ABSTRACT

Leptospirosis is a zoonotic disease with a high incidence rate in many parts of the world due to the presence of various hosts for the pathogenic Leptospira. Tropical, subtropical and humid regions are suitable for long-term survival of the bacterium. Because of the temperate and humid climate, northern areas of Iran are suitable for pathogenic Leptospira and outbreak of the disease. Therefore, identification of infected areas is important from a public health and economic point of view. Previous studies show that the incidence rate of leptospirosis is increasing every year. Therefore, accurate diagnosis, control and prevention of this disease seem necessary through vaccination and raising public awareness, especially among high-risk groups. Today, diagnostic methods including immunofluorescence assay, enzyme-linked immunosorbent assay, microscopic agglutination test (MAT) and polymerase chain reaction (PCR) are used to diagnose the leptospirosis. MAT is the gold standard test for the diagnosis of leptospirosis with extensive applications in Iran. Due to the importance of this disease and its high prevalence in recent years, the present study aimed to investigate the epidemiology and diagnosis of leptospirosis in Iran.

Keywords: Epidemiology, Leptospirosis, Diagnostic techniques, Iran
INTRODUCTION
Leptospirosis is one of the most common zoonotic diseases in humans and livestock, with high incidence rates around the world (1). The prevalence of this life-threatening disease is higher in tropical, subtropical and wet regions (2). According to reports, this disease is more prevalent during summer (late spring) and autumn (3). Leptospirosis is transmitted via direct or indirect contact with urine, blood or tissue of animals infected with pathogenic Leptospira. Several animal species are considered as natural hosts for Leptospira. The severity of this recurrent disease ranges from mild to acute, depending on the bacterium, its serotypes and host’s immune system (3-6). The World Health Organization (WHO) has recognized leptospirosis as the second most commonly transmitted infection from livestock to humans (7, 8). In addition to public health concerns, the disease imposes significant financial burden due to the reduction of milk production, animal weight loss, livestock abortion, production of weak calves and decreased livestock fertility (9-12). Evidence suggests that the rate of leptospirosis mortality is 5% to 30% around the world (9, 11). In developing countries, including Iran, the disease occurs more commonly among farmers, stockbreeders, slaughterhouse workers, butchers and fishermen during warm seasons. On the other hand, in developed countries, leisure activities, such as swimming, water skiing and other water sports as well as outdoor activities in contaminated areas, have been introduced as the main disease transmission routes (13). Port cities and towns near the Caspian Sea have a temperate and humid climate. In this region, many patients diagnosed with leptospirosis are hospitalized in emergency wards every year with a noticeable mortality rate (1). Generally, agricultural lands are ideal environments for rodents. Animals are the main reservoirs of pathogenic Leptospira in farms. Livestock are allowed to wander freely in farms for grazing. Therefore, humans and livestock are constantly exposed to the risk of leptospirosis. A set of conditions can lead to the spread of leptospirosis in Iran (14). With regards to the importance of this disease and its high incidence in recent years, the present study aimed to review the literature on leptospirosis in Iran.

Data collection
This study was conducted by reviewing several online databases including Google Scholar, PubMed, IranMedex, SID and Scopus. The searched keywords were “leptospirosis” and “Iran”. All published data before the winter of 2018 were used to extract epidemiologic and diagnostic data.

Epidemiology
No accurate information is available regarding the incidence of leptospirosis. However, based on a recent report by the WHO, annual incidence of leptospirosis varies from 0.1-1 per 100,000 in temperate regions to 10-100 per 100,000 in tropical and humid regions; it also occurs sporadically in industrialized countries (15). Generally, spread of the disease depends on factors, such as climate, population density and contact between the keeper host and random host (16). The most important hosts in rural and urban areas are rodents. If livestock come in contact with urine of infected rodents, the infection can spread to humans and other animals (17). Different terms have been proposed to describe leptospirosis in different parts of the world with regard to the infection source, type of involved serovars, clinical findings in humans and livestock and place of occurrence (Table 1) (18, 19). Water is recognized as the most common source of transmission. In people with normal skin, infection may develop due to swimming in contaminated water, although skin abrasion is possibly the cause of infection. Transmission through sexual intercourse has been also reported (20).
In a study conducted by Perret et al. in Chile, 70% of patients had positive history of contact with surface waters (21). In a study in Iran, 34.9% of patients had a history of contact with surface waters (22). In Costa Rica, the disease was transferred to humans through drinking contaminated river water (6). Leptospirosis is a seasonal disease, with the highest incidence during summer and autumn in temperate regions and during wet seasons in warm regions (23). Infection has been also reported during winter in areas where livestock are kept in enclosed spaces (16). Moreover, significant rainfall and flood increases the risk of contracting the disease (24).

