Dog-mediated Leptospirosis

27

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ABSTRACT

Leptospirosis is a waterborne zoonotic melody faced globally by both animals and humans. Based on the emergence and losses caused by leptospirosis, it is a recurring and neglected disease of public health importance all over the globe. Lack of public awareness and negligence on a massive scale combined with expeditious and unplanned urbanization in developing countries cause the re-emergence of this disease under unsanitary conditions. As most wild and domestic animals can be the carriers of the pathogen causing this acute febrile illness, everyone is at risk of getting infected including all the healthcare professionals, pet owners, farmers, workers and volunteers at animal daycare centres and shelters for stray animals, fishermen, sanitary workers, rodent catchers, sewage cleaners, etc. The clinical signs and symptoms include a variety of flu-like to acute kidney failure in severe or untreated cases. Typical cases of leptospirosis depict signs like pneumonia, pulmonary haemorrhages and jaundice but many cases are reported worldwide with very rare and uncommon clinical manifestations. This chapter will cover all the possible aspects of dog-mediated leptospirosis from the morphology of the pathogen, its transmission, occurrence, clinical signs, diagnosis and prevention of this disease.

Key word: Leptospirosis, Animal, Haemorrhages, Jaundice, Prevention, Awareness.

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1. INTRODUCTION

Leptospirosis is one of the most underrated tropical diseases. It is a waterborne malady that can spread from animals to humans by infection of spirochetes belonging to the genus Leptospira (Chatterjee et al. 2017; Esteves et al. 2018). The recently published reviews and reports have stated that leptospirosis is nominated as a major emerging and recurring disease by the World Health Organization (WHO). It is widespread among humans and animals all across the world (Wasiński and Dutkiewicz 2013; Chatterjee et al. 2017). More than 350 serovars and 30 serotypes of leptospiral strains both pathogenic and saprophytic have been identified till now (Trott et al. 2018). Leptospira has infected more than a million people worldwide. It is also responsible for about 60,000 deaths worldwide each year (Thibeaux et al. 2018). Researchers suspect that the actual load of the disease of leptospirosis could be greater than the value of the estimates about it (Herman et al. 2016). There are several reasons for that, some important factors in this regard for this situation are probably as follows:

(i) Fewer cases are reported about the actual number

(ii) Established point-of-care-test for diagnosis; therapies and prevention of the disease are not operating effectively

(iii) The inaccurate reporting has led to a seemingly doubtful epidemiologic situation

(iv) Poor hygiene practices, insufficient sanitation, and rapid urbanization of countryside regions without proper planning (Thornley et al. 2002; Kamath and Joshi 2003; Vijayachari et al. 2008; Bandara et al. 2014).

Reports by researchers show that Leptospira was first mentioned by DJ Larrey in 1812. He reported it in Cairo among the troops of Napoleon's army. However, in 1886 Adolph Weil reported the presence of the same organism in kidney tissues. Later on, he named the infection after himself calling it "Weil's disease." Arthur Stimson was the first one to describe and differentiate spirochetes from one another in 1907. He worked on differentiating the *Leptospira interrogans* from other types of spirochetes such as the *Spirochaeta interrogans* from the luminal portion of the kidneys. These kidney tissues were sampled from yellow fever patients. He studied leptospires extract from kidney tissues by using Levaditi silver deposition. The kidney tissues he mostly studied were sampled from people who had died of yellow fever. Hubener, Uhlenhuth, and Fromme in Germany and Noguchi and Inada in Japan simultaneously experimented with transmitting leptospirosis to a guinea pig in 1915. Later on, they were able to successfully isolate the leptospira from infected guinea pigs marking the success of the experiment. Ido et al., reported in 1917 that rats are the carriers of leptospires. Leptospira was identified as the etiological agent of cattle yellow fever in 1940. In the 1980s, leptospirosis was being thoroughly documented only as a disease of veterinary aspect with a major economic impact on cattle, dogs, swine, perhaps sheep, and horses. Many pieces of research regarding Leptospira and leptospirosis have been published starting from this era till the present. Latest advancements in terms of next-generation sequencing (NGS), DNA studies, and genome sequencing have helped researchers comprehend the Leptospira organism and its infection mechanism at the base molecular level (Stimson 1907; Levett 2001; Adler and de la Peña Moctezuma 2010; Trivedi and Kamath 2010; Taylor et al. 2015; Goarant 2016; Kim 2019). The disease was known by many names at the time before its proper identification in several regions. Different regions had different names for it. Chinese used to call it "rice field jaundice". On the other hand, Japanese called leptospirosis "autumn fever" or "seven-day fever." Some other names traditionally used for leptospirosis were "Cane-cutter's disease," "swine-herd's disease, and "Schlammfieber" (mud fever). These names were formed in association with the occupations of the infected patients. Generally, leptospirosis is also known as canicola fever, Fort Bragg fever, cane-field fever, 7 days fever, rat catcher's yellows, Weil's disease, nanukayami fever, harvest fever, field fever, mild fever, pretibial fever, etc. Leptospirosis is mostly prevalent in countries that are tropical and humid or have subtropical climates. It is known to be prevalent in regions of tropic climate. Currently, leptospirosis is also being seen in other areas such as temperate regions. Its appearance in other areas can be

