Original Article

Molecular Detection of *Leptospira* Infection in the Iranian Dromedary Camel Population of South Kerman, Iran

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ABSTRACT

Background: Leptospirosis is a zoonotic disease with global distribution. A wide variety of mammals, including camels, are affected by this disease. The most important complications of this disease comprise a severe drop in production, abortion, and kidney damage. The climatic conditions of southern Kerman Province in Iran, combined with the significant presence of camels, underscore the need for evaluating Leptospirosis in camels in this region.

Objectives: This study aimed to detect the presence of *Leptospira* bacteria in the camel population of South Kerman Province, Iran, using molecular methods.

Methods: For this purpose, 100 blood samples were taken from the jugular veins of seemingly healthy camels in the south of Kerman to perform molecular techniques. Then, the DNA from the samples was extracted using a DNA extraction kit, following the manufacturer's instructions. In the following, a polymerase chain reaction (PCR), technique using specific primers targeting the *16S rRNA* gene of *Leptospira interrogans* was employed to detect *Leptospira* bacteria.

Results: The DNA of the *Leptospira* bacterium was found in 5 out of 100 camel blood samples (5%) using the PCR molecular technique.

Conclusion: This study demonstrated that the *Leptospira* bacterium is present in the apparently healthy camel population in southern Kerman. Considering the ever-increasing use of camel meat and dairy products in humans, as well as the resulting economic losses in the country's livestock industry, this finding can serve as a warning to the Ministry of Health and Medical Education of Iran.

Keywords: Camel, Iran, Leptospira, Polymerase chain reaction (PCR), South of Kerman

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Introduction

Leptospirosis is a disease that can affect both humans and animals, with a global distribution. The cause of this disease is *Leptospira interrogans*. Different serovars of *Leptospira* have a remarkable ability to infect domestic animals, wild animals, rodents, and humans with this disease (Khalili et al., 2020) there is no accurate information on the overall prevalence of this disease in humans and animals. The aim of this systematic review and meta-analysis was to estimate the seroprevalence of leptospirosis among human and domestic and wild animals in Iran. (Khalili et al., 2020). Humans, as well as wild and domestic animals, can contract Leptospirosis directly after contact with the body secretions of infected animals or indirectly by being in contaminated environments. In the event of a struggle with this disease, camels can incur significant economic and health risks.

Leptospirosis in camels, like other ruminants, can be associated with complications such as abortion, reduced fertility, septicemia, anemia, jaundice, fever, anorexia, bloody urine, and necrotic injuries of the skin and mucous membrane. In recent years, the prosperity of the milk and camel meat market, due to its numerous advantages, has made the prevention and control of Leptospirosis in these animals very important. The results of studies conducted in Iran and the world clearly show that leptospiral infection with all common serotypes has a significant occurrence in camels (Marsh et al., 2014; Gyimesi et al., 2015; Zhang et al., 2016; Mohammadpour et al., 2020; Yeni et al., 2024). The clinical picture of Leptospirosis is similar to that of some bacterial and viral infections, and it does not exhibit pathognomonic and specific symptoms. For a definitive clinical diagnosis, laboratory diagnosis is of particular importance (Rostampour Yasouri et al., 2020). By using laboratory methods, it will be possible to determine the serovar causing the infection and identify the reservoirs of the disease, thereby controlling the disease. Leptospira detection methods in clinical samples are possible using both direct (including culture techniques, microscopic, and molecular observation) and indirect methods (serology) (Cucchi et al., 2019). The inability of serological methods to quickly diagnose this bacterium due to its slow growth and the absence of specific antibodies in the first week of the disease has made the polymerase chain reaction (PCR), technique a suitable alternative, given its high sensitivity and accuracy (Rafeei et al., 2012; Khalili et al., 2020)

Studies conducted by researchers have confirmed the relationship between weather conditions and the occurrence of Leptospirosis (Cucchi et al., 2019). Jiroft City is located in the south of Kerman Province, Iran. The climate of this city is hot and relatively humid. Therefore, according to the mentioned weather conditions, this city is favorable for the transmission of Leptospirosis. Additionally, this city holds the fifth rank in camel breeding nationwide. Therefore, in this research, molecular diagnosis of *Leptospira* bacteria was carried out in the camel population of South Kerman.

Materials and Methods

Study population

One hundred blood samples were taken from apparently healthy mature female and male camels (with an average age of 4 years and above) with an average weight of 370 kg for females and 300 kg for males (June–September 2022).

Sample collection

Five milliliters of blood were collected from the jugular vein of each camel. Then, the blood was immediately transferred to an EDTA-containing tube for PCR tests. Following collection, the samples were promptly preserved and transported to the laboratory for subsequent analysis. All of the camels were clinically healthy and showed no clinical symptoms of Leptospirosis at the time of blood collection.

