of leptospires and the PCR products were analyzed by DNA-DNA hybridization by using a 289-bp fragment internal to the amplified DNA. PCR amplicons also were obtained with DNA from the closely related nonpathogenic *Leptospira biflexa* but not with DNA from other spirochetes, such as *Borrelia burgdorferi, Borrelia hermsii, Treponema denticola, Spirochaeta aurantia,* or more distant organisms such as *Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis,* and *Proteus mirabilis.* Therefore, protocol of Merien and coworkers is a genus-specific assay which amplifies DNA from both pathogenic and non-pathogenic serovars (Figure 8). On the other hand, the approach described by Gravekamp et al. (1993) and evaluated by Brown et al. (1995), requires two sets of primers in order to detect all species containing pathogenic serovars. However, in recent times, lipL32 gene-based primers are one of the most commonly used targets for leptospires detection, with approximately 50% of researchers

using this marker. Real time PCR also has been developed for detection of pathogenic leptospires based on lipL32 gene with high specificity and sensitivity (Ahmed et al., 2020). PCR may yield false-positive results in case of contamination with DNA or give false-negative results due to inhibitors which are sometimes present in the clinical materials that are being examined.

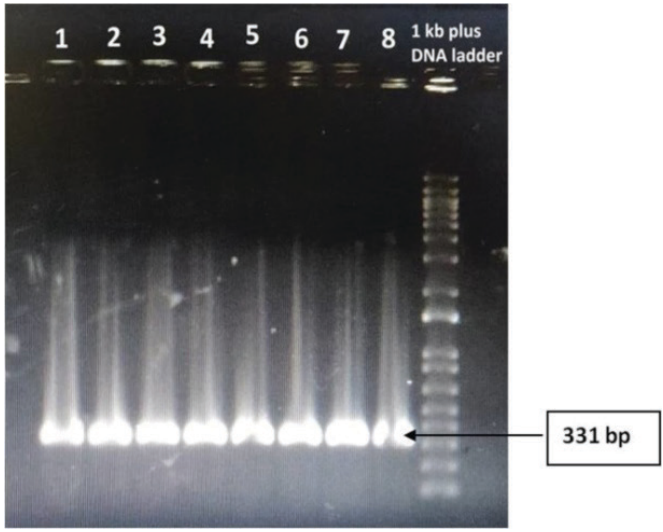


Figure 8. 16s rRNA gene-based PCR on reference serovars maintained in our lab showing positive amplicons in lanes 1-7. *Positive amplicons result in size of 331 bp specific to 16s rRNA gene (rrs). 1 kb plus ladder is run for the size estimation.*

7. Epidemiology: International and Indian Status

7.1 International Status

Leptospirosis occurs worldwide and has been reported in a number of countries of the South-East Asia Region from time to time. The magnitude of the leptospirosis differs from country to country and depends on awareness and attitude of public health care decision makers. Although, the global burden of leptospirosis is clearly unknown, more

than 500,000 human cases of leptospirosis are reported worldwide each year, with a fatality rate of up to 25% in some regions. Leptospirosis infects > 1 million people with around 6% mortality per year worldwide and has to be dealt with carefully for control and prevention (Chatterjee et al., 2017). The World Health Organization's (WHO's) Leptospirosis Burden Epidemiology Group estimated that there were 873,000 cases worldwide annually with 48,600 deaths in 2019.

There have been number of reports on leptospirosis worldwide, of which few latest are described in this monograph. A systemic review done by Munoz-Zanzi et al. (2020) reported a total of 318 outbreaks during 1970–2012 out of which 36% turned up in Latin America and the Caribbean region, 13% in Southern Asia followed by 11% in North America. Isolation is more abundant in South America, especially in Brazil and Argentina, due to the high animal species variability present in this geographic area. Another epidemiological study done by Galan et al. (2021) revealed that annually 3,810 cases of leptospirosis were reported with urban areas of Brazil having higher numbers.

Dogonyaro et al. (2020) randomly selected slaughterhouses to analyze the seroprevalence of leptospirosis in the cattle in South Africa and observed 27.6% positive samples by microscopic agglutination test (MAT). The highest detected serogroup in seropositive cattle was Sejroe (38.2%) whereas lowest was Canicola (1.8%).