attributed to various factors such as human migration and climate change due to deforestation, urbanization with poor and unplanned sanitation systems, improper waste disposal mechanisms, and lack of hygiene management (Desai et al. 2009; Costa et al. 2015; Dunay et al. 2016). Transmission of leptospira is favoured in regions with tropical climates. The biological load and spread of leptospirosis are still underscored due to underreporting incidence. Hence its re-emergence is often observed to occur notably more than other maladies and in various regions of the world (Holla et al. 2018). During the past two decades, leptospira infections have been on the rise in the southern region of Indian territory. These regions include areas of the Andaman Islands, Kerala, and Tamil Nadu (John 1996). Recently, leptospirosis infections have reached a score of DALY which is around 2.90 million per year. It has been reported worldwide that more males are infected by leptospira with numbers going over 2.33 million per year. The main reason for this increase is the existence of occupational risks. Leptospira infections also impact the economic status of developing countries burdening their financial resources. The biological burden of leptospirosis has been identified to be much greater than filariasis and rabies which carry a DALY score of 42/100000 as tropical and neglected maladies (Costa et al. 2015; Taylor et al. 2015; Torgerson et al. 2015; Goarant et al. 2019).

2. MORPHOLOGY & CLASSIFICATION OF PATHOGEN

Morphologically Leptospires have hair-like forms on their bodies and are slender commas or spiral in shape. Leptospira can be distinguished from other spirochetes based on their characteristic hooks on both ends of the body. The length of Leptospira ranges from 6 to 20 µm and they have an average diameter of around 0.15 µm. They possess a specific periplasmic endoflagella which contributes to its peculiar cork-screw-like movement differentiating it from other spirochetes and are also responsible for their pathogenicity (Slamti et al. 2011). High viscosity of fluids leads to high swimming speed of Leptospira species (Trueba et al. 2004). Their fragile structural makeup makes them best viewed under stain-free dark-field microscopy. Leptospires have characteristics of both gram-positive and gram-negative bacteria as they are gram-variable in appearance. Leptospira has lipopolysaccharides (LPS) on its surface, which adds to its virulence, in contrast to other important spirochete genera like Treponema or Borrelia which don't possess such layer. Leptospira virulence factors that may be involved in the infection include adhesion molecules, lipopolysaccharides (LPS), hemolysins, outer membrane proteins (OMPs), and other surface proteins (Adler and de la Peña Moctezuma 2010). Initially, due to being 8-azaguanine and low-temperature growth Leptospira were broadly categorized under the saprophytic biflexa and pathogenic interrogans groups. Later, as science advanced, serological and genetic traits were used to classify it. There are now three main clinically significant groups of Leptospira that cause leptospirosis in humans. There are 16 pathogenic strains in the interrogans group associated with human and animal infection. Among these strains, nine species of pathogens are linked to human leptospirosis. Mild or chronic infection-causing pathogens also known as host-mediated pathogens are included in the intermediate group (Chiriboga et al. 2015; Trott et al. 2018). There are fourteen nonpathogenic or saprophytic strains in the saprophytic or biflexa group, including *L. biflexa*; and *L. wolbachii* which are not involved in causing leptospirosis in animals or humans (Bharti et al. 2003; Trueba et al. 2004; De Brito et al. 2018; Trott et al. 2018; Escandón-Vargas et al. 2019).

