Academic Journal of Animal Diseases 9(1): 19-32, 2020
ISSN 2079-200X

DOI: 10.5829/idosi.ajad.2020.19.32

Epidemiology and Zoonotic Implication of Leptospirosis in Domestic Animals in Ethiopia

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Abstract: Leptospirosis is a re-emerging zoonotic disease of worldwide public health significance that affects domestic animals and humans. The disease is caused by various serovars of *leptospira interrogans* that belong to the genus *Leptospira.* Pomona, Canicola, Bratislava, Grippotyphosa, Hardjo and Icterohemorrhagiae are the common serovars of *L. interrogans.* Leptospirosis contains a large spectrum of the host that harbor and excretes the agent from their renal tubules. The principal reservoir hosts for many *Leptospira* serovars are rodents. However, cattle, sheep, goats, dogs, horses and pigs are reservoir hosts among domestic animals and they act as a carrier for several months. Leptospirosis occurs as acute, sub-acute and chronic forms. It is characterized by septicemia, hemorrhagic syndrome, abortion storm and stillbirth. Leptospirosis can be transmitted through direct and indirect contact. The urine of diseased or carrier animals, contaminates water, mud feed, aborted fetus and uterine discharge are the major sources of leptospirosis. Abattoir workers, sewage workers, veterinarians and recreational activities such as water sports and white rafting are the major risk groups of leptospirosis. Laboratory tests used for the detection of *leptospira* are microscope evaluation, culture, molecular method, serology and anima inoculation. The occurrence of leptospirosis is affected by factors related to management; host and environmental. Leptospirosis in a domestic animal might be controlled through vaccination, prophylactic treatment of exposed animals with antibiotics; quarantine introduced new animals, rodent management and improved environmental hygiene. As a result, it is important to conduct applicable control techniques and increasing the public awareness about zoonotic transmission of leptospirosis is recommended. Besides, any investigation and control effort should be conducted by collaboration among human, animal and environmental health professions.

Key words: Domestic Animals · Epidemiology · Leptospirosis · Zoonosis

INTRODUCTION

Leptospirosis is a worldwide important zoonotic disease caused by genus *Leptospira* [1]. *Leptospira interrogans* are pathogenic species that cause leptospirosis whereas *L. biflexa* is nonpathogenic [2]. Leptospirosis is most common in temperate regions during late summer and early rainfall and in tropical regions during rainy seasons [3]. The disease can affect both humans and various animals' species resulting in morbidity and mortality. It can be directly transmitted through interaction with secretions, blood or urine of diseased animals or indirectly through water contaminated mainly with the urine of reservoir animals [1].

Leptospirosis has occurred as acute, sub-acute and chronic forms. It is characterized by septicemia, hemorrhagic syndrome, abortion storm, stillbirth and reduced milk production [4]. The diagnosis of leptospirosis is based on the availability of the sample and the temporal stage of the disease. Laboratory tests used for the detection of *leptospira* are microscope evaluation, culture, molecular method, serology and anima inoculation [5]. Leptospirosis can be treated by antibiotics such as tetracycline, penicillin, ampicillin, doxycycline, streptomycin and erythromycin [6], while prevention is characterized by sanitary control and decrease in the risk of infection occurring due to interaction with contaminated environments, infected wild animals as well

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as with synanthropic animals and rodents [3]. Control measures of leptospirosis are aimed at limiting the occurrence of clinical disease based on integrated action in several links of the transmission chain [7].

Leptospirosis is recognized as a re-emerging global public health problem due to the increased incidence in both developing and developed countries [8, 9]. Global warming that leads to extreme weather events such as cyclones and floods, increased rainfall and increased population and urbanization are considered as the factors associated with the upsurge in the incidence of leptospirosis as well as the magnitude of outbreaks [10]. The global burden of leptospirosis is unknown due to the paucity of data [11]. Although the incidence of leptospirosis appears to have decreased in developed countries, it is rising as a major public health problem in developing countries [12].

In tropical areas where humans and animals live in close interaction, warm and humid conditions favor environmental survival and transmission of the pathogen [10]. Leptospirosis is recognizing as an important cause of renal failure and febrile disease in south-east Asia and Latin America [13]. However, in Africa there is no or very little is understood concerning the extent of human zoonosis or the epidemiology of *Leptospira* infection in several animal species [14]. This is why it remains as one of the neglected tropical zoonotic diseases and further evaluating its impact on livestock health and productivity needs priority for prospective study in Africa [15]. Generally, the battle against leptospirosis can be considered an excellent example of 'One Health', where the relationship between humans, animals and ecosystems need to better understand and manage the disease. Subsequently, any study and control effort requires a truly multi-disciplinary and coordinated approach [16].