DNA extraction

DNA was extracted from each blood sample using a blood DNA extraction kit (Parstous, Iran) according to the manufacturer's instructions (Parstous, 2025). The quality and quantity of DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Then, the DNA samples were stored at -20 ˚C until they were required for molecular analysis.

Conventional PCR

The PCR reaction was carried out in a final volume of 25 µL, containing 12.5 µL of PCR Master Mix (Ampliqon, Denmark, 2024), 1 µL of each primer (LP, 0.4 µM) (Pishgam Biotech, Iran), 8 µL of nuclease-free water, and 2.5 µL of template DNA. The information on primers is described in Table 1. PCR reactions were performed using a Thermal Cycler (Bio-Rad, USA). The PCR conditions were as follows: initial denaturation

Table 1. Primers used in the study

Primer Name	Primer Sequence	Product Size (Bp)	Ref.
Lp-F Lp-R	5′-GCGCGTCTTAAACATGCAAG -3′ 5′-CTTAACTGCTGCCTC CCGTAG-3′′	306	Doosti et al. (2012)

at 95 ˚C for 3 minutes, followed by 35 cycles of denaturation at 95 ˚C for 30 seconds, annealing at 57 ˚C for 30 seconds, and extension at 72 ˚C for 1 minute, with a final extension at 72 ˚C for 10 minutes. Distilled water and leptospiral DNA from a commercial Lepto PCR kit (Intron Biotechnology Company, Korea) were used as negative and positive controls, respectively. The PCR products were visualized on a 1% agarose gel stained with 7 µL DNA Green Viewer (Parstous, Iran), and the amplicon size was compared with a 100 bp DNA ladder (Ampliqon, Denmark).

Results

The molecular results of this research detected the DNA of *Leptospira* bacteria in 5 out of 100 camel blood samples (5%) (Figure 1).

Discussion

Epidemics of Leptospirosis in various locations over the last decade indicate the resurgence of this disease (Sakhaee et al., 2007). Worldwide, 7 to 10 million people are infected with Leptospirosis every year (Mohammadpour et al., 2020). A wide range of mammals, including cattle, sheep, goats, horses, dogs, camels, wild ruminants, rodents, and humans, are affected by this disease. Climate changes all over the world, an increase in torrential rains, frequent floods, an increase in population density in urban areas, the close relationship between humans and domestic animals, poverty, and, in parallel, the inability of human society to observe hygiene, among other things, have increased the frequency and extent of this disease all over the world. In other words, hot and humid climatic conditions, as well as rainfall, are among the key factors in increasing abundance. Due to the hot and humid climate and the presence of several rivers in the south of Kerman, this area is favorable for the prevalence of Leptospirosis. Paraclinical tests typically aid in the diagnosis of diseases. Bacterial culture methods are expensive, time-consuming, difficult, and unsuccessful in most cases (Cucchi et al., 2019). The PCR technique is used for the early detection of the disease, especially during the first week of the disease and in the absence of antibodies (Rafeei et al., 2012; Khalili et al., 2020). Therefore, according to the aforementioned explanations, this study investigated the molecular diagnosis of *Leptospira* bacteria in the camel population of South Kerman, yielding an infection frequency of 5%, which indicates the presence of this bacterium in the camels of South Kerman. Probably the reason for the low frequency of infection in camels in South Kerman is the following.

First of all, since there are several important rivers in the south of Kerman, it was assumed that the frequency

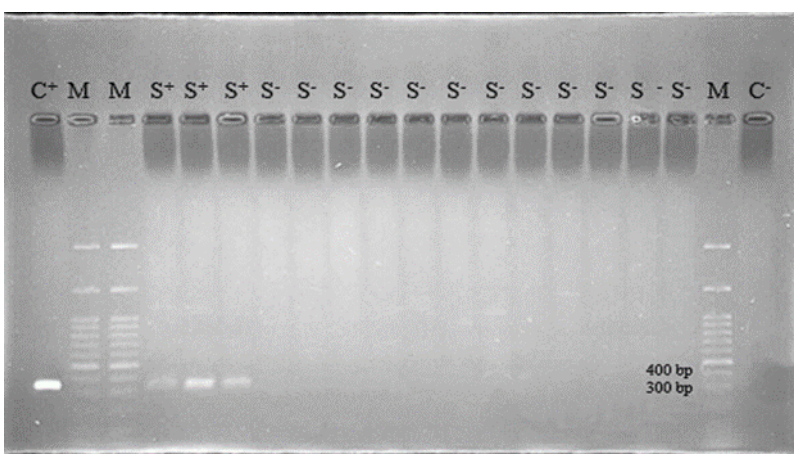

Figure 1. Electrophoresis photo of PCR products

Note: S^+: Positive samples for *16S rRNA* gene of *L. interrogans*; S^-: Negative samples for *16S rRNA* gene of *L. interrogans*; C^+: Positive control (leptospiral DNA of commercial Lepto PCR kit); C^-: Negative control (sterile distilled water); M: 100bp DNA ladder.

of this disease is high in this region. However, due to consecutive droughts in the last few years, these rivers have become severely depleted. So some of these rivers dried up. This problem of drought can be an important reason for reducing the frequency of disease.