3. TRANSMISSION

Direct contact is the main route of transmission of Leptospira rather than indirect contact. Indirect contact of infection is transmitted by contaminated water or soil by incidental and/or by carrier mammals, from which humans get Leptospira infection (De Brito et al. 2018). Many wild, domestic, and

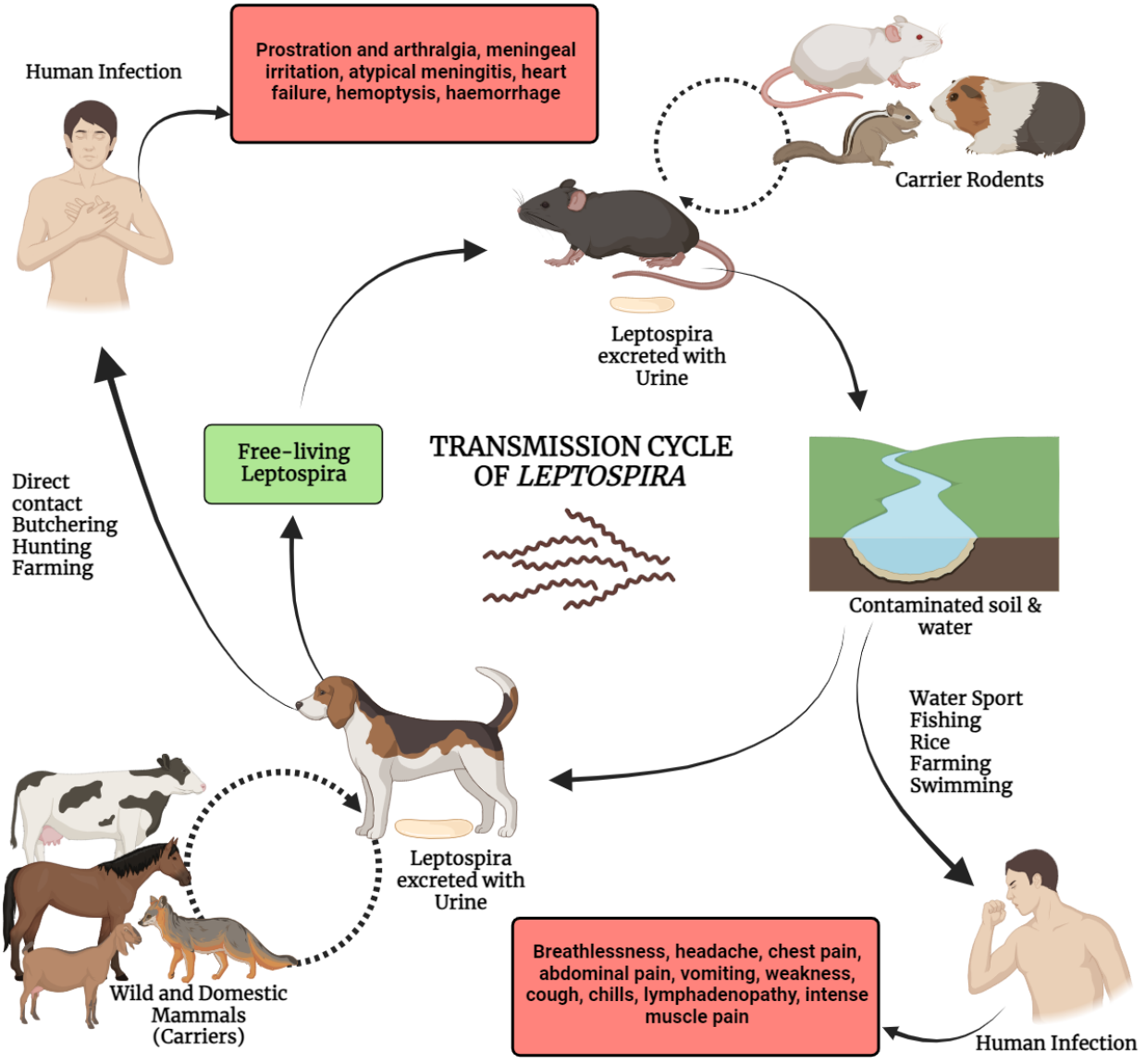

Fig. 1: Transmission cycle of *Leptospira*.

pre-domestic animals are asymptomatic carriers of Leptospira (Fig. 1). They carry a variety of Leptospira species in their renal tubules and within 2 weeks to a few months release them in urine. Leptospira may survive for a lifetime in the environment (Witchell et al. 2014; Herman et al. 2016). In urban slum areas, the infection mainly spreads via rats. They behave as asymptomatic carriers and contaminate soil and water sources with their urine. According to a study carrier rats disseminate infection at the rate of approximately 104-107 Leptospires/1 thus helping in the infection cycle as a reservoir (Witchell et al. 2014). Outbreaks of leptospiral infections are often linked to storms and floods after periods of intense rainfall when leptospires are swept into water bodies from the urine-contaminated soil. Leptospires can be kept far from natural water systems such as lakes, rivers, ponds, puddles, water dams, springs, cosmetic fountains, sewage, wet soil, and agricultural fields (Rawlins et al. 2014; Escandón-Vargas et al. 2019). Rain produces an alkaline pH that promotes leptospira growth and survival in the environment by washing away the fertilizer from the soil during floods etc. (Shekatkar et al. 2010).

For weeks to years, pathogenic leptospires may thrive in freshwater and damp soil, particularly when the pH is mildly alkaline (Trueba et al. 2004). Leptospires' deleterious effects on soil are highly underestimated as it is considered only a waterborne disease (Costa et al. 2012; Casanovas-Massana et al. 2018). The main route of infection in humans is pre-cutaneous from contaminated water bodies and soil and less commonly through inhalation and consumption during work-related or recreational pursuits. Seldom are reports of indirect transmission via animal bites and interhuman transmission (Sharma and Kalawat 2008; Musso and La Scola 2013). The following are the risk factors associated with Leptospira:

