PCR DETECTION OF LEPTOSPIRA IN STRAY CATS, PROBABLY RESERVOIR

DéTECTION DE LA LEPTOSPIROSE PAR PCR CHEZ LE CHAT ERRANT, PROBABLE RÉSERVOIR

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(Note presented by A. Philippon)

Leptospirosis is an important spirochetal disease in terms of public health worldwide. Numerous wild and domestic animal species are major reservoirs of Leptospira, and sources of infection for humans. Transmission to humans occurs by direct contact with infected animals, or by contact with soil or water contaminated with the urine of an infected animal, generally asymptomatic. The objective of this study was to determine the frequency of Leptospira by PCR in stray cats in Isfahan province, Iran. Amplicons of 793 bp were detected in the blood (buffy coat) of 28 out of the 132 stray cats examined (21.2%), as all of them showed no clinical signs of leptospirosis. Stray cats seem to constitute a non-negligible reservoir of Leptospira spp. for humans.

Key words: leptospirosis, cat, reservoir animals, PCR.

INTRODUCTION

Leptospirosis is a worldwide, zoonotic disease that causes by Leptospira spp, a gram-negative spirochete organism. More than 200 serovars of pathogenic Leptospira are isolated from human beings and various wild and domestic animals (Leighton & Kuiken, 2001; Leveret 2005; Majed et al. 2005; Thongboonkerd, 2008). This disease is important in public health. Outbreaks of leptospirosis are reported from different countries such as India, Japan and Brazil during the last few years (John, 1996; McBride et al. 2005; Nakamura et al. 2006). A wide range of animals are source of infection for humans and act as important reservoirs for Leptospira spp. Direct contact with infected animals or exposure to contaminated soil and water with the urine of reservoir animals are routine ways in transmission of leptospirosis into the human. After leptospiremia, this orga-
nism localizes in the kidneys of infected animals and excreted in their urine (Faine et al. 1999; Vijayachari et al. 2008; Koizumi et al. 2008; Adler & de la Peña Moceteuma, 2010).

However different studies showed that several species of animals were responsible for distribution and transmission of leptospirosis in the environment but the role of felines is unknown. Cat may serve as a source of Leptospira infection for human (Everard et al. 1979; Agunloye & Nash, 1996; Mosallanejad et al. 2011). In Iran, there is a high population of stray cats that live near the human habitats for obtaining their dietary requirements. They may also be exposed to Leptospira spp. by hunting the infected prey such as rodents, which are considered as important Leptospira spp. reservoirs and contaminate the environment. In addition, cats have contact with stray dogs that may be affected to leptospirosis (Everard et al. 2010). Therefore, it is necessary to investigate the possible role of cats in the epidemiology of Leptospira spp.

The present study was undertaken for detection of Leptospira spp. frequency in the blood of stray cats by polymerase chain reaction (PCR) method.

**MATERIALS AND METHODS**

**Sample collection**

From autumn to winter of 2009, 132 stray cats were captured with the special iron cage at different areas of Isfahan province, central part of Iran. The used cage has a hook for hanging the bait. When the cat enters the cage for eating the bait, the door of cage is closed automatically. The cats were examined for clinical signs of leptospirosis and all of them were clinically normal. They were sedated by injection of ketamine (10 mg/kg) and acepromazine (0.15 mg/kg). Three ml of blood was collected from the jugular vein of each cat in the tubes containing EDTA anticoagulant, centrifuged at 10000 x g for 10 minutes. The plasma on the top of tube was removed and discarded. The buffy coat was aspirated and resuspended in 4 volumes of sterile 0.2% NaCl to lyse the erythrocytes. After one min, 7.2% NaCl was added to reconstitute isotonicity. The cells were further washed in phosphate-buffered saline and stored at -20ºC until the processing (Muller-Doblies et al. 1998).

**DNA extraction and PCR amplification**

DNA was extracted from each 132 buffy coat samples using genomic DNA purification kit (Fermentas) according to the manufacturer’s instructions. PCR technique was performed using the primers previously described by Krishna et al. (2008). The forward flabB primer was 5’TCTCACCCTCTCTAAAAGTTCAAC3’ and the reverse was 5CTGAATTTCCGTTTCAATTTGCC3’. The reaction was incubated at 94ºC for 6 min in one cycle, followed by 34 cycles of denaturation at 94ºC for 50 sec, annealing at 58ºC for 5 sec, extension at 72ºC for 45 sec, and a final extension at 72ºC for 10 min. A negative control (sterile water), and a positive control DNA from Leptospira interrogans (Razi Institute, Karaj, Iran), were included in each amplification run. In the negative extraction control, an equal volume of sterile deionised water was used. As positive controls, sterile water was artificially inoculated with 106 cells obtained from cultures of Leptospira interrogans. The amplified samples were analyzed by electrophoresis (120 V/208 mA) in 2% agarose gel. The gel was stained with 0.1% ethidium bromide (0.4 µg/mL) and viewed on UV transilluminator. A sample was considered positive when the 793 bp fragment was obtained.

**Statistical analysis**

Statistical analysis was performed using SPSS/18.0 software for significant relationship between the presence of Leptospira in male and female cats. Chi-square test was performed and differences were considered significant at P< 0.05.