In rural areas, outbreak of leptospirosis has been regularly observed due to torrential rainfall (25). According to a study in Thailand, the highest incidence rate of the disease was reported during wet seasons (late summer) (26). In urban areas, sewage and garbage waste is regarded as the main source of transmission, while water and environment can be contaminated with urine of infected mice (27). In these areas, the risk of infection is high in sewage workers as well as municipality workers but still not as high as farmers (28).

The prevalence of leptospirosis is limited to northern areas of Iran, especially the Guilan Province where the disease is endemic. It also occurs sporadically in neighboring provinces, including the Golestan (29), Tehran, Qom, Jahrom, Sabzevar and Ardabil provinces (30).

In recent years, other studies have reported the outbreak of the disease in Shiraz (31), Uremia (32), Zanjan (33), Hormozgan (34), Mashhad (35), Sistan and Baluchestan (36), Lorestan (37), Khuzestan and Tabriz (38). A major health concern is the long-term survival of many pathogenic species of *Leptospira* in water and wet soil, as they can enter the host body (human or livestock) through dermal abrasions (39).

Since simultaneous outbreak of leptospirosis in humans and livestock has not been studied in Iran, we aimed to investigate the prevalence of this infection and its common serovars in different provinces of Iran. The climate varies widely in different areas of Iran; therefore, surveying leptospirosis outbreak based on the isolated serovars is necessary in different areas of the country (Table 2).

### Diagnostic techniques

Symptom-based diagnosis of leptospirosis is not feasible since the clinical signs and symptoms of this infection are similar to those of influenza and other febrile disease. If the disease is not diagnosed or treated promptly, it can progress into acute phase and cause serious and long-term damages. Therefore, alongside clinical examination, laboratory examinations are of great importance for the diagnosis of leptospirosis (16, 40).

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**Table 1. Features of leptospiral disease**

| Diffusion/spread | Clinical findings | Disease in humans | Host | Infection source | *Leptospira interrogans* |
|------------------|-------------------|-------------------|------|-----------------|-------------------------|
| Through the world | Fever, an eruption in the pretilial region - jaundice | Infectious jaundice | Human | Dog’s urine | Canicola |
| Europe, America, Africa | Pseudo-influenza disease, aseptic meningitis | Mouse & Skunk | Cattle | | |
| All around the world | Fever, sweating, aseptic meningitis | Marsh fever | Cattle | Rodents/ water | Grippotyphosa |
| Europe, America, Australia | Fever, jaundice | Seven-day fever | Swine & Human | Mouse’s urine/ water | Icterohaemorrhagiae |
| All around the world | Jaundice, hemorrhage, aseptic meningitis | Weil’s disease | Cattle-dig Swine | | Icterohaemorrhagiae |
| Europe, America, Australia | Fever, sweating, aseptic meningitis | Swineherd’s disease | Human | Pig/ Cow | Pomona |

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Table 2. Frequency of livestock and human serum samples suspected of leptospirosis based on the dominant serovars and categorization of Iranian provinces according to laboratory assessments by the microscopic agglutination test (MAT) technique.