(1) Not taking precautionary measures while travelling to an area where Leptospira is endemic

(2) Exposure while working in the veterinary profession, agriculture, animal caretakers, fishermen, abattoirs, gardeners, rice mill workers, and sewage workers.

(3) Freshwater sports participants during canoeing, surfing, and caving.

(4) Volunteers who assist in disaster relief operations in flood-hit areas (Londeree 2014; de Sainte Marie et al. 2015; Desai et al. 2016; Pissawong et al. 2020). Leptospirosis in slum areas is related to exposure to infected rats and improper hygiene and drainage systems (Costa et al. 2014; Hagan et al. 2016; Santos et al. 2017).

4. PATHOGENESIS

The pathogenesis and mode of transmission of Leptospirosis is not well understood although it was reported two centuries ago. However, a lot of genes have been checked to understand their function and their role in the pathogenicity and immunogenicity of the causative agent. This was achieved through research using bioinformatics and molecular approaches. The virulent genes of Leptospira are unique to it and are not found in any other bacterial species and also possess a distinctive virulence system. The genetic analysis and comparison of pathogenic and saprophytic leptospira species expressed approximately 900 distinct genes to pathogenic leptospira species. Important proteins like H-binding proteins that attach to sphingomyelinase, extracellular matrix laminin, hemolysins etc, proteins needed for motility, flagella, and chemotaxis, and virulence-producing proteins like OMP and LPS are all encoded by these genes (Picardeau et al. 2008; Adler et al. 2011). Leptospira manifests its infection in two stages:

(i) mild anicteric phase.

(ii) classical icteric phase.