In Ethiopia, *Leptospira* antibodies were detected four decades ago in domestic animals with prevalence as high as ranging from 8.30 to 91.20% using a microscopic agglutination test [17]. The first human leptospirosis in Ethiopia was reported from Wonji hospital, central and southern Ethiopia, where 47.50% of febrile patients were positive for *Leptospira* infection [18]. Ethiopia stands in the first place as a hot spot of leptospirosis in the world. Therefore, an investigation should be conducted, to estimate its prevalence, risk factors and geographical distribution in the country and to set its appropriate prevention and control measurements [19].

A zoonotic disease prioritized was conducted to prepare a national strategy to prevent and control the most important zoonotic disease in the country [20]. The prioritization was done based on the severity in humans, the proportion of human diseases attributed to animal exposure, the impact of animal disease at the household level, the availability of intervention methods and the existence of collaboration among the sector. Leptospirosis is one of the first five selected diseases to be tackled through the establishment of the One Health-focused zoonotic diseases in the coming five years in the country [21]. Even though, there is few documented information concerning the occurrence of leptospirosis in domestic animals in Ethiopia, socioeconomic, warm and humid conditions of the areas are favorable for the survival and transmission of the pathogen [22].

Leptospirosis is one of the zoonotic diseases that can easily be transmitted through contact with contaminated areas by rodent urine and other excreta, where there is easy access of the communities to such areas with no knowledge about its transmission and prevention mechanism. Its investigation and creating awareness about its risk to exposed groups is very important to safeguard the public health and livelihoods of society. Therefore, this paper aimed to review the epidemiology and zoonotic implication of *Leptospira* infection in domestic animals.

Leptospirosis in Domestic Animals

Etiology: Leptospirosis is caused by pathogenic spirochaetes of genus *Leptospira* occurring in almost all the mammalian species [23]. Genus *Leptospira* is categorizing under order Spirochaetales, family Leptospiraceae, class Spirochaetes and it is dividing into two species: *Leptospira interrogans*, including all pathogenic strains and *Leptospira biflexa* involving the saprophytic strains isolated from the environments [2]. *Leptospira interrogans* have more than 250 serovars (serovars varieties) that are arranged into 23 serogroups [1]. Pomona, Canicola, Bratislava, Grippotyphosa, Hardjo and Icterohemorrhagiae are the common serovars of *L. interrogans* [24]. The bacteria are highly motility, thin, flexible and filamentous, made up of fine spirals with hook-shaped ends [25]. It is 0.1 µm wide and 6-20 µm long. In tissue and inside phagocytes, the organism will assume a spherical or granular look. Their narrow helical type permits *Leptospira* to burrow into a tissue. *Leptospira* have two periplasmic flagella, one attached sub terminally at each end that extends toward the cell center without overlapping, although the flagella lie inside the spirochaete outer membrane, they are integral to cell form and motility [26].

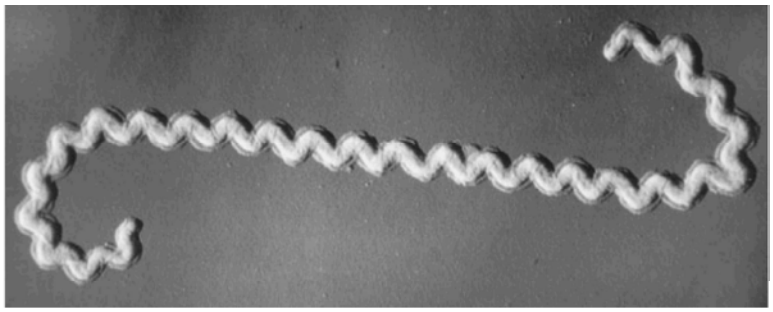

Fig. 1: *Leptospira* species showing characteristic helical shape, periplasmic flagella and outer membrane [25]

Table 1: Leptospira serovars and their maintenance as well as the accidental host

Serovar	Maintenance host	Accidental host
Leptospira hardjo	Cattle	Sheep, human
Leptospira pomona	Pig	Sheep, cattle
Leptospira icterohaemorrhagiae	Cat	Domestic animals and human
Leptospira grippotyphosa	Rodent	Cattle, sheep, pig, horse, dog
Leptospira brastislava	Pig	Horse, dog
Leptospira canicola	Dog	Pig, cattle

The *Leptospira* genome has two circular chromosomes and the genome is larger compared with the genomes of the other spirochetes. This indicates that *Leptospira* species can live in a diverse environment like animal hosts and freely in the environment [28]. *Leptospira* is an obligate aerobe with an optimum growth temperature of 28-30°C and PH 7.2-7.6 [28]. The media used for isolation and cultivation of *Leptospira* are liquid or solid enriched with rabbit serum or bovine albumin. Fletcher's semisolid medium, Korthof's liquid medium and Ellinghausen-McCullough-Johnson-Harris medium are the most commonly used media. On the other hand, no growth occurs on blood agar and other routines and some produce urease [29, 30].