In a recent study published from Australia, approximately one fourth of unvaccinated and some of the vaccinated pig-hunting dogs were found positive to leptospiral antibodies in serological examination using MAT (Orr et al., 2022). Most human cases from India, Indonesia, Thailand and Sri Lanka have been reported during the rainy season.

7.2 Indian Status

The overall seroprevalence of 12.7% in dairy cattle was observed in Maharashtra, Gujarat, Punjab, Tamil Nadu, Haryana, Telangana, Jharkhand, Chhattisgarh and Karnataka with highest prevalence of 30.4% in Maharashtra in a study conducted in 2016 (Balamurugan et

al., 2016). In another study, Balamurugan et al. (2018) reported the seropositivity of 70.51% in cattle having reproductive problems from different states which included Maharashtra, Gujarat, Andhra Pradesh, Telangana, Karnataka Tamil Nadu, Punjab, Haryana, Chhattisgarh, Sikkim and Uttarakhand and concluded that Hardjo is the most prevalent serovar among cattle in endemic states of India.

In south Gujarat seroprevalence in bovines was checked by using MAT as a diagnostic test and 12.81% seropositivity was reported (Patel et al., 2014). Pandian et al. (2015) found 9.11% seroprevalence of *L. serovar* Hardjo in cattle from nine districts of Bihar. A cross sectional survey done in the North Eastern states of India indicated high seroprevalence of *Leptospira interrogans* serovar Hardjo in cattle in the states of Bihar with 4.5% and Assam 1.2% in small scale dairy farms (Leahy et al., 2021). Alamuri et al (2019) studied 426 random serum samples from Prakasam, Kurnool, Guntur, Chittoor, Srikakulam, Visakhapatnam, and Godavari districts of Andhra Pradesh by screening Leptospira-specific antibodies by microscopic agglutination test which showed 68.08% (290/426) overall seropositivity with 70.8% (75/106) in cattle and 67.18% (215/320) in buffaloes.

In a 4-year-retrospective study in human beings to determine the seroprevalence of leptospirosis in Northern states of India by qualitative determination of anti-leptospira-IgM using ELISA kit, 100 out of total 1545 patients were seropositive. Interestingly, there was significant decline in leptospirosis seroprevalence from 26.90% in 2000–2010 and 20% in 2011–2014 to 6.47% in 2014–2018 (Agrawal et al., 2018).

In a recent study, recombinant leptospiral surface antigen (Lsa27) of pathogenic *Leptospira* were evaluated as a diagnostic antigen for the detection of anti-leptospiral antibodies in the human sera using latex agglutination test (Alamuri et al., 2021). A diagnostic sensitivity of 90.48% and diagnostic specificity of 91.35% with an accuracy of 90.98% was observed in a substantial agreement against MAT which is the gold standard test for leptospirosis.

8. Treatment

Treatment with effective antibiotics should be started as soon as onset of disease. In adults doxycycline 100 mg twice a day for seven days is given. Severe cases can be treated with intravenous penicillin. Minor cases can also be treated with amoxycillin or erythromycin. In case of children, amoxycillin or ampicillin for 7 days should be given @30–50 mg/kg/day in divided doses. In animals, tetracycline, erythromycin, enrofloxacin, may be given. In cattle streptomycin/dihydrostreptomycin intramuscularly has also been tried.

9. Prevention and Control

1. Field workers should wear rubber shoes and gloves to avoid direct contact with contaminated mud and water.
2. During rainy season, 200 mg of doxycycline once a week for 6 weeks should be taken by at risk individuals.
3. As rodents are the natural reservoirs of leptospirosis, adopting control methods of rodents before rainy season can cause significant difference.
4. Discharge of urine of animals in water bodies should be avoided because it may be the source of infection to other species.