The former exhibits a slight infection that is usually self-limiting and treatment is also not needed in most cases. Anicteric infection covers approximately 80-90% of leptospira infections (Levett 2001). It consists of an incubation period of 1 to 2 weeks with a range of 2 to 30 days after which characteristic clinical signs begin to appear (Lau et al. 2010). It is present in the environment and enters the body through cuts, abrasions, or contact with mucous membranes. Followed by its entry into the host body, it passes through the membranes and enters the bloodstream with the help of chemotactic factors (Fig. 2). It usually occurs within 2 to 7 days of inoculation.

The main chemoattractants include haemoglobin, long-chain fatty acids, pyruvates, and sugars nervous system, and lungs. However, the main diagnostic factor of Leptospira infection is the infection in the hepatorenal system. According to studies, various Leptospiral proteins are involved in virulence and pathogenicity (Yuri et al. 1993; Lambert et al. 2012; Affroze et al. 2016). They attack the defense mechanism of the host cell by attachment to the fibrinogen network and extracellular matrix (ECM) proteins. According to studies, some toxic substances and proteins are produced by the pathogen which damages the cell membrane and destroys the host cells' vascular network (Martinez-Lopez et al. 2010; Evangelista et al. 2014; De Brito et al. 2018). They are also involved in the production of several virulence factors which play a major role in pathogenesis. These factors include phospholipase, immunoglobulin-