Epidemiology of Leptospirosis: Leptospirosis has worldwide spread because of the large spectrum of hosts that harbor and excrete the agent from their renal tubules [31]. The vital purpose of the epidemiology of leptospirosis is in states of the renal carrier, the animal that has its renal tubules inhabited by *Leptospira*, which in turn is excreteted in the urine infecting the environment [29]. Because *Leptospira* survive longer in warm and environments like in tropical climates, occupations that involve indirect contact with contaminated wet soil or water (example, rice and taro farming, sewer workers) are at higher risk [32]. Additionally, to occupationally exposed teams, urban slum dwellers in areas with poor sanitation are communities at significantly high risk [33]. Infection-related to outdoor recreational exposure, international travel, especially to endemic areas in the tropics and flooding is increasing [34].

Host: All mammals seem to be prone to at least one of *Leptospira*. The principal reservoir hosts for most *Leptospira serovars* are wild mammals, specifically rodents. Cattle, sheep, goats, dogs and pigs are reservoir hosts among domestic animals and they act as a carrier for several months (temporary carrier) while rodents usually remain carriers throughout their life (permanent carrier). Thus, rodents are considered the main reservoir of infection [22]. The particular reservoir hosts differ with the serovar and the geographic region (Table 1). *Leptospira* infection in reservoir hosts is more probable to be asymptomatic, mild or chronic [35].

Source of Infection and Modes of Transmission: The major sources of infection of leptospirosis are the urine of diseased or carrier animals, contaminated surface water, mud feed, soil, aborted fetuses and uterine discharge [36]. Then, the organism enters the body through mucous membranes of the eyes, nose, vagina, or abraded skin [37]. Leptospirosis can be transmitted through direct and indirect contact based on the immediate source of infection. When the immediate source of infection is animal tissue, body fluid, urine, transplacental, or venereal the transmission is terms as direct, while the source of infection is an environment

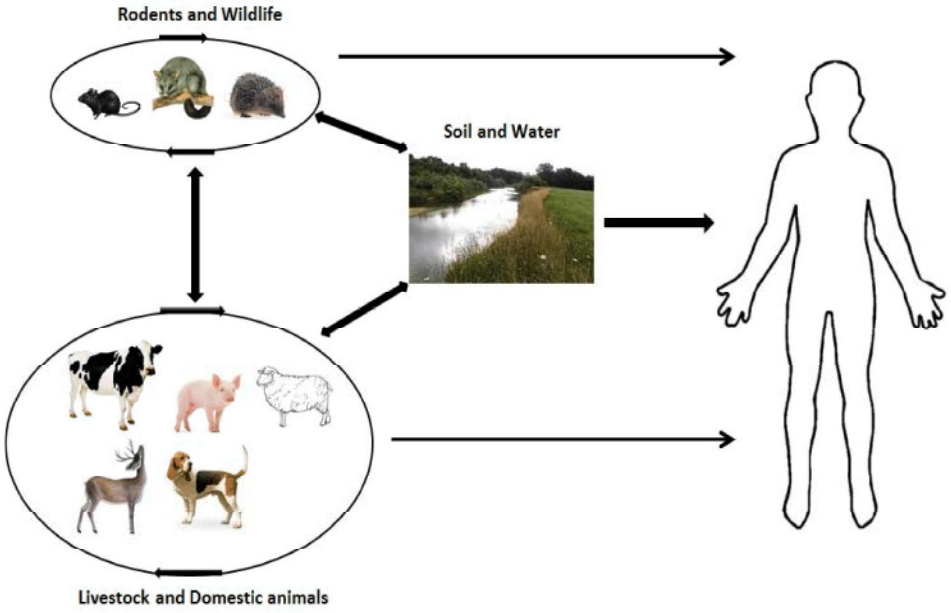

Fig. 2: Flow diagram of the epidemiology of leptospirosis in animals and humans [40].

contaminated with the urine of carrier animals transmission is termed as indirect [30]. Infected milk and semen of diseased bull may contain *Leptospira*; as a result the transmission via milk and natural breeding or artificial insemination can occur but is unusual [38]. Transmission of *Leptospira* infection to humans is through direct or indirect contact of mucous membranes or abraded skin with urine from disease animals or contaminated freshwater surfaces including mud or water in lakes, rivers and streams. Ingestion or inhalation of contaminated water or aerosols could as well cause infection [39]. Transmission among humans is occurs through blood transfusion, organ transplantation, breast-feeding and sexual intercourse [36].

Risk Factors

Host and management risk factors: All age groups of animals can be affected by *Leptospira* infection. However, the leptospirosis is high in young animals with higher morbidity. Although leptospirosis nearly occurs in all mammalian species, it occurs commonly in cattle, sheep, goats, dogs, horses and pigs, but the disease appears to be rare in cats [36]. Certain management factors that pose risks of infection are diseased animals introduced into herds, common grazing with the infected one, access to contaminated water provides like streams, rivers, flood or drainage water and buying or loan of infected male animals for natural insemination [24].