10. Referral Laboratories

Various referral laboratories at national level are:

1. Regional Medical Research Center (ICMR), Port Blair (A&N), Tel: 03192- 251158/251159
2. National Centre for Disease Control, 22-Sham Nath Marg, Delhi, Tel: 011- 23971272/23971060

3. National Institute of Epidemiology, Chennai,Tel: 044-26820517, 044- 26821600
4. ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, Karnataka. Tel:080 2309 3110
5. Bacteriology & Mycology Division, IVRI, Izatnagar, UP, 243122 Tel: 0581- 2301865.
6. Zoonoses Research Laboratory, Tamil Nadu Veterinary& Animal Science University, Chennai, Tel: 044- 25362787; 044-2530 4000

References

1. Adler, B., & de la Peña Moctezuma, A. (2010). Leptospira and leptospirosis. *Veterinary Microbiology, 140(3-4)*, 287-296.
2. Agrawal, S. K., Chaudhry, R., Gupta, N., Arif, N., & Bhadur, T. (2018). Decreasing trend of seroprevalence of leptospirosis at All India Institute of Medical Sciences New Delhi: 2014-2018. *Journal of Family Medicine and Primary Care, 7(6)*, 1425–1428. https://doi.org/10.4103/jfmpc.jfmpc_198_18.
3. Ahmed, A.A., Goris, M.G.A. & Meijer, M.C. (2020). Development of lipL32 real-time PCR combined with an internal and extraction control for pathogenic Leptospira detection. *PLoS ONE 15(11)*: e0241584. https://doi.org/10.1371/journal.pone.0241584.
4. Alamuri, A., Thirumalesh, S., Kumari, S.S., Kumar, K.V., Roy, P., & Balamurugan, V. (2019). Seroprevalence and distribution of serogroup-specific pathogenic Leptospira antibodies in cattle and buffaloes in the state of Andhra Pradesh, India. *Veterinary World, 12(8)*, 1212–1217. https://doi.org/10.14202/vetworld.2019.1212-1217.
5. Alamuri, A., Vinod K.K., Varghese, B., Palkhade, R., Mahadeviah, S.N., Chaudhari, S., Roy, P., & Balamurugan, V. (2021). Evaluation of recombinant leptospiral surface antigen (Lsa27) lipoprotein for

serodiagnosis of human leptospirosis by latex agglutination test. *Indian Journal of Medical Microbiology, 39(2),* 212–217. https://doi.org/10.1016/j.ijmmb.2021.01.006.

6. Asensio-Sánchez, V. M., Haro-Álvarez, B., Herreras, J., & Martín-Prieto, A. (2018). Unusual ocular clinical manifestation of leptospirosis. *Archivos de la Sociedad Española de Oftalmología (English Edition), 93(7)*, 342-346.
7. Balamurugan, V., Alamuri, A., Veena, S., Bharathkumar, K., Patil, S.S. & Govindaraj, G. (2016). Investigation on the prevalence of *Leptospira* serovar Hardjo in organized cattle dairy farms of India. *Indian Journal of Animal Sciences, 86,* 1145-1147.
8. Balamurugan, V., Alamuri, A., Bharathkumar, K., Patil, S.S., Govindaraj, G.N., Nagalingam, M., Krishnamoorthy, P., Rahman, H., & Shome, B.R. (2018). Prevalence of Leptospira serogroup-specific antibodies in cattle associated with reproductive problems in endemic states of India. *Tropical Animal Health and Production, 50(5)*, 1131–1138. https://doi.org/10.1007/s11250-018-1540-8.
9. Benacer, D., Who, P.Y., Zain, S.N.M., Amran, F.& Thong, K.L. (2013). Pathogenic and saprophytic Leptospira species in water and soils from selected urban sites in peninsular Malaysia. *Microbes and Environments, 28,* 135–140.
10. Brown, P.D., Gravekamp, C., Carrington, D.G., van de Kemp, H., Hartskeerl, R.A., Edwards, C.N., Everard, C.O., Terpstra, W.J., &Levett, P.N. (1995). Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. *Journal of Medical Microbiology, 43(2)*, 110–114. https://doi.org/10.1099/00222615-43-2-110.
11. Charon, N.W., Greenberg, E.P., Koopman, M.B.H.&Limberger, R.J.(1992). Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. *Research in Microbiology, 143,* 597–603.
12. Chatterjee, P., Bhaumik, S., Chauhan, A. S., &Kakkar, M. (2017). Protocol for developing a database of zoonotic disease research in India (DoZooRI). *BMJ open, 7(12)*, e017825.