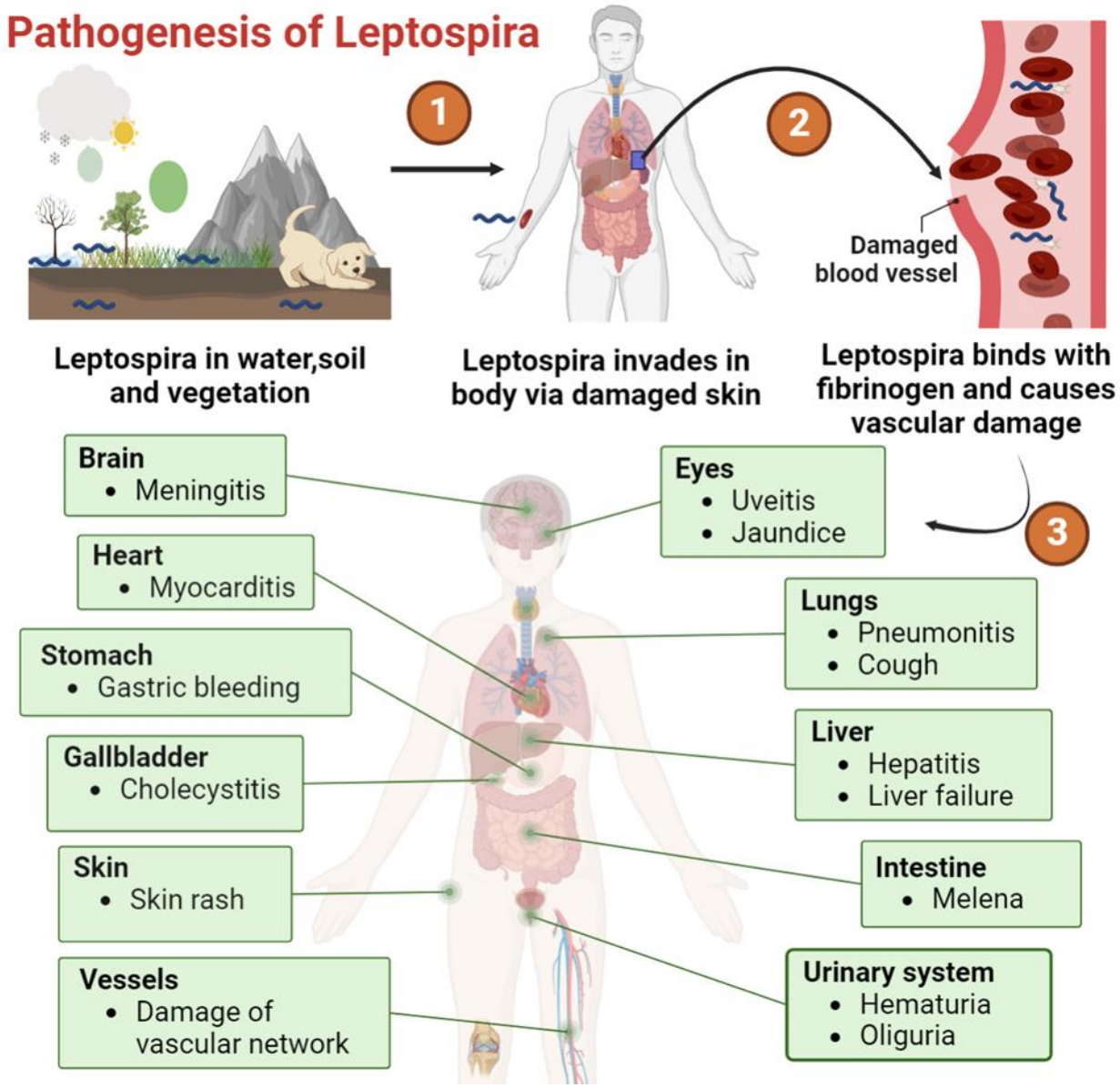

Fig. 2: Pathogenesis of *Leptospira*.

like proteins Lig A and Lig B, lipoproteins, hemolysin, sphingomyelinase, other putative outer proteins, hyaluronidase, collagenase, surface adhesion proteins (del Real et al. 1989; Segers et al. 1990; Atzingen et al. 2008; Gomez et al. 2008; Figueira et al. 2011; Shylaja et al. 2011; Kassegne et al. 2014). The acute septicemic stage in patients includes leptospiremia and septicemia 90% of patients diagnosed with anicteric leptospirosis normally recover. But the infection is remittent and appears again causing haemorrhages of vital organs like kidneys, liver, intestines, lungs, etc, and extreme infection after 2 to 3 days although the patient might have recovered from other signs and fever. Patients in later stages of infection exhibit uveitis, meningitis, and rashes (Rajapakse et al. 2015). The second form of leptospirosis called the classical form is also known as Weil's disease named after the scientist who discovered it. It mainly affects the liver and affects 5-10% of individuals approximately. In this severe form of infection,

erythrocytes can be seen in urine resulting in anuria and oliguria due to the invasion of hepatics and alteration in the function of aminotransferases and leukocytes by the pathogens. There is an increase in the concentration of creatinine and urea which serve as major indicative of Leptospira infection. The infection is systemic and involves major systems like the gastrointestinal tract, and hepato-renal system organisms (Natarajaseenivasan et al. 2012). In Leptospiruria, pathogens appear in urine between 7 to 30 days of infection. Leptospires inhabit and multiply within the renal tubules and continue to be within blood circulation adding to the longevity of infection. Patients mostly recover with proper treatment. Treatment will improve after completely understanding the pathogenesis (Tullu and Karande 2009; De Brito et al. 2018).