Pathogenic risk factors: Virulent *Leptospira* resist the anti-bacteria action of complement and neutrophils in non-immune hosts, however, it is rapidly killed by either mechanism within the existing of particular epithelial and endothelial antibody [38]. The ability of *Leptospira* to invade Vero cells and to reduce apoptosis in macrophages was correlated with virulence. Nevertheless, the organism must penetrate host epithelial and endothelial cell barriers for both hematogenous spread and localization in target organs, such as liver and kidney. A cytotoxic glycolipoprotein fraction is showing to inhibit host ATPase with the activity ascribed to the presence of long chain fatty acid. *Leptospira Pomona* in cattle causes intravascular hemolysis due to hemolytic exotoxin [41].

Zoonotic Implication of *Leptospiral* Infection

Risk Factors in Human: *Leptospira* infection risk groups that are exposed to animal reservoirs or contaminated environments, such as abattoir and sewage workers, salver workers, coalmines, plumbers, farmworkers, veterinarians, slaughter house employees, meat handlers, military personnel and employees within the fishing industry [42]. Recreational activities that increase the risk of *Leptospira* infection are husbandry and water sports like canoeing, swimming and white rafting residents of some urban areas [24]. Men are more frequently diagnosed with leptospirosis compared with women and this has been traditionally attributed to the representation of men in high-risk occupations [43].

Pathogenesis and Clinical Signs of Leptospirosis: The *Leptospira* organism enters the body through mucous membranes (mouth, nose, eyes and vagina) or skin with lesions and scratches [31]. Via lymphatic vessels from the infection site, the *Leptospira* enter the bloodstream. In the bloodstream, the bacteria will multiply and spread to organs such as kidneys, spleen, central nervous system, liver, eyes and reproductive organs. There are 3 attainable pathways once the systemic circulation. If the animal has a high and adequate antibody titer, the body will clear from *Leptospira* and no clinical signs can be seen. An animal with a moderate antibody can present with a mild or short leptospiremia followed by mild clinical signs. The *Leptospira* are then eliminating through the kidneys and after the elimination, the animals will not continue to shed *Leptospira*. If the animal has a low or absent antibody titer there will be a multiplication of *Leptospires* in the bloodstream [44].

The endothelium will be damaged that can result in ischemia in different organs such as the kidneys (renal tubular necrosis), liver (hepatocellular damage) or lungs. Neutrophils and thrombocytes are stimulated by lipopolysaccharides (LPS) in the outer membrane of the *Leptospira* and this contributes to inflammation and coagulatory abnormalities. The LPS can contribute to renal and hepatic damage. Meningitis can develop if the *Leptospira* enter the nervous system or cerebral spinal fluid in the acute phase of the disease. If *Leptospira* organism persists despite the antibody response, then immune-complex-mediated meningitis occurs. When this phenomenon occurs in the eyes it causes uveitis [29].

The incubation period of leptospirosis is based on dose, infectious strain and host but is on average 7-14 days [45]. Antibodies become detectable 5-7 days once infection [36]. It takes concerning 2 weeks for the *Leptospira* to reach proximal tubular cells and also the tubular lumen within the kidneys. In the best-case scenario, the antibodies will clear the blood and tissues from *Leptospira.* The bacteria can also become eliminated from the kidneys and no *Leptospira* shed in the urine. In some animals, despite an increased antibody liter, the bacteria can replicate and persist in the renal tubular cell. This may result in chronic shedding of *Leptospira* in the urine for days to months, even years [46].

Leptospirosis is characterized by a broad range of clinical symptoms in animals with slight variations among species: acute, sub-acute or chronic. Clinical signs of acute or sub-acute disease are detected in the leptospiremic phase and it is characterized by septicemia, high fever and anorexia, petechiation of mucosa, depression and acute hemolytic anemia with hemoglobinuria, jaundice and pallor of the mucosa [29].

Clinical signs related to chronic infections in animals are generally associated with reproductive losses through abortion, stillbirth, infertility and mastitis and milk drop syndrome. Abortion usually occurs during the last trimester of pregnancy [47, 48]. Infertility and milk drop happens solely in pregnant or lactating cows as a result of *Leptospira* organisms like the pregnant uterus and lactating mammary gland to proliferate [24]. A sudden drop in milk production may affect up to 50% of the cow at one time and precipitate fall in the herds' milk yield, the decline may last for up to 8 weeks but individual cow's milk production will return to normal with 1-14 days [38].