| Sample   | Province   | Autumnalis Number (%) | Canicola Number (%) | Grippotyphosa Number (%) | Icterohaemorrhagiae Number (%) | Pomona Number (%) | Sejroe hardjo Number (%) | Sejroe Sejroe Number (%) | Ballum Number (%) | Australis Number (%) | Tarassovi Number (%) | Reference |
|----------|------------|------------------------|---------------------|--------------------------|-------------------------------|-------------------|--------------------------|--------------------------|-------------------|---------------------|----------------------|-----------|
| **Livestock** |            |                        |                     |                          |                               |                   |                          |                          |                  |                     |                      |           |
|           | Gilan      | 5 (17.8)               | 1 (3.5)             | 16 (21.4)                | 8 (57.1)                     | 0 (0)                          | 14 (50)                    | 5 (17.8)                  |                   |                     |                      | 35        |
|           | Mazandaran | 0 (0)                  | 0 (0)               | 0 (0)                    | 6 (20)                       | 0 (0)                          | 0 (0)                     | 0 (0)                     |                   |                     |                      | 35        |
|           | Ardabil    | 1 (2.3)                | 3 (7.1)             | 0 (0)                    | 0 (0)                        | 0 (0)                          | 26 (61.9)                 | 0 (0)                     |                   |                     |                      | 35        |
|           | Qom        | 3 (7.5)                | 1 (2.5)             | 0 (0)                    | 8 (20)                       | 1 (2.5)                       | 2 (5)                     | 0 (0)                     |                   |                     |                      | 35        |
|           | Hormoghan  | 0 (0)                  | 1 (2.8)             | 1 (2.8)                  | 16 (45.7)                    | 0 (0)                          | 6 (17.1)                  | 0 (0)                     |                   |                     |                      | 35        |
|           | Uromia     | 9 (6.92)               | 2 (1.53)            | 2 (1.53)                 | 7 (5.38)                     | 0 (0)                          |                           |                           |                   |                     |                      | 39        |
|           | Tabriz     | 17 (22.3)              | 25 (32.9)           | 5 (6.6)                  | 26 (34.2)                    | 0 (0)                          | 0 (0)                     | 3 (3.9)                   |                   |                     |                      | 41        |
| **Human** |            |                        |                     |                          |                               |                               |                          |                          |                   |                     |                      |           |
|           | Gilan      | 1 (2.4)                | 1 (2.4)             | 1 (2.4)                  | 5 (12.1)                     | 1 (2.4)                       | 2 (4.8)                   | 2 (4.8)                   |                   |                     |                      | 35        |
|           | Mazandaran | 0 (0)                  | 0 (0)               | 2 (7.6)                  | 3 (11.5)                     | 2 (7.6)                       | 2 (7.6)                   | 6 (23.07)                 |                   |                     |                      | 35        |
|           | Lorestan   | 22 (36.67)             | 38 (63.33)          | 0 (0)                    | 0 (0)                        | 0 (0)                          |                           |                           |                   |                     |                      | 38        |
|           | Azarbaijan | 0 (0)                  | 0 (0)               | 0 (0)                    | 4 (13.33)                    | 0 (0)                          | 0 (0)                     |                           |                   |                     |                      | 42        |
|           | Mazandaran | 5 (3.25)               | 1 (0.65)            | 3 (1.95)                 | 10 (6.5)                     | 6 (3.9)                       | 6 (3.9)                   | 22 (14.29)                | 26 (16.87)         | 18 (11.69)         | 21 (13.64)          | 43        |
|           | Ahwaz      | 16 (42.1)              | 7 (18.4)            | 3 (7.9)                  | 11 (28.9)                    | 1 (2.6)                       |                           |                           |                   |                     |                      | 44        |

Figure 1. Photomicroscopy of *Leptospira* showing its morphology and hooked ends (photo was taken under 100× magnification)
Dark-field microscopy of body fluids, such as blood, urine and cerebrospinal fluid can indicate the possibility of early diagnosis; however, sensitivity of this method is low. In case of blood samples, bacteria can be detected only within few days after the onset of the disease. Direct examination of blood samples via dark-field microscopy can lead to misdiagnosis given the sensitivity of 40.2% and specificity of 61.5% (42). Thus, sole use of this method is not recommended and other methods should be applied concomitantly. Direct investigation can be conducted through immunofluorescence staining, immunoperoxidase staining and silver staining, which are not used extensively due to limited availability and their relatively low sensitivity (43). Furthermore, *Leptospira* can be detected in tissues with the aid of histopathological staining and immunohistochemistry (20).

**Culture studies**

*Leptospira* can be isolated from clinical samples, including blood, cerebrospinal fluid, urine and tissue. Blood culture must be carried out immediately after the onset of disease and before antibiotic administration. Urine samples are also appropriate for the isolation of *Leptospira* during the Leptospirosis phase, almost one week after the occurrence of disease (bacteria enter renal ducts and are excreted through urine. This phase can last long, during which the disease is transmitted. Blood and urine samples are inoculated into specific culture media and incubated at 30°C. Cultures must be investigated regularly using dark-field microscopy (44).

It is known that pathogenic *Leptospira* grow slowly and their isolation through culture is very difficult and time-consuming. Considering the initial enrichment of pathogenic *Leptospira* in the Ellinghausen-McCullough-Johnson-Harris (EMJH) medium, culturing in a solid EMJH medium earlier than two months does not produce positive results (one-month culture in liquid EMJH medium, transfer and inoculation of bacterium from the medium and re-culturing in solid EMJH for observing colonies). On the other hand, some *Leptospira* cannot be cultured; in other words, they are not capable of forming a colony (45). However, isolation and determination of their characteristics should be considered in epidemiological studies (44).

