PCR detection of Leptospira in stray cat and possible role as reservoir

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Introduction

Leptospirosis = **worldwide, zoonotic disease** that causes by *Leptospira* spp. = **spirochete**. > 200 serovars of pathogenic *Leptospira* are isolated from **human beings and various wild and domestic animals**. This disease is important in **public health**. **Outbreaks** of leptospirosis are reported from different countries (India, Japan and Brazil) during the last few years.

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 organism localizes in the kidneys of infected animals and **excreted in their urine**.
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. In addition, cats have contact with stray dogs that may be affected to leptospirosis (Greene *et al.* 2006).

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 captured with the iron cage (Isfahan province). When the cat enters to 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. These were sedated (ketamine, 10 mg/kg, acepromazine 0.15 mg/kg). 3 ml of blood collected from the jugular vein + EDTA, centrifuged at 10000 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 1 min, 7.2% NaCl was added to reconstitute isotonicity. The cells were further washed in phosphate-buffered saline and stored at -20°C 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). PCR technique was performed using the primers previously described by Krishna et al. (2008). The forward *flabB* primer was 5’TCTCACCGTTCTCTAAAGTTCAAC3’ and the reverse was 5CTGAATTTCGTTTCATATTTTGCC3’. The reaction was incubated at 94°C for 6 min in one cycle, followed 34 cycles of denaturation at 94°C for 50 sec, annealing at 58°C for 5 sec, extension at 72°C for 45 sec, and a final extension at 72°C 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 observed.
Statistical analysis
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

Fig. 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).
RESULTS

No significant difference was found between the male and female cats ($P > 0.05$).

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>PCR positive</th>
<th>Number</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>53.6</td>
<td>15</td>
<td>72</td>
<td>Female</td>
</tr>
<tr>
<td>46.4</td>
<td>13</td>
<td>60</td>
<td>Male</td>
</tr>
<tr>
<td>21.2</td>
<td>28</td>
<td>132</td>
<td>Total</td>
</tr>
</tbody>
</table>
Discussion

Cats have a **high risk of exposure** to *Leptospira* spp. The frequency of *Leptospira* was estimated by *flabB* gene of *Leptospira* by PCR (Krishna et al. (2008) detecting pathogenic leptospirosis.

The **review of literature** reveals that **microscopic agglutination test** (MAT), **dark field microscopy** and **ELISA** conventional methods in blood have **some disadvantages** (Dey et al. 2004; Liu et al. 2006).

**PCR** is a simple, rapid and valuable method that has ability to detect small number of organism (Cespedes et al. 2007). Hernández-Rodríguez et al. (2011) **compared PCR technique with** culture and dark field microscopy and reported **100% sensitivity** and **99% specificity** for PCR, and 95% and 89% for MAT method in compare to microbiologic culture.
In our study, **21.2% of samples** were positive for *Leptospira* spp. Jamshidi *et al.* (2009) reported **27%** prevalence of leptospirosis in 111 stray and household cats in Tehran province. Larsson *et al.* (1984) determined occurrence of 172 leptospiral infection in cats: **12.8%** were positive with titers $\geq 100$ and the most frequent serovar was pomona. In Spain, the prevalence of leptospirosis in cats was reported **4.5 to 14.0%** (Millan, 2009).

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.

**Cats may be a serious candidate in transmission of infection.** 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 leptospiro cortical epidemiology.