Leptospira infection in goat and sheep will be severe or subclinical and will manifest as reproductive problems like infertility, abortion and stillbirth [49, 50, 51]. In several study anorexia, lethargy and vomiting were the three most common clinical signs of leptospirosis in the dog. Besides, weight loss, polyuria, diarrhea, abdominal or lumbar pain, musculoskeletal pain and dehydration were also common [52]. The clinical features of equine leptospirosis are similar to those detected in other animals such as cattle, with low-grade fever, listlessness and anorexia the most common presentation in milder disease. In more severe forms leptospirosis a range of typical signs might occur, together with conjunctiva suffusion, jaundice, anemia, petechial hemorrhages on the mucosa and general depression. Renal failure may also occur, particularly in foals. The infection of pregnant mares may cause placentitis, abortion and stillbirths [53].

The majority of cases may have a subclinical disease or show very mild symptoms and do not require medical treatment [26]. The mild symptoms of leptospirosis in both animals and humans are not disease-specific. For instance, clinical symptoms in animals mimic other infectious abortifacient diseases (brucellosis, neosporosis, bovine virus diarrhea, porcine circa virus) [10], while clinical symptoms in humans mimic many other diseases with febrile syndromes (dengue fever, influenza, hepatic disease, Hantavirus infections) [26]. Thus, leptospirosis is often misdiagnosed, which contributes to the underestimation of the occurrence of the disease [54].

Diagnosis of Leptospirosis: Because of the several clinical and often "flu-like" symptoms, human leptospirosis is frequently undiagnosed or misdiagnosed

as other diseases with febrile syndromes (aseptic meningitis, influenza, hepatic disease, Hantavirus infections) [26]. This is also the case in domestic animals, where most cases are difficult to diagnosis clinically, due to non-specific clinical presentation or unapparent clinical signs with host-adapted serovars [29]. Hence, the diagnosis of leptospirosis in both humans and animals cannot be made with confidence without laboratory confirmation [55].

Leptospirosis is, as usual, a biphasic disease, with the acute phase about 4-7 days. During this phase, leptospiremia occurs and *Leptospira* can be present in high numbers in multiple body fluids including blood and cerebrospinal fluid [56]. The immune (convalescent) phase usually occurs during the second week after onset of symptoms and is characterizing by excretion of *Leptospira* in the urine and appearance of antibodies in the serum [36]. Antibodies usually reach maximum levels within 2-3 weeks then slowly withdraw but may remain detectable for 2-10 years in humans and similar period or for a lifetime at low levels (particularly in reservoir hosts) in animals [57].

Several laboratory tests described for the detection of *Leptospira* include; direct examination of clinical specimens for organisms (microscopic evaluation), the culture of *Leptospira* from clinical specimens, detecting the presence of Leptospiral DNA (molecular method), detection of *Leptospira* antibodies (serology) and animal inoculation [58]. The tests available have different capacities for diagnosis and this is based on the kind of specimen that is available, the course of the disease and the purpose for testing [59].

Direct Examination for Leptospires and Antigen

Dark Field Microscopic Examination: Dark field microscopic examination (DFM) of body fluids like blood, urine, CSF and dialysate fluid will use to identify rapidly the presence of *Leptospira* [55]. This method can be applied to tissues removed for surgical or experimental reasons from animals, or necropsy specimens, tissues from carcasses or abortion products [57]. Although the dark field microscope is useful in situations where laboratory resources are limited, this method has low sensitivity and specificity [57]. The risk of false positives due to misinterpretation of fibrin or protein threads, cell debris and other artifacts can be high, even for experts [51]. Dark field microscope also suffers from other disadvantages like technical with obtaining suitable specimens (specimens should be taken aseptically and sent to a laboratory without delay), the requirement of a high level of operator skill and this method provides no information of infecting serovars [1]. A variety of staining methods either Geimsa stain or silver impregnation on air-dried smears have been used to increase the sensitivity of direct microscopic examination of *Leptospira* in veterinary specimens, including immunofluorescence staining of bovine urine [52].

Culture: *Leptospira* organisms could be isolated from the body fluid, mainly urine. However, tissue from dead animals is giving a better chance of successful isolation, if target tissue is not autolyzed. Such target tissue is the kidney, liver, lungs and brain. If the agent is suspected for abortions, isolation could be attempted from non autolyzed abortion materials or tissue samples from a freshly aborted fetus. Isolation of *Leptospira* organisms from tissue (kidney, liver, lungs) confirms maternal infection [29]. Isolation needs expensive and properly prepared and kept culture media. Inoculated media are incubated at 28-30°C for several weeks or months. Cultures are incubated in a dark and quiet environment. Time of incubation based on the serovar such as Pomona and grippotyphosa require the least time incubation up to 10 days. Regardless of the time required for isolation, the inoculated culture media should be protected from contamination, thus require the addition of antimicrobial agents selected to inhibit the growth of contamination [31].