5. CLINICAL SYMPTOMS

Because leptospirosis produces varied signs, it might be challenging to distinguish it from other etiologies that cause acute undifferentiated febrile illness (AUFI). Leptospirosis can produce a wide range of clinical signs from flu-like symptoms to multiple organ failure. Acute febrile illness, breathlessness, headache, chest pain, abdominal pain, vomiting, weakness, cough, chills, lymphadenopathy, distinct and intense muscle pain that is limited to thighs, back or calf muscle, prostration and arthralgia, accompanied by any of the following: meningeal irritation, atypical meningitis, heart failure, hemoptysis, haemorrhages (from the lung, intestines, etc.) and less commonly rashes on the skin are the symptoms that usually appear during the initial stages of infection that are related to leptospiremia and lasts for approximately 7 days (Ding et al. 2001; Lin et al. 2008; Bhatia et al. 2015; Holla et al. 2018). The symptoms of the acute phase, observed after weeks or even years of illness include conjunctival suffusion or uveitis (Verma and Stevenson 2012). Leptospiral uveitis is linked with Lru A and B gene product proteins. The protein products of Leptospiral genes A and B are responsible for causing Leptospiral uveitis (Faber et al. 2000; Shylaja et al. 2011). In equines, uveitis is intermittent and occurs more frequently (Pearce et al. 2007). Because of the proximity of endemic geographic locations the symptoms are sometimes misdiagnosed as other causes of acute febrile syndrome, including dengue, hepatitis, and malaria (Esteves et al. 2018). These symptoms fade in 5-7 days, and the patient is capable of recovering even without medications and therapy, or it simply retreats to asymptomatic. A transient recovery phase begins after it, during which IgM is detectable in the blood due to the immunological response. The infection seems to reappear 1-3 days following the initial phase. This is the second phase. The infection is serious at this stage and affects vital organs. Meningitis symptoms such as stiffness of the neck are evident in the second phase. In some rare cases, encephalitis is also observed. The unusual involvement of the circulatory, pulmonary, neurological, ophthalmic, gastrointestinal, and various other systems results in clinical manifestations. Hematologic characteristics such as reticulocytopenia, normocytic normochromic anaemia, thrombocytopenia, hypogonadism, etc. have also been recorded in conjunction with pulmonary haemorrhage that does not outcome kidney failure or jaundice, transverse myelitis, and rhabdomyolysis (Bracho et al. 2010). By binding to the chemotactic factors Leptospires also result in the manifestation of thrombocytopenia and a disease known as TTP or thrombotic thrombocytopenic purpura (Lin et al. 2008; Musso and La Scola 2013; Şükran et al. 2013; Herman et al. 2016). In 20-70% of infected individuals, respiratory system is involved resulting in pneumonia, respiratory distress syndrome (ARDS), severe pulmonary hemorrhagic syndrome (SPHS), acute alveolar haemorrhage, and other complications. Pulmonary Leptospirosis results from delayed antibiotic therapy which is more deadly than other types of leptospirosis (Singh et al. 1999; Dolhnikoff et al. 2007; Gulati and Gulati 2012; Vijayachari et al. 2015; Schönfeld et al. 2019). The most common manifestations of hepatic and renal involvement in icteric infection are anuria and/or oliguria. There is also a rise in the

blood creatinine and urea levels. It may cause a variety of complications in pregnant women from abortion and stillbirth to various other complications and eventually leading to the death of the foetus. It can potentially kill both the mother and the foetus in rare situations. However, the birth of healthy infants can occur with the proper use of antibiotics (Rahimi et al. 2018).

6. IMMUNITY

The basic way through which immunity responds against leptospirosis is the humoral response (Adler and Faine 1977). The main targeting agent for the protective antibodies is LPS. The levels of agglutinating LPS-specific antibodies describe the passive transfer of immunity in the transferred sera (Adler and Faine 1978). Other than these antibodies, LPS-specific monoclonal antibodies also provide passive immunity against leptospirosis in healthy animals (Jost et al. 1986). This is not confirmed if there are any other types of antibody-antigen responses against leptospirosis except LPS. However, the latest studies reveal that the humoral response of immunity is not the only way of protection against leptospirosis. The activation of intact TLR2 (Chassin et al. 2009) and TLR4 (Viriyakosol et al. 2006) are necessary to avoid lethal infection in case of mice. There is a variation of immune response in the case of hosts which are prone to acute leptospirosis while the bovine reservoir host has the cell-mediated response against the *L. borgpetersenii* serovar Hardjo. This cell-mediated response was also confirmed by the immunization trails in cattle, using whole leptospire-based vaccines, which proved that it is a T-helper 1 cell response other than the agglutinating antibody titers (Naiman et al. 2002; Brown et al. 2003; Blumerman et al. 2007).

