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Prevalence of Canine Visceral Leishmaniasis in Dogs at Adrestan District Detected by PCR

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Abstract: The present study was to identified a incidence of visceral leishmaniasis in owned dogs using Polymerase Chain Reaction (PCR) in Ardestan district of Isfahan. In this cross-sectional study, 184 owed dogs was sampled randomly. To determine the prevalence of visceral leishmania infection in dogs was used the Direct Agglutination Test (DAT) and Polymerase Chain Reaction (PCR). In this study, plasma samples from 184 dogs were examined using the direct agglutination. A total of twenty dogs (8.10%) have had positive titers. Four dogs (4.2%) of asymptomatic dogs and 16 dogs (8.8%) of dogs with symptoms had positive serum. Also statistically significant between visceral leishmaniasis with impotence, hair loss was seen ($p<0.05$). By using PCR, KDNA leishmania parasite identification and species of *L. infantum* was determined. The study results showed that peripheral blood by PCR with primers RV1 and RV2 on methods for early detection of disease in humans and dogs and also all cases are asymptomatic especially in endemic areas.

Key words: Visceral lieshmania, dogs, Aedestan district, Polymerase Chain Reaction (PCR), humans, Iran

INTRODUCTION

Now-a-days, leishmaniasis is a serious problem of public health and affects around 12 million of people in the world and the World Health Organization has named Visceral Leishmania (VL) as one of the top six infectious diseases of humans (Tavares *et al*., 2003; Bettini and Gradeoni, 1986). Canine Visceral Leishmaniasis (CVL) is a zoonotic disease in endemic areas which is more common in the Mediterranean and Middle East (Edrissian *et al*., 1996). Infected dogs are the main reservoir for *Leishmania infantum* and can be used as a potential sentinel for human VL in the endemic areas (De Paiva *et al*., 2007; Christensen and Herrer, 1973). Accurate detection of canine leishmaniasis is valuable to prevent transmission to humans. Due to the variable signs of canine leishmaniasis, the clinical diagnosis is not easy (Paradies *et al*., 2011). It has been demonstrated that both symptomatic and asymptomatic dogs infected with the parasite are the sources of infection for humans transmitted by the bite of sand fly (Farajnia *et al*., 2004; Molina *et al*., 1994). Sand fly vectors belonging to *Phlebotomus* sp. and *Lutzoumyia* sp. are responsible for transmission of *Leishmania* sp. between humans and animal reservoirs (Abranches *et al*., 1991; Belazzoug, 1992). Asymptomatic dogs are the most important source of sand fly vectors for parasite transmission to humans (Mohebali *et al*., 2005). Therefore, surveillance of canine *L. infantum* infection in endemic areas is very important to control VL in humans and animals. Serological methods have been widely used to investigate canine infection with *L. infantum* and PCR method are more sensitive than Serological methods (Maia and Campino, 2008; Edrissian *et al*., 1996).

The aim of the present study are to determine the seroprevalence of CVL in owned dogs of the Ardestan area and to identify the species of *Leishmania* isolated from dogs in this region.

MATERIALS AND METHODS

Study site: Ardestan district is located in Northeast Isfahan province with warm and dry climate. It covers an area of approximately 11591 km^2 including 306 villages and its population is estimated to be 45,150. Most of the inhabitants of Ardestan district are involved in agriculture and animal husbandry. The city of Ardestan is situated at an altitude of 1206 m above the sea level.

Dog and sampling: Researchers tested 184 dogs in endemic cases. The household dogs were randomly collected at the capital town of the county and adjacent

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villages in May and August, 2010 and examined for the external clinical signs of the diseases including weight loss, dry exfoliative dermatitis, ulcers, periorbital alopecia, diffuse alopecia and ocular signs. Oral consent was obtained from the owners of dogs 5 mL of blood samples were taken from the forearm vein of each dog in EDTA-coated polypropylene tubes for isolation of parasite DNA.

DNA extraction: Briefly, logarithmic phase promastigotes were disrupted inlysis buffer (50 mM NaCl, 50 mM EDTA, 1% SDS, 50 mM Tris-HCl, pH 8.0) and then incubated overnight with proteinase K (100 mg mL^{-1}, Sigma-Aldrich) at 37°C. DNA was purified further by phenolchloroform extraction and ethanol precipitation. An Eppendorf DNA thermal cycler and Taq DNA polymerase (Roche, Mannheim, Germany) were used to amplify the desired gene. The reaction mixture included 10 pmol of each primer, 200 mM dNTPs and 1.5 mM $MgCl_2$. PCR conditions were as follows: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min (Farajnia *et al*., 2004). The purified DNA, respectively was stored at -20°C.