13. Dehkordi, F.S., &Taghizadeh, F. (2012). Prevalence and some risk factors associated with brucellosis and leptospirosis in aborted fetuses of ruminant species. *Research Opinions in Animal and Veterinary Sciences, 2,* 275-281.

14. Dogonyaro, B.B., van Heerden, H., Potts, A.D., Kolo, B.F., Lotter, C., Katsande, C., Fasina, F.O., Ko, A.I., Wunder, E.A., Jr, & Adesiyun, A.A. (2020). Seroepidemiology of Leptospira infection in slaughtered cattle in Gauteng province, South Africa. *Tropical Animal Health and Production, 52(6),* 3789–3798.

15. Dos Santos, L.F., Guimarães, M.F., de Souza, G.O., da Silva, I.W.G., Santos, J.R., Azevedo, S.S., Labruna, M.B., Heinemann, M.B. & Horta, M.C. (2017). Sero-epidemiological survey on Leptospira spp. infection in wild and domestic mammals in two distinct areas of the semi-arid region of northeastern Brazil. *Tropical Animal Health and Production, 49,* 1715–1722.

16. Fraga, T.R. Barbosa, A.S. & Isaac, L. (2007). Leptospirosis: Aspects of innate immunity, immunopathogenesis and immune evasion from the complement system. *The Scandinavian Journal of Immunology, 73,* 408–419.

17. Galan, D.I., Roess, A.A., Pereira, S.V.C., & Schneider, M.C. (2021). Epidemiology of human leptospirosis in urban and rural areas of Brazil, 2000–2015. *PLoS ONE, 16(3)*, e0247763. https://doi.org/10.1371/journal.pone.0247763

18. Gangadhar, N.L., & Rajasekhar, M. (1998). A modified silver impregnation staining for leptospires. *Indian Veterinary Journal, 75,* 349-351.

19. Gravekamp, C., Van de Kemp, H., Franzen, M., Carrington, D., Schoone, G. J., Van Eys, G. J., Everard, C. O., Hartskeerl, R. A., & Terpstra, W. J. (1993). Detection of seven species of pathogenic leptospires by PCR using two sets of primers. *Journal of General Microbiology, 139(8),* 1691–1700. https://doi.org/10.1099/00221287-139-8-1691.

20. Haake, D.A., & Levett, P.N. (2015). Leptospirosis in humans. *Current Topics in Microbiology and Immunology, 387,* 65–97.

21. Hartskeerl, R.A., Collares-Pereira, M., & Ellis, W.A. (2011). Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clinical Microbiology and Infection, 17(4)*, 494-501.

22. Johnson, D. I., & Beck. (2018). Bacterial pathogens and their virulence factors. *Springer* (pp. 363-379).

23. Kurilung, A., Chanchaithong, P., Lugsomya, K., Niyomtham, W., Wuthiekanun, V. & Prapasarakul, N. (2017). Molecular detection and isolation of pathogenic Leptospira from asymptomatic humans, domestic animals and water sources in Nan province, a rural area of Thailand. *Research in Veterinary Science, 115*, 146-154.

24. Lau, C.L., Smythe, L.D., Craig, S.B., & Weinstein, P. (2010). Climate change, flooding, urbanisation and leptospirosis: fuelling the fire?. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 104(10)*, 631-638.

25. Levett, P.N., Morey, R.E., Galloway, R.L.&Steigerwalt, A.G. (2006). Leptospira broomii sp. nov., isolated from humans with leptospirosis. *The International Journal of Systematic and Evolutionary Microbiology, 56,* 671–673.

26. Leahy, E., Shome, R., Deka, R.P., Grace, D., Sahay, S. & Lindahl, J.F. (2021) Leptospira interrogans Serovar Hardjo Seroprevalence and Farming Practices on Small-Scale Dairy Farms in North Eastern India; Insights Gained from a Cross-Sectional Study. *Dairy, 2*, 231–241. https://doi.org/10.3390/ dairy2020020.

27. Libonati, H. A., Santos, G. B., Souza, G. N., Brandão, F. Z., &Lilenbaum, W. (2018). Leptospirosis is strongly associated to estrus repetition on cattle. *Tropical Animal Health and Production, 50(7)*, 1625-1629.