**Serological tests**

Detection of *Leptospira* antigens in clinical samples is not performed extensively. Recently, antigens have been identified in urine via dot-blot enzyme-linked immunosorbent assay (ELISA) or dot ELISA, using monoclonal antibodies against the unspecified 35-kDa pathogenic *Leptospira* (46). These tests are used to identify antigens in the urine of individuals who are serum IgM negative. The majority of leptospirosis cases are diagnosed with the aid of serological tests. Antibodies can be detected within 5-7 days after the emergence of blood signs. The highest antibody titer is observed within the second week, and the infection can be diagnosed first using blood and then cerebrospinal fluid.

Microscopic agglutination test (MAT) is the gold standard serological test for diagnosis of leptospirosis as it identifies agglutinated antibodies in the patient’s serum. In order to perform this test, the patient’s serum is placed adjacent to a live bacterial suspension of various *Leptospira* serovars. The highest titer of the serum, which leads to the agglutination of 50% of bacteria, is determined using dark-field microscopy. Considering the difficulties in the diagnosis of agglutination in almost half of *Leptospira* bacteria, the final titer can be practically controlled by evaluating the presence of 50% of non-agglutinated free leptospires against the suspension. In various laboratories, different final titers (from 1:100 to 1:800 dilutions) are used for diagnosis, which can influence the final diagnosis.

On the other hand, MAT has several limitations that restrict its extensive application including the need for a large spectrum of live leptospiral serovars to have a live antigen resource, presence of standard antisera, dark-field microscopy, skilled laboratory technicians, serum samples causing delay in diagnosis and false negative results mainly due to lack of a *Leptospira* serotype(s) in the panel of bacterial cultures. In addition, due to the high rate of cross-reactions in various serogroups, especially in the acute phase, interpretation of the results is difficult (47). Moreover, using live antigens have health hazards for laboratory technicians. It should be noted that MAT cannot distinguish between agglutinated IgM and IgG antibodies, and the antigen panel must include the representative serovars of all subgroups as
well as common serovars. Therefore, the WHO has suggested 19 serovars for 16 subgroups (44). Because of its low sensitivity, different samples including serum (a serum sample represents the acute phase and the convalescence period of the disease) are required for MAT to confirm the diagnosis. Therefore, rapid screening tests including ELISA, complement fixation test (CFT), dipstick ELISA, lateral flow test, indirect hemagglutination test and latex agglutination test have been taken into consideration for detection of antibodies in acute infections (48). Considering the complexity of MAT, different screening tests have been developed for detecting leptospiral antibodies during the infection. In this regard, CFT has been used extensively although it is not a standard method. This method is used for diagnosis in veterinary medicine based on which differences between various species can be determined (49). Accordingly, CFT has been replaced by ELISA methods. Since the IgM antibody is only produced at the end of the first week of disease onset, diagnosis is based on the identification of this antibody (50). If the first sample is collected at the beginning of the acute phase, IgM will be more sensitive than MAT. In specific dot-ELISA of IgM using nitrocellulose filters, a lower volume of reagents is needed. Due to advances in ELISA, this method can identify IgM and IgA by using fabric polyester resin instead of nitrocellulose. Although this test is a simple, fast and inexpensive approach, which can be used in laboratories with limited facilities, its results must be confirmed by MAT due to the possibility of false positives. Dipstick dot-ELISA is conducted using commercial kits, which show similar sensitivity to common IgM-ELISA test (43).

Use of LipL32 recombinant antigen in ELISA is a sensitive, specific and accurate approach for detection of anti-\textit{Leptospira} antibody in serum samples (51). On the other hand, serological tests are the most commonly used methods for laboratory diagnosis of leptospirosis. However, considering the delayed emergence of specific \textit{Leptospira} antibodies in the blood, they cannot be used for rapid diagnosis in the first week or in the acute phase of the disease; these problems can be eliminated by using molecular diagnostic methods.

**DNA detection**

In recent decades, detection of \textit{Leptospira} DNA using polymerase chain reaction (PCR) assay has been used for early diagnosis of leptospirosis. Sensitivity of this method in blood samples ranges from 28% to 98% in severe cases (52). The test also has a specificity of >90%, which is higher than that of MAT (53). Numerous genes, such as secY, rrs, Fla13, and rr1 as well as the LA3521 genomic position in the serovar of \textit{Leptospira interrogans} are targets for PCR diagnosis (54). \textit{Leptospira} can be reproduced in the blood, urine, cerebrospinal fluid, aqueous humor and tissues. Recently, real-time PCR has been introduced as a fast and sensitive approach for detecting \textit{Leptospira} DNA. This method can identify rrs, LigA, LigB, lipL32, secY and gyrB genes as well as LA0322 genomic position in the serovars of \textit{L. interrogans} using Taqman probes or fluorescence SYBR green stain. Both standard PCR and real-time PCR are applied for early diagnosis when antibodies are not produced (55).

