Domestic Dogs As Main Reservoir Host For Visceral Leishmaniasis In Boyer-Ahmad District, South Of Iran

Hossein Ansari [1], Abdolali Moshfe [2*], Pooya Khodadadi [3], Bahador Shahryari [4]

[1.] M.S,in Microbiology, Departement of Biology, Yasuj Payamenoor University, Yasuj, Iran
[2*.] Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[3.] Departement of Biology, Faculty of Basic Sciences, Jahrom Azad University, Jshrom, Iran
[4.] Departement of parasitology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
*Corresponding Author: amoshfea@yahoo.com

Abstract: Visceral Leishmaniasis (VL) is an endemic disease in some parts of Iran. *Leishmania infantum* is the main agent of disease in endemic areas. The aim of the present study was finding the main reservoir host of visceral leishmaniasis in Boyer-Ahmad district, south of Iran. In five VL endemic villages of Boyer-Ahmad district in 2010, fifteen infected dogs with symptoms of canine visceral leishmaniasis were found. At the first, all cases were tested by DAT for evaluation of anti leishmanial antibodies and then after necropsy, parasitological study was conducted by use of impression smear of liver and spleen. Nested PCR was use to determination of the parasite in the liver and spleen tissues. From fifteen cases, fourteen dogs had antibody titer above of 1:320 while one of the cases was seronegative. *Leishmania* amastigotes was seen in 13 smears of liver and spleen (13 cases). The agent of disease in 14 dogs determined as *Leishmania infantum* by nested PCR. This study confirmed that domestic dogs are the main reservoir host for VL in this area and they are infected with *Leishmania infantum* as the causative agent of canine VL.

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1.Introduction

Visceral leishmaniasis in Iran, a common disease among humans and animals (Canine) is agent according to studies reported in most parts of the country is *Leishmania infantum*, which is transmitted by various species of sandflies *Phlebotomus*(Gavgani AS, 2002). The disease approximately has prevalenced in 66 countries and annually incidence is estimated at around 500,000 peoples, with clinical symptoms such as enlarged spleen, liver, anemia and loss of all cellular elements, especially the white blood cells and increase gamma globulin (Desjeux P, 2001. Desjeux P, 2004. WHO 2007).

In studies conducted in Iran, the owner dogs, especially dogs with the disease have been identified as the main reservoir and *Phlebotumus* as major vectors of disease have been determinate. Visceral leishmaniasis is an endemic disease in some regions of Iran as the annual number of children is commonly affected. In Iran, the main focus of disease is in the provinces of Ardebil, Fars, East Azarbaijan and Bushehr, but in the other region the disease have been reported but the prevalence is less(.Mohebali M, 2011). Seroprevalence rate of infection in children in Boyer Ahmad township was 3.1% and in the ownership dogs was 10% (Sarkari B, 2010). Disease in this region of the country seems to have a good platform for expansion. The initial study to identify causes and conditions of the transfer is necessary in order to prevent the spread of disease among animal and human (Mohebali M, 2011, Sarkari B, 2010).The aim of the present study was finding the main reservoir host of visceral leishmaniasis in Boyer-Ahmad district, south of Iran (Khalili M, 2009).

2.Material and Methods

Experimental study was conducted in 2010. Five endemic villages in Boyer Ahmad district were selected and 15 dogs were diagnosed as Visceral Leishmaniasis. These dogs had clinical signs of VL including; impotence, hair loss, abnormal nail growth, skin sores, and having difficulty walking. The protocol of the study under international law on animal experiments were conducted and approved at the University Ethic Committee.

Fifty dogs of this study included privately owned the endemic area of Zilaie and Maregon, province of Kohgiluyeh and Boyer-Ahmad, in Iran. All dogs tested for leishmaniasis by polymerase chain reaction, serological (direct agglutination test) and microscopic study methods in epidemiological survey performed. 10 ml blood from the cephalic vein was prepared, then by ketamine anesthetized and killed. From their carcasses caught both impression smear and tissue sample (from liver and spleen) and were placed in 70% alcohol. Serum samples were taken from dogs' blood and tested by direct agglutination test (DAT) for anti-Leishmania antibody detection

