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PCR DETECTION OF LEPTOSPIROSIS IN IRANIAN CAMELS

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Summary

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Leptospirosis is a zoonotic infectious disease caused by pathogenic members of the genus *Leptospira* with a worldwide distribution, reported in humans and over 150 mammalian species. The camels reared in Iran are animals with a unique physiological constitution, resistant to many infectious diseases, but affected with leptospirosis. The aim of present study was to determine the prevalence of *Leptospira* infection in camels in Iran by molecular methods. One hundred and thirty camel blood samples were collected and genomic DNA was extracted. PCR reaction was performed for detection of *Leptospira* DNA using specific primers for *Leptospira 16s rRNA* gene. The frequency of leptospiral DNA in camel blood samples was 19 of 130 (14.61%). Leptospirosis may become more important in camels with the increasing trend towards intensive dairy camel production in some countries like Iran. Considering that the majority of leptospirosis cases in men were due to association of men with animals and disease-infected environment and according to present findings, the examination of camels for *Leptospira* infection seems to be necessary for control and prevention of leptospirosis.

Key words: *16s rRNA* gene, camel, Iran, *Leptospira,* PCR

INTRODUCTION

Leptospirosis is a zoonotic infection with a worldwide distribution, caused by spirochaetes of *Leptospira* genus. This thin, motile spirochaete with a hook-shaped end is usually spread through direct contact via injured skin mucosal membrane and causes emerging infectious disease in humans and camels (Doosti & Hoveizeh Tamimian, 2011; Sykes *et al*., 2011). Leptospirosis is caused by *Leptospira interrogans* sensu lato (Mosallanejad *et al*., 2011). Leptospirosis has been reported in over 150 mammalian species and men (Sykes *et al*., 2011).

The genus *Leptospira* contains at least 18 species classified on the basis of DNA relatedness and more than 300 serovars based on agglutinating LPS antigens (Esfandiari *et al*., 2011). Both saprophytic and pathogenic species exist in nature. Saprophytic species, such as *Leptospira biflexa*, live in water and soil and do not infect animals. Leptospires phylogenetically and pathogenically intermediate to these 2 groups have also been identified in humans and animals (Sykes *et al*., 2011). All pathogenic leptospires were formerly classified as members of *Leptospira interrogans*; the genus has recently been

reorganised and pathogenic leptospires are now identified in several species of *Leptospira* (Hassanpour *et al*., 2011).

Leptospira organisms could be isolated from body fluids, mainly urine; and target tissues as kidney, liver, lungs, and brain. If the agent is suspect for abortions, isolation could be attempted from non-autolysed abortion materials or tissue samples from a freshly aborted foetus. Isolation of the microorganism from foetal tissue (kidney, liver, lungs) confirms maternal infection (Burriel, 2010). Abortion may occur several weeks later, but may also occur as the only evidence of the disease in this form (Doosti & Hoveizeh Tamimian, 2011).

The prevalence of leptospirosis among animals, in sheep and goats, cattle, rats was 2-46% (Ciceroni *et al.*, 2000; Epsi *et al.*, 2000; Faria *et al.*, 2007) and indicates that for minimising its economic impact, the infection must be controlled mainly among food producing animals (Burriel, 2010). Long-term survival of pathogenic leptospires outside the host requires a warm and moist environment with near-neutral pH (Mosallanejad *et al*., 2011). Despite the presence of leptospiral antibody titres in feline populations, clinical reports of leptospirosis in camels and cats are infrequent (Mosallanejad *et al*., 2011). Serological evidence of camel leptospirosis has been reported in Egypt, Somalia, Ethiopia, Tunisia, United Arab Emirates, Iran, India, Afghanistan and the former USSR (Mansour & Gar El Nabi, 2009).

Direct methods of investigating leptospirosis are the isolation of the causative agent and the identification of *Leptospira* spp. antigens in tissue and body fluids using methods as immunofluorescence, immunochemistry, and molecular techniques (Burriel, 2010). The microscopic agglutination test is based on the use of live *Leptospira* cultures and this method may take up to eight weeks with weekly inspection and examination. Moreover, in other methods many factors may cause false positive and negative results. Polymerase chain reaction (PCR) has been used to detect a large number of microorganisms of clinical significance including leptospirosis pathogens (Doosti & Hoveizeh Tamimian, 2011).