**Ethical consideration**

The study was approved by the local ethics committee of our faculty, in accordance with the ethics standards of “Principles of Laboratory Animal Care”.

**RESULTS**

Based on PCR technique, amplicons of the expected size (793 bp) were detected in 28 out of 132 (21.2%) buffy coat samples of stray cats (figure 1). In this study, the serovars of positive samples were not identified. No significant difference was found between the male and female cats (P> 0.05) (table 1).

![Figure 1: Detection of leptospiral infection in blood samples of stray cats. M: 1000 bp molecular weight markers, lane: 1 positive control, lane: 2 negative control, lanes 3-7: positive amplification (793 bp).](image)

<table>
<thead>
<tr>
<th>Sexe</th>
<th>Number</th>
<th>PCR positive</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Female</td>
<td>72</td>
<td>15</td>
<td>20.8</td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>13</td>
<td>21.6</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>28</td>
<td>21.2</td>
</tr>
</tbody>
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*Table 1: The proportion of PCR positive of buffy coat samples collected from stray cats.*
DISCUSSION

Leptospirosis causes important public health problems in human and is a significant economic disease in animals which provide food source for humankind (Vanasco, 2000; Victoria et al. 2008; Shivara et al. 2009). In the nature, Leptospira spp. is preserved by various animal reservoirs such as dog, cattle, sheep, horse, pig and rodents (Koizumi et al. 2008; Pinto Jorge et al. 2011). Each animal species may be responsible in transmission cycle of leptospirosis. Cats have a high risk of exposure to Leptospira spp. These animals can be hosts for some serovars of this organism but due to lack of manifestation of clinical signs, not be attended properly. Many people are interested in keeping the cats as a pet. The household cat may go out and contact with stray dogs. In according to the close contact between human and cat, it needs the role of this species in the epidemiology and transmission of leptospirosis is more investigated.

In present study, the frequency of Leptospira in cat was estimated by flabB gene of Leptospira by PCR. Krishna et al. (2008) used PCR-RFLP based flabB gene for diagnosis of leptospirosis in rats and patients suspected to clinical signs of leptospirosis. They showed flabB PCR has capable in detecting of pathogenic leptospirosis.

In this study, we used PCR technique because the review of literature reveals that microscopic agglutination test (MAT), dark field microscopy and ELISA are conventional methods in identification of Leptospira spp. in blood sample but each of them has some disadvantages (Dey et al. 2004; Liu et al. 2006). In recent studies, PCR technique is more considered for diagnosis of Leptospira spp. in different clinical samples (Gumussoy et al. 2009; Shivara et al. 2009, Hernández-Rodríguez et al. 2011). PCR is a simple, rapid and valuable method that has ability to detect small number of organism (Cespedes et al. 2007). Amplification of leptospiral DNA from blood can be useful in the diagnosis of acute infection.

In our study, 21.2% (n=28/132) of buffy coat samples were positive for Leptospira spp. in the cat population. The frequency of infection in the present study was higher than that the previous report by Mossallanejad et al. (2011) from Iran, which obtained 4.9% serologic positive in 102 stray cats in Ahvaze province by MAT. They described that low prevalence of feline leptospirosis may be due to relatively hot and dry weather in all seasons specially in summer that reach up to 50°C. Climate may be an important factor affecting the prevalence of Leptospira in the each area. Tropical climate is suitable climate for surviving Leptospira spp. and the highest prevalence of Leptospira has been reported in the rainy season (Ward et al. 2002). In a second study in Iran, Jamshidi et al. (2009) reported 27% prevalence of leptospirosis in 111 stray and household cats in Tehran province. They concluded that cats can be exposed to leptospires and infect the environment or transmit the disease to contact people. Larsson et al. (1984) determined occurrence of leptospiral infection in cats by microscopic agglutination test and attempted to isolate leptospires from the kidneys of these animals. Of 172 serum examined samples, 22 (12.8%) were positive with titers greater than or equal to 100 and the most frequent serovar was pomona. Natarajaseenivasan et al. (2002) investigated leptospirosis in the rice mill workers, and the animal and rodent populations living in the same area by MAT. They stated intermediary hosts including cattle, dogs, and cats (66.6%, n=6 out of 9 cats) reared in the same area, can be an epidemiological niche for transmission of leptospires. In Spain, the prevalence of leptospirosis in cats was reported from 4.5 to 14.0% (Millan, 2009). Agunloye & Nash (1996) investigated 87 cats to determine the prevalence of leptospiral infection and possible resultant disease. Eight cats (9.2%) reacted serologically with the antigens of three serovars. Andre-Fontaine (2006) in a survey on 98 ill cats in France showed that 48% were positive in microagglutination test (MAT) to Leptospira spp. and stated that this infection is also frequent in the feline species.

The present results show preliminary data of leptospirosis in cats. We suggest that the role of cats in dispersion of Leptospira spp. in environment should be more considered. Epidemiology of Leptospira spp. chain is complex and cats may be a candidate in transmission of infection. Amplification of leptospiral DNA from blood would only be positive in acute stage of infected animals. Animals that have cleared the organisms from the bloodstream, but still harbor it in their organs such as liver or kidney are not detected with this approach. Further studies should be conducted for detecting Leptospira spp. in urine and kidney for assessment of chronic shedding and better understanding the role of cat in leptospirosis epidemiology.

REFERENCES