Molecular method

Polymerase Chain Reaction (PCR): The PCR can be detecting *Leptospira* DNA in clinical samples like the serum, urine and aqueous humor. The PCR involves the enzymatic amplification of target deoxyribonucleic acid sequences specific to the organism through a series of polymerizations that are applied by heat-stable DNA polymerase enzymes using primers, which are short DNA fragments and they bind specifically to the sequence of interest. The amplified DNA produced by this reaction is visualizing agarose gel electrophoresis. Modern methods such as fragment length polymorphism (FLP), pulse field gel electrophoresis (PFGE) and other methods are currently being assessed [5].

Compared with culture, PCR assays are reported to be more sensitive for detecting *Leptospira* in clinical material from both humans and animals [63]. The high sensitivity of PCR assays may be due to the fact that these assays detect both viable and dead bacteria, while

culture requires sufficient numbers of viable bacteria for sample preparation (which is difficult to ensure due to the limited survival time of *Leptospira*) and may be affected by poor sample quality [64]. The PCR assays also provide a considerable time advantage, compared with the long incubation time for culture and the long reporting times for MAT (involves diagnosis with paired sera) [65]. The use of PCR for early leptospirosis diagnosis, that is before antibodies appear in the blood, can provide a major improvement in disease management, since treatment should be started as soon as possible after disease onset.

Compared with the tests like MAT and ELISA that identify antibodies in blood serum instead of the presence of *Leptospira* directly, the direct detection of *Leptospira* DNA by PCR can differentiate current infection from past exposure. For animals, the PCR has additional advantages over serological tests, such as avoiding false-positive serological results due to vaccination induces antibodies and PCR informs the carrier status of the host [66].

The most recognized limitation for the PCR - based mostly diagnosing of *Leptospira* infection is that the inability of most assays to detect the infecting serovar [67]. Though this is often not crucial for individual patient/animal management, the identity of the infecting serovar has necessary epidemiological and public health worth. Strategies designed to overcome this drawback include restriction endonuclease digestion of PCR products, analyzing the amplification products by melting curves and direct sequencing of amplification [68].

Moreover, DNA extracted from clinical specimens may contain inhibitors to PCR and lead to false-negative results. In blood samples, substances such as urea, creatinine and hemoglobin derivatives are likely to inhibit DNA amplification [69]. The chemical components in the blood collection systems may also interfere with PCR. A study comparing the results from some standard blood collection systems demonstrated that the collection tubes containing lithium heparin interfered with the PCR [70].

Serology Techniques: Serology is the most frequently used diagnostic approach for leptospirosis [71]. The serological tests available are mainly base on the detection of immunoglobulin IgM and IgG class antibodies. The response patterns of IgM and IgG class antibodies found in human and animal cases are similar [72, 73]. IgM antibodies appear first (as early as the second day of onset of symptom) during the infection, followed by IgG class antibodies [74]. Both IgM and IgG antibodies commonly persist after infection, but the persistence of IgM antibodies is generally shorter than IgG antibodies [75]. Therefore, tests for the detection of IgM antibodies can identify infections at an earlier stage than those for the detection of IgG antibodies. Tests for the detection of IgG antibodies are more appropriate for detecting residual antibodies from past infections.

Microscopic Agglutination Test (MAT): Microscopic agglutination test (MAT) is the standard method for the serological diagnosis of leptospirosis [76]. It determines agglutinating antibodies within the blood serum of a patient by mix it in varied dilutions with life or killed formalized *Leptospira*. An anti-leptospiral antibody presence within the blood serum causes *Leptospira* to stay along to create clumps. This agglutination is observed by using dark field microscopy [45]. Agglutinating antibodies can be either IgM or IgG. Approximately 7 to 10 days after the onset of symptoms, antibodies can also be detected by the MAT [77].

For each human and animal cases, the quality criterion for confirming clinical *Leptospira* infection cases by the MAT is seroconversion or a fourfold or additional increase in antibody titer between paired sera [1]. The specified interval between paired sera to detect rising titers depends on the delay between onset of symptoms and presentation of the individual. If the first serum is collected while the symptoms of overt leptospirosis are present, an interval of three to five days may be adequate to detect rising titers. Although the MAT is in common use for leptospirosis diagnosis in animals, this test detects antibodies in serum, rather than the presence of *Leptospira* directly in urine and kidney and therefore does not reflect the carrier status of the host [78].

The MAT will offer a general impression of that serogroups/serovars are circulating a population and is taken into account because the most acceptable test to employ in epidemiological sero surveys. The titer cut-off for determining exposure to *Leptospira* is different from that determining clinical disease. It is suggested that a titer cut-off of 50 should be used to indicate exposure to *Leptospira spp.* for both humans and animals [79]. When applied to early infections, the high degree of cross-reaction that occurs between serovars from the same or different serogroups prevents MAT from being a reliable test to predict the infecting serogroup/serovar [80].