28. Lim, V.K.E. (2011). Leptospirosis: a re-emerging infection. *The Malaysian Journal of Pathology, 33(1)*, 1.

29. Loureiro, A.P., &Lilenbaum, W. (2020). Genital bovine leptospirosis: A new look for an old disease. *Theriogenology, 141,* 41-47.

30. Mérien, F., Amouriaux, P., Perolat, P., Baranton, G., & Saint Girons, I. (1992). Polymerase chain reaction for detection of Leptospira spp. in

clinical samples. *Journal of Clinical Microbiology, 30(9),* 2219–2224. https://doi.org/10.1128/jcm.30.9.2219-2224.1992

31. Mori, M., Bakinahe, R., Vannoorenberghe, P., Maris, J., de Jong, E., Tignon, M., Marin, M.&Desqueper, D. et al. (2017) Reproductive disorders and leptospirosis: a case study in a mixed-species farm (cattle and swine). *Veterinary Science, 4*,64.
32. Munoz-Zanzi, C., Groene, E., Morawski, B.M., Bonner, K., Costa, F. & Bertherat, E. (2020). A systematic literature review of leptospirosis outbreaks worldwide, 1970–2012. *Revista Panamericana de Salud Pública*, *44*: e78. https://doi.org/10.26633/RPSP.2020.78
33. Nally, J.E., Hornsby, R.L., Alt, D.P., Bayles, D., Wilson-Welder, J.H., Palmquist, D.E., & Bauer, N.E. (2018). Isolation and characterization of pathogenic leptospires associated with cattle. *Veterinary Microbiology, 218*, 25-30.
34. O'Toole, S.M., Pathak, N., Toms, G.C., Gelding, S.V., & Sivaprakasam, V. (2015). Fever, jaundice and acute renal failure. *Clinical Medicine, 15(1)*, 58.
35. Office International des Épizooties (2021). OIE Terrestrial manual. Chapter 3.1.12: 1-13.
36. Orr, B., Westman, M.E., Malik, R., Purdie, A., Craig, S.B. & Norris, J.M. (2022) Leptospirosis is an emerging infectious disease of pig-hunting dogs and humans in North Queensland. *PLoS Neglected Tropical Diseases 16(1)*: e0010100. https://doi.org/10.1371/journal.pntd.0010100.
37. Patel, J., Vihol, D., Prasad, M., Kalyani, I., Raval, J., Patel, K., Assadi, S. & Balamurugan, V. (2014). Seroepidemiological pattern of leptospirosis in bovine of South Gujarat, India. *Veterinary World. 7.* 999-1003. 10.14202/vetworld.2014.999-1003.
38. Pandian, S.J., Ray, P.K., Chandran, P.C., & Kumar, M. (2015). Seroprevalence of Brucella abortus and Leptospira hardjo in cattle. *Veterinary World, 8(2)*, 217–220. https://doi.org/10.14202/vetworld.2015.217-220

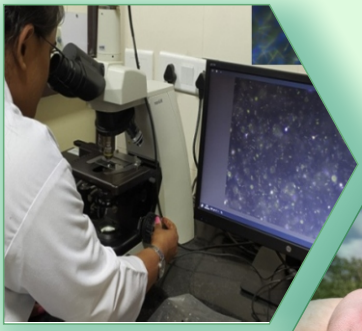
39. Tilahun, Z., Reta, D., & Simenew, K. (2013). Global epidemiological overview of leptospirosis. *International Journal of Microbiology Research, 4(1)*, 9-5.

40. Waggoner, J.J., & Pinsky, B.A. (2016). Molecular diagnostics for human leptospirosis. *Current Opinion in Infectious Diseases, 29(5)*, 440.

❑❑❑

Monograph

Leptospirosis (An Emerging Zoonosis)





Scan this QR to view all our books

IP Innovative Publication Pvt. Ltd.

A-2, Gulab Bagh, Nawada, Uttam Nagar
New Delhi-110059
Web: www.ipinnovative.com

For Joining us on facebook.com/InnovativePublicationIndia

ISBN 978-93-91208-72-1