7. VACCINES

It was first demonstrated in 1916 by Ido et al. that immunization can be done using killed leptospires in case of experimental infection (Ido et al. 2005). After that, all the livestock, domestic animals, and human population have been vaccinated with whole-leptospire-based vaccines routinely6. However, the use of these vaccines causes serious side effects and produces immunity only for short-term defense which is serovar-specific-1. In the case of circulating serovar agents polyvalent vaccines are used which give complete coverage and they should be formulated at affordable prices if there is an emergence of new serovars (Gonzalez et al. 2005). Also, the whole-leptospire-based vaccines do not completely control the transmission of the infection and prevention of the disease which limits the use of these vaccines. Due to all the above-mentioned reasons, the main effort is the production of subunit vaccine candidates to identify surface proteins associated with the bacterial surface that are reserved among the bacterial serovars and also the targets for immune responses concerned with the killing of bacteria. The prime evidence of using this method was seen in the past use of the outer membrane of E. coli. One is vesicles containing recombinant Lip41 and OmpL1 for immunization in hamsters which protects them against some lethal types of leptospires (Haake et al. 1999) Later on, it was seen that the LipL32 produced the best immunoprotection when it is delivered through naked DnA136, Mycobacterium bovis bacille Calmette-Guérin (bCG) (Seixas et al. 2007) and adenoviral systems (Branger et al. 2001). When used in experimental animals the efficiency of these candidate vaccines is low ranging between 40-70%. The high level of protection is shown to be produced by the Lig proteins subunit vaccine candidates which are almost 100% in mice (Viriyakosol et al. 2006) and hamsters (Palaniappan et al. 2006; Silva et al. 2007; Yan et al. 2009). The Lig proteins produced the maximum cross-protective immunity against a range of serovar agents that has been determined also due to the amino acid sequence of these proteins which is 70-100% similar to Leptospira spp. (McBride et al. 2009). The presence of this multiple genome

sequence helps in using different techniques to find out new vaccine candidates (Gamberini et al. 2005). The main purpose is to generate a single vaccine candidate which protects against a wide range of Leptospira species. The genome of *L. interrogans* and *L. borgpetersenii* have 2780 same open reading frames out of which 656 are not shown in *L. biflexa* genome (Bulach et al. 2006; Picardeau et al. 2008). Techniques to purify several target candidates are done by sequencing the genome of a larger number of Leptospira species specifically pathogenic and also the bioinformatics analysis of that genome, selection of open reading frames and specifically those genomes which encoded the outer membrane proteins (Yang et al. 2006). The major hurdle to implementing these strategies is the lack of in vitro correlates for immunity against leptospirosis. The screening at a high throughput level is not feasible in experimental animals given the expected number of antigen candidates. The major purpose of the production of a vaccine is to find out if the infection with leptospira protects against reinfection in the population with a high risk of disease and to find out the mode of action of immunity that is involved. As far as the epidemiologically produced immune correlates are found, different types of vaccine candidates are produced to get new virulence factors and outer membrane proteins.

8. CONCLUSION

Leptospirosis has become one of the biggest public health concerns all across the globe especially in the tropical, temperate, and subtropical regions. An emergence and then re-emergence of this disease has been observed due to rapid unplanned urbanization, improper sanitation, poor surveillance programs, and unhygienic waste management practices and control plans. Additionally, the negligence of this disease added fuel to the fire. As mentioned earlier, there are more than a million people infected with leptospirosis. The mortality rate has been observed to be around 6% per year worldwide. This situation necessitates careful planning for its control and prevention. Leptospira infection is one of the most neglected 17 diseases. These diseases have been categorised by WHO as maladies getting the least consideration from local and international health institutes. Regions of South Asian areas are being reported as the center points of rampantly endemic leptospira infections. The reports indicating an increase in the prevalence of leptospirosis have been published recently regarding the South-Indian regions including the states of Tamil Nadu and Kerala. Despite the systemic availability of control and prevention programs against the infection. A troublesome and hard-to-eliminate portion of sequelae left by leptospirosis is the kidney tissue carrier state. It can last several months or even years. Leptospira also co-aggregate with other environmental bacteria to make the conditions suitable for their survival, hence helping them to persist for a long time. This phenomenon requires the study of the bioburden of Leptospira, especially the pathogenic strains from the environment with extensive research to comprehend transmission patterns. This research can also help us in the development of appropriate prevention and control strategies against leptospirosis.

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