The MAT is extremely specific, however, has the subsequent disadvantages: (i) facilities for culturing and maintaining live *Leptospira* are needed; (ii) the method is technically strict and long, notably once the panel is large; (iii) antibodies might not be detectable once the causative strain is not represented within the panel or

solely low titer is found with a serovar that antigenically resembles the absent causative serovar (the finding of no or low titer within the MAT does not exclude *Leptospira* infection in these circumstances, it is never potential to make sure that the panel is complete since new unidentifiable *Leptospira* could cause disease and for this reason, it is ought to contain a genus-specific screening test, like ELISA using a generally reactive antigen); (iv) the MAT can't be standardized consequences of live *Leptospira* are used as antigen [81].

Enzyme Linked Immuno Sorbent Assay (ELISA): Conventional microtitre plate ELISA and dot-ELISA can detect IgM-class antibody in the early phase of the disease, 24-48 hours before it can be detected by MAT, so that current or recent infections may be indicated. Whereas, whenever no antibody is detected or low titer is found, a second sample should examine for seroconversion or a significant rise in titer. The test (antigen) can be standardized and commercial kits are available so there is no need for facilities for the culture of *Leptospira* in local laboratories to provide antigen [81]. This ELISA works on the principle that any *Leptospira* IgM antibodies present in patient serum will bind to the *Leptospira* antigen attached to the polystyrene surface of the micro wells. The residual serum is removed from these micro wells by washing with 1% buffer (provided in the kit). The peroxidase-conjugated anti-human IgM is there after added to the wells and the plate is re-incubated allowing the bound antigen antibody complexes to bind to the conjugate. Wells are washed again and a colorless substrate system, tetramethylbenzidine hydrogen peroxide added. The substrate is hydrolyzed and the chromogen turns blue. The TMB turns yellow once the reaction is to stop using phosphoric acid. Color development indicates the presence of IgM antibodies to *Leptospira* in the serum sample [81].

Enzyme-linked immunosorbent assay (ELISA) is often used as an alternative to MAT for screening for *Leptospira* infection in both humans and animals [83]. As well as being easier to perform, ELISA is inexpensive, safer (as it uses killed antigen and therefore reduces the risk of infection for laboratory personnel) and gives a less subjective result than MAT [84].

Another advantage of ELISA over MAT is the serological response of IgM and IgG can detect separately. The earliest time post-infection that the antibody may be detected by ELISA is affected by which class of antibody the ELISA is testing [55]. The IgG antibody was detected regarding constant time as IgM, however, persisted for much longer. As IgM antibodies are detected earlier in the acute phase of infection and persisted for shorter periods than IgG, the IgM-ELISA is deemed as a more suitable method for detecting acute infection in humans and animals and is more commonly used [1].

The IgM-ELISA is less specific than MAT test, hindering its use as a single test of diagnosis of leptospirosis. A limitation of the use of single serum samples for the IgM-ELISA test is the persistence of IgM antibodies. Compared with the MAT, false positive reactions detected in animals due to vaccination by IgM-ELISA may happen with higher frequency. Due to the lower specificity of ELISA compared with the MAT, a positive result from a single sample can only be considered as presumptive evidence of infection. Subsequent confirmation of a positive test is required by testing a convalescent sample with an alternative method, preferably MAT [70].

Most of the ELISA assays use whole-cell lysates, usually the saprophytic strain *L. biflexa* serovar Patoc because the antigen, which shares many surface antigens with infective strains [67]. Recently, a recombinant lipoprotein-based ELISA test has been accessed, with improved specificity and reproducibility. Recombinant cell-surface lipoprotein antigen lipL32 has proved to be a helpful antigen for the enzyme-linked-immunosorbent serologic assay test in humans, cattle and dogs [85].

The enzyme-linked-immunosorbent serologic assay has the subsequent disadvantages: (i) solely one antigen is employed particularly the genus-specific antigen that is shared by infective and saprophytic *Leptospira* alike; (ii) since it is based on genus-specific antigen, the enzyme-linked-immunosorbent serologic assay test does not provide a sign of infecting serovar [86].

Other Serological Methods: Several other serological tests have been used as screening tests for antibodies, including macro-agglutination, complement fixation reaction, indirect immunofluorescence, indirect hemagglutination assay, lepto dipstick test, lateral flow assay test and latex agglutination. Nevertheless, these tests are rarely used due to their lack of sensitivity or specificity [67].

Animal Inoculation: Laboratory animals are useful for isolating the organisms from contaminated material and for maintaining recent isolates and may be used to recover a single serotype from a mixed culture. Young animals ideally weanlings ought to be used that should be free from endemic *Leptospira* infection; guinea pigs, hamsters, gerbils, young rabbits, Swiss white mice, albino

American deer mice and 1-3 day old chicks may be used. The material ought to inoculate intraperitoneally through one of the lower quadrants of the abdominal wall. The animals should examine twice daily and a drop of peritoneal fluid can examine with dark field microscopy for active *Leptospires* from the third to the seventh day. On the death of the animal hemorrhagic lesions with spirochetes are found in many organs [2].

