# PCR on of

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## Introduction

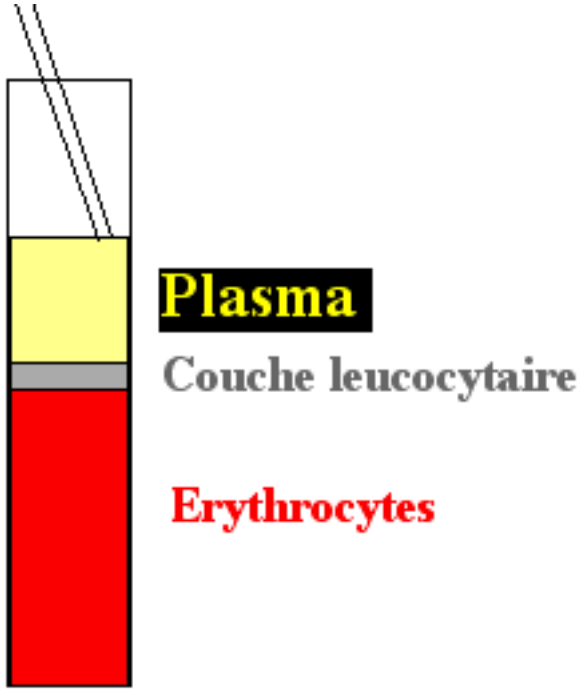
Leptospirosis = **worldwide, zoonotic disease** that causes by *Leptospira* spp.= **spirochete**. > 200 serovars of pathogenic *Leptospira* are isolated from **human beings and various wild and domestic animals**. This disease is important in **public health**. **Outbreaks** of leptospirosis are reported 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 [illegible] captured with the iron cage (Isfahan province [illegible] 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 -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). PCR technique was performed using the primers **previously described by Krishna *et al*. (2008)**. The forward ***flab*B primer** was 5'TCTCACCGTTCTCTAAAGTTCAAC3' and the reverse was 5CTGAATTCGGTTTCATATTTGCC3'. **The reaction** was incubated at 94ºC for 6 min in one cycle, followed 34 cycles of denaturation at 94ºC for 50 sec, anneal-ing 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 Institue, 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. **ed positive** when the 793 bp frag-ment was ob

## 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

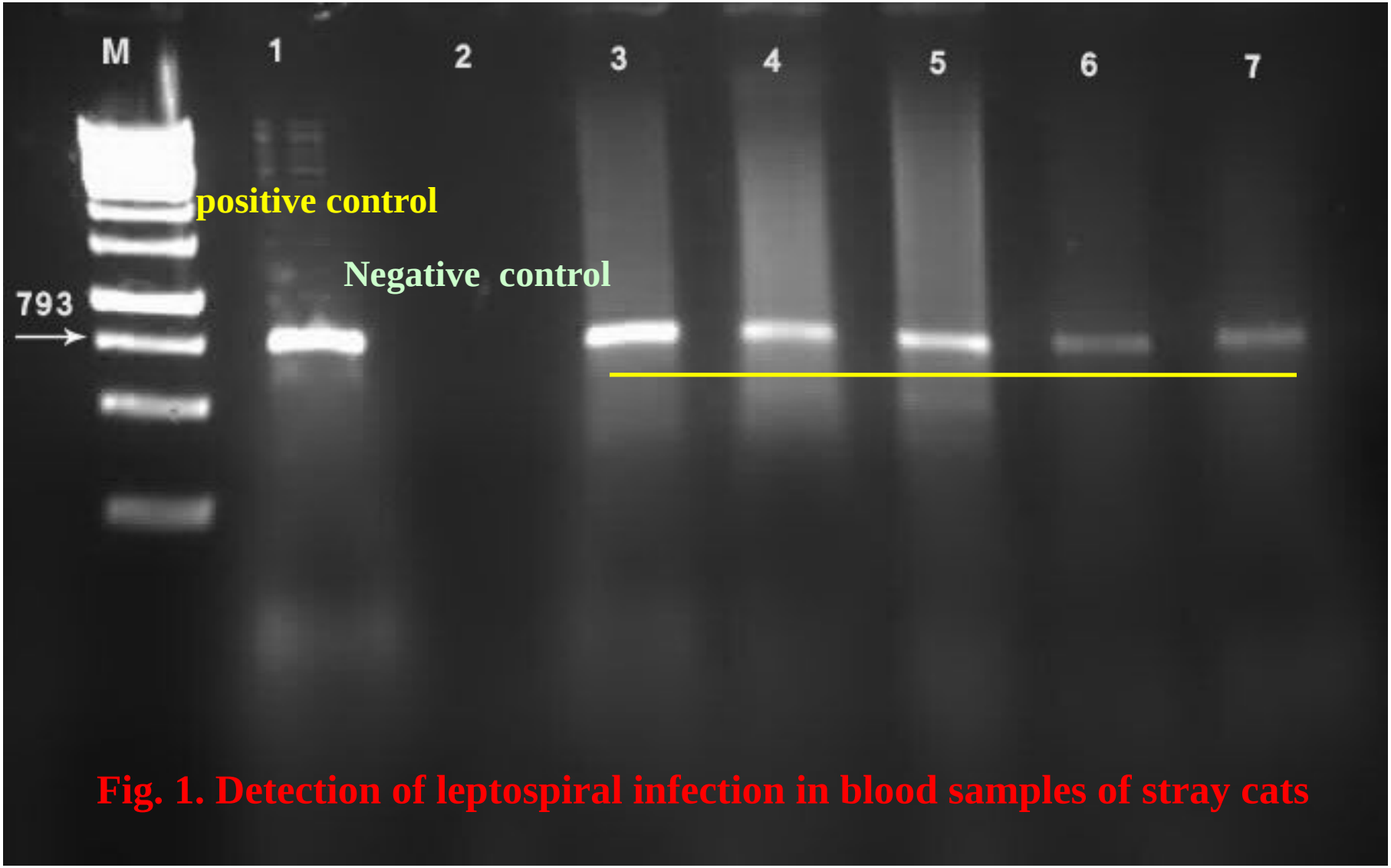



**Fig. 1. Detection of leptospiral infection in blood samples of stray cats**

**M: 1000 bp molecular weight markers, lane: 1 positive control, lane: 2 negative control, lanes 3-7: positive amplification (793 bp).**

# RESULTS

| Percentage (%) | PCR positive | Number | Sex |
|---|---|---|---|
| 53.6 | 15 | 72 | **Female** |
| 46.4 | 13 | 60 | **Male** |
| 21.2 | 28 | 132 | **Total** |

Table 1. The proportion of PCR positive of buffy coat samples collected from 132 stray cats.

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

# Discussion

Cats have a **high risk of exposure** to *Leptospira* spp. The frequency of *Leptospira* was estimated by ***flab***B 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 leptospiral epidemiology.