PCR: Leishmania DNA in peripheral blood was detected by PCR using SYBR® Green Real-time PCR Master Mix-Plus (Toyobo Biotechnology) as previously described. The PCR was carried out using the primers (forward: 5'-CCTATTTTACACCAACCCCCAGT-3'; reverse: 5'-GGGTAGGGGCGTTCTGCGAAA-3') that amplify the 120 bp fragment of the minicircle kinetoplast DNA of leishmania. The limit of detection was 0.1 parasite per PCR reaction. Genomic DNA from leishmania reference strain MHOM/CN/92/SC10H2 was used as a positive control and the negative control was established with deionized water instead of DNA extract then PCR products were analysed by electrophoresis.

Statistical analysis: The results of each group were analyzed using the Pearson Chi-square (χ^2) test. The difference between the results was considered significant with $p<0.05$.

RESULTS AND DISCUSSION

In this study, twenty dogs were seropositive. The Seroprevalence Rate (SPR) of CVL was 10.8%. Out of 166 (90.2%) asymptomatic dogs, 4 (2.4%) were seropositive and out of 18 (9.7%) symptomatic dogs, 16 (88.8%) were seropositive. About 113 (61.4%) dogs were male and 71 (38.5%) were female of these, 14 (12.8%) male dogs and

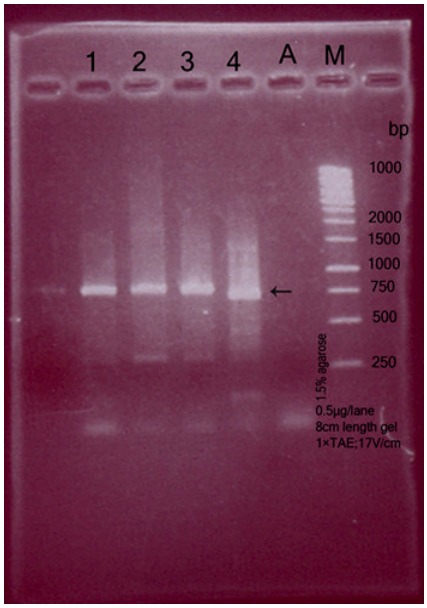

Fig. 1: Amplification of parasite DNA of PCR extracted from cultures of parasite isolates was used for PCR. Lane M: Marker, 100 bp ladder; Lane A: Negative control; Lane 4 *L. infantum*, Lanes 1-3: DNA (1 ng) *L. infantum* isolated from parasite cultures (750 bp)

6 (8.4%) female dogs were seropositive. Significant statistical differences between sex and seroprevalence were not observed ($p>0.05$) also statistically significant between visceral Leishmaniasis with impotence, hair loss was seen ($p<0.05$). Of the studied dogs, the largest age group was 2-3 years with 130 (70.6%) dogs. However, only 13 dogs (10%) from mentioned age group were seropositive (Fig. 1).

The overall seroprevalence rate of CVL was 10.8% in the Ardestan district. Similar results were found in Portugal, Italy and Iran (Molina *et al*., 1994; Gradoni, 1995; Ikonomopoulos *et al*., 2003; Gavgani *et al*., 2007; Bokaei *et al*., 1998). Because the annual prevalence of CVL in endemic regions frequently fluctuates and can vary among adjacent villages additional surveys are needed to establish the trend of CVL in the region studied. Nevertheless, the results imply that the prevalence of CVL may be an important risk factor for human disease in this region (Gavgani *et al*., 2002). The most important serological result was a high proportion of seropositivity for leishmaniasis 90.2% among asymptomatic dogs. These data are extremely important because owned asymptomatic dogs can play a significant role in the epidemiology of this zoonotic disease. Furthermore, the domestic dog population could be helpful sentinels to follow the progress of the disease in

endemic areas. Epidemiological studies on the reservoir hosts of VL have been discouraged by the lack of sensitive and practical methods to detect infections in the various species. Negative parasitological results do not rule out leishmania infection in dogs therefore, a combination of clinical, parasitological. PCR has been shown to be as good as or better than these diagnostic methods with the advantage that it provides a more rapid result. A number of PCR assays for the diagnosis of VL due to *L. infantum* have been developed over the past few years (Kazemi *et al*., 2008). Blood has been used as source of parasite for CVL diagnosis in recent studies. In a previous study in Bahia, Brazil used serology to detect leishmania infection in dogs and suggest that PCR might serve as gold standard to define leishmania infection than culture or hamster inoculation (Ashford *et al*., 1995). Pilatti *et al*. (2009) compared four PCR assays for the detection of Leishmania DNA in conjunctival swab samples and showed that DNA based methods had significantly higher sensitivity (Paradies *et al*., 2011).

Studies have shown that PCR testing is significantly more sensitive than conventional parasitological methods. In agreement with previous reports, the present study found PCR testing of peripheral blood to be particularly encouraging allowing for routine diagnosis of CVL (Ikonomopoulos *et al*., 2003).

CONCLUSION

This study shows that CVL is a problem in Ardesatn district and that infected dogs may play a role in the incidence of human disease. Finally, PCR testing with using peripheral blood can be used as sensitive, specific and rapid screening test for CVL.

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