Treatment, Prevention and Control of Leptospirosis: The primary aim of treatment is to control the infection before irreparable damage to the liver and kidneys occur. Treatment with antibiotics counseled as before long as doable when signs seem. The results of treatment are often disappointing because in most instances animals are present for treatment only when the septicemia has subsided. The secondary aim of treatment is to regulate the leptospiruria of carrier animals and render them safe to stay within the group. Other antibiotics used to treat leptospirosis include tetracycline, penicillin, ampicillin, doxycycline, streptomycin and erythromycin [6]. The efficacy of treatment may depend on the serovar. Fluid therapy, blood transfusion and other supportive care may also be necessary. These supportive treatments depend on the animal and needed if the animal is severely affected and in shock, it will need fluid therapy. In beef herds, further abortions prevented by vaccination and treatment of all animals with antibiotics and in dairy cattle, only infected animals usually treated due to the potential loss of milk sales [87]. The primary treatment for equine recurrent uveitis in horses is anti-inflammatory drugs such as corticosteroids and medications to decrease discomfort due to topical atropine, surgery and other therapies that may also be used [24].

Understanding the epidemiological features of leptospirosis is a critical step in designing interventions for reducing the risk of disease transmission [36]. Intervention methods will target several points within the transmission cycle of *Leptospira* infection. Although little can do in wild animals, leptospirosis in domestic animals controlled through vaccination, prophylactic treatment of exposed animals with antibiotics, quarantine introduced of new animals of regardless of the species for a minimum of four weeks, rodent control, regular serological testing, improved environmental hygiene, separating young animals from adults and safe AI [24].

Occupational hygiene, taking care of animal bite, vaccination, drinking clean water, early treatment, prophylactic therapy, acquisition of information for people coming to high-risk areas, is fundamental for preventing human leptospirosis [88]. In herds, the disease is usually introduced by an infected animal, through the environment or by contact with other infected animals in the mixed pasture. Animal re-position must be select according to the non-reactivity of herds to leptospirosis. Leptospirosis vaccines are available for pigs, cattle and dogs. Although the vaccines prevent disease, they do not completely prevent infection or the shedding of the organisms. Immunity is basically serovar specific: vaccines are protecting solely against the included serovars or closely associated serovars [3].

So far, few documented information regarding the occurrence of leptospirosis in domestic animals in Ethiopia, climate, socioeconomic and other leptospirosis factors are mainly favorable for the occurrence and spread of the disease in the country. In Ethiopia, leptospirosis has been reported to occur in domestic animals [17] with a prevalence of 70.7% in cattle, 47.3% in goats, 43.4% in sheep, 91.2% in horses, 57.1% in pigs and 8.3% in dogs. In humans [89] reported from a total of 59 patients admitted the outpatient of Wonji Hospital, 47.5% of the patients were positive of leptospirosis and the occurrence of the disease was more common in males than females. According to [90, 91], a total of 184 out of 418 horse samples had antibody titers of 1:100 or greater to at least one of 16 serovars, indicating the presence of 16 serovars of *leptospira* species in horses in central and southern Ethiopia. This indicated that 44% of sampled horses were seropositive to at least one serovars.

CONCLUSIONS

Leptospira infection is a re-emerging zoonotic disease of worldwide public health significance that affects domestic animals and humans. The disease is caused by various serovars of *leptospira interrogans* that belong to the genus *Leptospira*. The incidence of this zoonotic disease is that the most typical in each temperate and tropical regions. It can be directly transmitted through interaction with secretions, blood or urine of diseased animals or indirectly through water contaminated mainly with the urine of carrier animals. The urine of diseased or carrier animals, contaminated water, mud feed, aborted fetus and uterine discharge are the major source of *Leptospira* infection. Abattoir workers, sewage workers, veterinarians and recreational activities such as water sports and white rafting are the major risk groups of leptospirosis. The occurrence of leptospirosis is affected by factors related to management, host and environmental factors. This zoonotic disease in domestic animals could be controlled through vaccination, prophylactic treatment of exposed animals

with antibiotics, quarantine introduced of new animals, rodent management, improved environmental hygiene, separating young animals from adults and safe AI. As a result, it is important to conduct applicable control techniques and increasing the public awareness about zoonotic transmission of leptospirosis is suggested. Besides, any study and control strength should be conducted by collaboration among human, animal and environmental health professions.

ACKNOWLEDGMENTS

The author would like to say thanks to all my friends who help me in this review and also thank the Ethiopian Institute of Agricultural Research for logistic support.

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