Camels are raised basically for meat consumption which is of better quality and less expensive. Leptospirosis may become more important in camels as there is an increasing trend towards intensive dairy camel production in some countries like Iran. So, the purpose of present study was to determine the frequency of *Leptospira* infection in camels using PCR technique in the Isfahan province, southwest Iran.

MATERIALS AND METHODS

Sampling and DNA extraction

In present study, 130 camel blood samples were collected from slaughterhouses in Isfahan province (southwest Iran) in 2011. Genomic DNA was isolated from specimens using DNA Extraction Kit (Qiagen, Crawley, UK) according to the manufacturer's protocol. The extracted DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell (Sambrook & Russell, 2001). The extracted DNA of each specimen was kept frozen at –20°C until used.

16S ribosomal RNA gene amplification

Species-specific oligonucleotide primers Lp-F: 5'-GCGCGTCTTAAACATGCAAG -3' and Lp-R: 5'-CTTAACTGCTGCCTC CCGTAG-3' designed from the *16S*

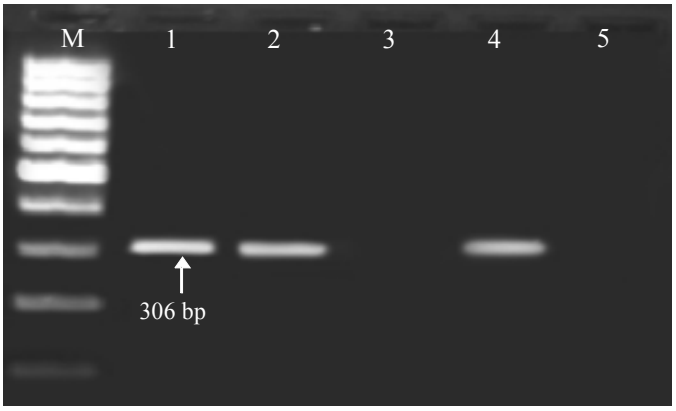

Fig. 1. Gel electrophoresis for detection of leptospiral infection in blood samples. Lane M – 100 bp DNA ladder (Fermentas, Germany); lane 1 and 2 – positive samples, lane 3 – a negative sample; lane 4 – positive control; lane 5 – negative control.

ribosomal RNA gene of *Leptospira* (accession number: JF460977.1) were used for gene amplification. PCR was performed in a 50 µL total volume containing 1 µg of template DNA, 1 µM of each primer, 2 mM $MgCl_2$, 200 µM dNTP, 5 µL of 10× PCR buffer and 1 unit of *Taq* DNA polymerase (Roche Applied Science). The following conditions of PCR were used for gene amplification for the first round: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min. The programme was followed by a final extension at 72 °C for 5 min. The PCR amplification products (10 µL) were subjected to electrophoresis in a 1% agarose gel in 1× TBE buffer at 80 V for 30 min, stained with ethidium bromide, and images were obtained in UVIdoc gel documentation systems (UK). A negative control (sterile water) and a positive control DNA from *Leptospira* ATCC 43642 strain were included in each amplification run.

RESULTS

One hundred and thirty camel blood samples were examined for presence of leptospiral DNA. The primers used were derived from the *16S rRNA* gene of *Leptospira.* Agarose gel electrophoresis of positive samples revealed a 306 bp fragment. An example of PCR amplification of blood samples is shown on Fig. 1.

Leptospiral DNA was found in 19 of 130 (14.61%) camel blood samples The results showed a high frequency of *Leptospira* infection in camels reared in the Isfahan province.

DISCUSSION

Leptospirosis is a global public health problem as it causes increased mortality and morbidity in different countries (Shafighi *et al*., 2010). Leptospirosis was first described in Malaysia as early as 1926 by Fletcher who was responsible for the initiation and establishment of leptospirosis research in this country (Bahaman *et al*., 1988). The earliest recognised report