Recently, loop-mediated isothermal amplification (LAMP) has been used for identification of pathogenic \textit{Leptospira} species. Unlike PCR, this method can duplicate the sequence of target DNA in isothermal conditions with high specificity and efficiency for almost one hour. This method does not require a thermal cycler in order to establish a thermal gradient. The results of LAMP can be assessed by naked eye (56). This method has been implemented to detect \textit{Leptospira} DNA in kidney of mice; however, its sensitivity and specificity have not been evaluated in clinical human samples (57).

**DISCUSSION**

Leptospirosis is a common multifactorial disease in Iran, particularly in the northern areas. Without laboratory tests, accurate clinical diagnosis of leptospirosis can be challenging (37). In order to diagnose this disease, various methods including MAT, ELISA, fluorescent antibody test, culture and PCR have been introduced (45). Serological tests, including MAT, which is regarded as the gold standard test in epidemiological studies, are among the most common diagnostic approaches (58).

Studies on leptospirosis rely on three principles: humans, livestock and environment (59).
Studies carried out in Iran and other countries suggest the presence of serum contamination with *Leptospira* in livestock; however, the results are controversial regarding the frequency and type of involved serotypes (Table 2). In a study by Yasouri et al. (2013), the frequency of pathogenic *Leptospira* was 33.04% among 115 water, soil and animal feces samples collected from rice fields of Tonekabon, northern Iran (60). In Italy, contaminated water or soil (81.8%), followed by animals and animal urine (18.2%) were identified as the main source of infection. Moreover, running water (51.2%) and stagnant water (27.9%) were the most common sources of infection (61).

The most common serotypes were Ballum, Sejroe, Tarassovi and Australis in a study in the Mazandaran Province, Iran. House mice and rats were the reservoir hosts of Ballum serogroup, while farm animals were the hosts of Sejroe, Australis, and Tarassovi serogroups. In another serological investigation, antibodies against *L. interrogans* serogroups Icterohaemorrhagiae, Grippotyphosa and Canicola were detected in suspected cow flocks in the Mazandaran Province, Iran (62). These serotypes were less common in humans. Furthermore, Ebrahimi et al. reported the rate of *Leptospira* serotypes to be 48.5% in tribal and the highest rate of incidence was related to serotype Hardjo (54.12%), followed by Pomona, Icterohaemorrhagiae and Canicola (63).

The rate of leptospirosis differs widely depending on the country and serotypes. For instance, Autumnalis was found as the dominant serotype in Nadu, India (64). In Brazil, Copenhageni from the Icterohaemorrhagiae serogroup was reported as the predominant serotype in an epidemic area of Salvador (65). In Italy, Icterohaemorrhagiae, Poi, Copenhageni and Bratislava were identified as the most abundant serotypes (61).

Fakheri et al. reported that the most important contributing factors for leptospirosis are the occupational exposure of farmers, livestock breeders, slaughterhouse workers, hunters and fishers, contact with infected animals and recreational activities, such as swimming, boating and skiing (66). In industrialized countries, leptospirosis occurs in a single-caught format and is mostly related to recreational boating, swimming in rivers and hunting. In addition, leptospirosis has been reported in mine workers, sewage workers and fishers (67, 68). In this regard, in a study by Zakeri et al., contact with animals, contaminated water and farming were reported as the main risk factors for leptospirosis (69).

**CONCLUSION**

Since *Leptospira* have multiple specific hosts, eradication of leptospirosis can be challenging. Leptospirosis is known to resemble influenza and cannot be easily diagnosed due to variability of clinical signs. The disease is underreported due to the shortage of diagnostic samples and difficulties in diagnosing and differentiating the clinical signs of the disease from other endemic diseases. Today, there is no reliable reference/resource for estimation of leptospirosis in humans. A major problem in evaluating the occurrence of leptospirosis in humans is identification of its sources. Therefore, further studies are required to investigate the sensitivity of leptospirosis tests. Despite the economic burden of leptospirosis and the associated public health concerns, this disease has not been taken seriously in Iran. To control this disease, it is important to educate people about the modes of transmission and encourage the use of protective equipment among rice farmers and livestock breeders. It is also essential to determine prevalence and dominant serovars/serogroups in different areas, and provide polyvalent vaccines.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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