Secondly, the space for keeping camels was very suitable, allowing a small number of animals to be accommodated in a large area, thereby eliminating any crowding issues. This issue significantly reduced the likelihood of healthy animals coming into contact with the urine or infected secretions of sick animals or disease reservoirs.

Studies with similar frequency percentages to the present study's results (low frequency) have been conducted, as follows.

A study was conducted in Saudi Arabia in 2009 on urine samples from 36 locally slaughtered camels. The samples were examined using a dark-field microscope and then cultured. All samples were negative. Additionally, the serum of 90 Arabian camels was tested using a microagglutination test. Six camels (6.7%) were positive for *Leptospira autumnalis* antibodies. This log was the first serological record of camel Leptospirosis in Saudi Arabia (Hussein & El-Nabi, 2009).

In 2019, a study in the Republic of Kazakhstan examined the prevalence of Leptospirosis in cattle, pigs, and camels using serological and bacteriological methods. Most positive results were observed in cows and pigs, but among camels, they were sporadic (Ilyasov et al., 2019).

In 2015, a study was conducted in Egypt, involving 1250 animals (270 rats, 168 dogs, 625 cows, 26 buffaloes, 99 sheep, 14 horses, 26 donkeys, and 22 camels). The animals were tested using microagglutination and PCR techniques to investigate Leptospirosis. The isolation rate of *Leptospira serovars* for rats, dogs, and cows was 6.9%, 11.3%, and 1.1%, respectively. Additionally, PCR results revealed 24%, 11.3%, and 1.1% positivity rates for rats, dogs, and cows, respectively. Camels and other species were negative in both techniques (Brasileira et al., 2015).

Additionally, various studies have been conducted with different frequency percentages of the results from the present research, which are as follows.

In a study in Iran, blood samples were collected from 60 camels in Ardabil City, Iran. Their serum was analyzed by the microagglutination method. The frequency of contamination was 20%. The frequency of infection with serovar Pomona (8.3%), serovar Hardjo (3.8%), and serovar Canicola (3.3%) was announced (Afkhamnia et al., 2014).

In a study conducted by Doosti et al. to determine the prevalence of *Leptospira* infection in Iranian camels by molecular method. One hundred thirty camel blood samples were collected, and their genomic DNA was extracted. The PCR reaction was performed to detect *Leptospira* DNA using specific primers targeting the *16S rRNA* gene of *Leptospira*. The abundance of *Leptospira* DNA was 14.61% (Doosti et al., 2012).

A study conducted by Hassani and colleagues (2012) in Isfahan evaluated the presence of Leptospira in 49 camels. The frequency of contamination by bacterial culture and multiplex PCR was 14.29% and 6.33%, respectively (Hassani, 2021).

Among the reasons for the difference between the results of the mentioned research and the results of the present research, we can point out things such as disease diagnosis with different techniques, the use of other clinical samples such as urine, geographical differences as a result of climate differences, and also the difference in the level of hygiene.

Conclusion

The results of the present research and studies conducted worldwide clearly indicate the presence of leptospiral infection in camels, including those in southern Kerman. Therefore, due to the increasing use of camel meat and milk in humans, necessary measures should be taken to control this disease in camels in various regions of the world, including the southern region of Kerman, as well as to raise public awareness among the general population. Additionally, according to the results of the present research, the presence of this bacterium in apparently healthy camels raises the alarm for public health and economic losses. Some recommendations for better control of this infection in the camel population are as follows: Controlling rodents and humidity in camel housing, Improving the sanitary conditions of camel slaughterhouses, paying closer attention to the presence of this zoonotic bacterium in apparently healthy camels and implementing practical programs to prevent this issue (done by the Ministry of Health and Medical Education of Iran).

Ethical Considerations

Compliance with ethical guidelines

All implementation phases of this study were approved by the Animal Care Committee of Veterinary College of Shahid Bahonar University of Kerman, Kerman, Iran.

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This study was extracted from the masters's thesis of Reza Dorri, approved by the Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Authors' contributions

Experiments and writing the original draft: Reza Dorri; Data analysis: All authors; Supervision, review and editing: Elham Mohammadi and Mehdi Golchin.

Conflict of interest

The authors declared no conflict of interest.

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