of leptospirosis in Iran was published by Rafyi and Magami (Shafighi *et al*., 2010). The incidence of leptospirosis has been reported to vary significantly in different seasons, and to be higher in winter months (Cetinkaya *et al*., 2000). Headache, myalgia, nausea, and vomiting are common complaints; however, neurologic, respiratory, cardiac, ocular, and gastrointestinal manifestations can occur (Hassanpour *et al*., 2011). Leptospires enter the body through cuts and abrasions, mucous membranes or conjunctivae, or aerosol inhalation of microscopic droplets and invasion of the central nervous system and aqueous humour of the eye (Libraty *et al*., 2007). Infection may also arise from bathing or accidental immersion in the fresh water of lakes, rivers or canals contaminated with the urine of the infected livestock. Leptospirosis was thought to be primarily an occupational disease. Miners were the first occupational risk group to be recognized (Zavitsanou & Babatsikou, 2008). Other high risk groups reported are the farmers, veterinarians, abattoir workers, rodent control workers, soldiers, sewer workers, fish farmers, rice field workers, banana farmers, and others. Understanding the epidemiological features of leptospirosis is a critical step in designing interventions for diminishing the risk of the disease transmission (Zavitsanou & Babatsikou, 2008). Human leptospirosis can be highly variable, ranging from asymptomatic infection to sepsis and death (Hassanpour *et al*., 2011). Rats and other rodents are the most important sources for human infection (Zavitsanou & Babatsikou, 2008). The accumulation of information in recent years on leptospirosis has shown that serovar distribution around the world is neither uniform nor stable (Burriel, 2010). Leptospirosis has been identified as a re-emerging infectious disease in tropical and subtropical climates and this has been demonstrated by the large outbreaks in Nicaragua, Brazil, India, Southeast Asia, Malaysia and in the United States (Zavitsanou & Babatsikou, 2008; Burriel, 2010).

Leptospirosis is one of the most representative zoonotic diseases, with great economic importance for livestock causing significant economic losses (Lucheis & Ferreira, 2011). Diagnosis of leptospirosis is based on laboratory confirmation because its clinical signs are nonspecific and may be mistaken with other febrile diseases (Vado-Solis *et al.,* 2002). Previously the diagnosis of leptospirosis had relied mainly on the detection of antibodies with either serological techniques (ELISA) or microscopic agglutination test (Aghaiypour & Safavieh, 2007), which are time consuming and difficult. In addition, detection of camel abortion cause is very important and it is better to use the molecular methods such as PCR technique (Doosti & Hoveizeh Tamimian, 2011). The practical value of PCR in diagnosis of leptospirosis is its ability for rapid detection of the bacteria in early phase of disease, which is very important with regard to its treatment and control (Aghaiypour & Safavieh, 2007). Studies have proved that PCR is faster and more sensitive than the conventional tests (Cetinkaya *et al*., 2000).

The percentage of leptospiral infection among cattle in west Malaysia was 14.4% (32/222) (Bahaman *et al*., 1987). The same study in west Malaysia showed that out of the 3377 serum samples from domestic animals tested, the observed prevalence of *Leptospira interrogans* was 40.5% in cattle, 31% in buffaloes and 16% in pigs using MAT assay (Bahaman *et al*., 1987). The study of Bal *et al*.

(1994) showed that 26 of the 29 urine samples from patients with leptospirosis were positive by PCR. Incidence of leptospiral abortion in Brazilian dairy cattle was studied in 1999, in total, 72 (60%) of 120 aborted foetuses had evidence of leptospiral infection (Doosti & Hoveizeh Tamimian, 2011).

In our study the frequency of *Leptospira* pathogens was investigated in camels by PCR technique and *16S rRNA* gene of this infectious agent was amplified by species-specific primers. DNA of leptospires was detected in 19 out of 130 camel blood samples (14.61%). The results of this study showed a high frequency of this microorganism in camel in west of Iran. The study by Mansour & Gar El Nabi (2009) provided serological evidence of leptospirosis in camels in Saudi Arabia and reported 6.7% of the serologically tested camels to be positive for *Leptospira*. A study performed in 2011 on frequency of leptospiral DNA in liquid rennet samples of aborted bovine and established that the frequency of this microorganism was 17 of 120 (14.16%) (Doosti & Hoveizeh Tamimian, 2011). Cetinkaya *et al.* (2000) showed that the seroprevalence of disease in cattle populations in the UK varied between 35% to 76% in the different regions. The prevalence of leptospiral infection in Ahvaz (Southwest Iran) in horses, donkeys and sheep was 27.88%, 40% and 14.9%, respectively (Haji Hajikolaei *et al*., 2005; 2007). Also, the frequency of pathogenic leptospiral DNA were 28.46% in ewes, and 27.47% in humans in Khoy and Mazandaran respectively (Esfandiari *et al*., 2011; Hassanpour *et al*., 2011).

In conclusion, the results of present study showed the importance of leptospiral infection among Iranian camels. Leptospiral antibodies detected in the serum of indigenous camels (*Camelus dromedarius*) in Iran, provided evidence for the existence of camel leptospirosis in the country and stressed the need for further studies on the prevalence and epizootiology of this important zoonotic disease in Iranian camels.

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