(Fakhar M, 2004. Moshfe A, 2009a). The direct agglutination test (DAT) antigen for this study was prepared in the parasitology department of the School of Health at Tehran University of Medical Sciences. Antigen for DAT was performed as described by Harriet et al 1989(Harith A, 1998). In brief, serums, placed in a microtiter plate with V-shaped wells, and normal saline was added for elution. The plates were left at ambient temperature overnight (12-h). The next morning a fresh solution of normal saline containing 0.2% gelatine was prepared by heating in hot water on a charcoal fire to 37°C; after cooling, 2-mercaptoethanol (0.1M) was added. The solution (75 µL aliquots) was pipetted into the microtitre plate and 50 µL of eluate were added to each well, followed by 50 µL of antigen (incubation). Initially, for screening purposes, serial dilutions were made from 1:20 to 1:20480. Negative control wells (antigen only; on each plate) and known negative and positive controls were tested. The titre was defined as the highest dilution at which agglutination was still visible, as a blue mat, enlarged dot with frayed edges, or enlarged blue dot, compared with negative control wells which had clear blue dots(9,10). Incubation was for 18 h, after which samples with titres 1:320 were diluted further to give end-point titres 1: 20480. In This study for the animal sample for anti-Leishmania antibody titer of the 1:320 and higher as visceral leishmaniasis infection and lower than 1:320 were considered negative (Mohebali M, 2006). Impression smears were prepared from the spleen and liver of the animals, stained with Giemsa, and examined under a compound light microscope for the amastigotes of Leishmania. The variable segment on minicircles of kinetoplast DNA from any Leishmania parasites present in the Impression smear and tissue samples were amplified using two-round, nested PCR (Moemenbellah-Fard MD, 2003).The DNA in each smear and sample tissue produced was extracted by digestion in lysis buffer and phenol chloroform (Motazedian MH, 1996).The primers were CSB1XR (CGA GTA GCA GAA ACT CCC CTT CA) and CSB2XF (ATT TTT CGC GAT TTT CGC AGA ACG) for the first round and LiR (TCG CAG AAG GCC CTT) and 13Z (ACT GGG GGT TGG TGT AAA) for the second. Reference strains of L. major and L. infantum were used as standards. The first-round reaction mixture contained 250 µM deoxynucleoside triphosphate (dNTP), 1.5 mM MgCL2, 1.0U Taq polymerase, 50 mM Tris-HCI (pH 7.6), 1 % Tween 20, and 40 ng each of primers CSBIXR and CSB2XF, in a final volume of 25 µL.The DNA was amplified for 25 cycles in a Progene thermo cycler (Techne, Cambridge, U.K.) set to run at 94°C for 1 min, 54°C for 1 min, and then 72°C for 1 min in each cycle. The first round product (1.0µL of a 9: 1 dilution in distilled water) was used as template for the second round, in a total volume of 25µL and under similar conditions to those for the first round except using LiR and 13Z as the primers (Moemenbellah-Fard MD, 2003. Motazedian MH, 1996. Moshfe A, 2009a).A 3µL sample of each second-round per product was subjected to electrophoresis in 1.5% agarose gel. The separated DNA was stained with 1 % ethidium bromide, visual-ized on a ultra-violet transilluminator and compared with molecular-weight markers and the corresponding second-round products for the L. infantum and L. major standards.

3.Results

In serological method 14 dogs out of 15 (between 3- 4 years old) had antibody titers of 1/320 and higher, and one dog was negative. In microscopic study from 15 spleen impression smear case, 11 of them have amastigote and 4 were negative and so 15 liver impression smear case, 10 of them have amastigote and 5 were negative. In summery impression smear for 13 dogs, Leishmania amastigotes were observed, and 2 dogs were negative. Using PCR method, parasite DNA has found in spleen and liver biopsies and in impression smear from 14 dogs out of 15 dogs, that was diagnosed as *L.infantum* and in one dog as *negative* (Fig.1). For each PCR positive sample, the second-round products of the nested PCR were identical to those of the *L. major* reference strain (with a main band of 560 bp) and distinct from those of the *L.infantum* standard (with a main band of 680 bp) (Moemenbellah-Fard MD, 2003).

4. Discussions

Visceral leishmaniasis is one of the most important parasitic diseases that are endemic in some parts of Iran. The various forms of human leishmaniasis are an important public-health problem in many tropical and subtropical countries (Mohebali M, 2011). The spread of leishmaniasis depends on the distribution of vectors and reservoir animals. Unfortunately, however, control of the vector or reservoir is difficult to implement and, in most endemic regions, the cost of such a program would exceed the resources available (Gavgani AS, 2002). In the mammal reservoirs, the parasites were detected to be restricted to the skin, except for sloths, of which the places of isolation were the spleen and liver (Mohebali M, 2011c, Gavgani AS, 2002). In this study we investigated 14 cases of 15 dogs were diagnosed with *Leishmania infantum*, which causes the disease known in other parts of the country (Mohebali M, 2011c. Fakhar M, 2004. Moshfe A, 2009. Moshfe A, 2008. Mohebali M, 2001a.

Mohebali M, 2001b). The carrier (vectors) of this disease should also be determined with precision study. Cases of VL have been reported from different parts of Kohgiloyeh and Booyerahmad province in the southwest of Islamic Republic of Iran but the prevalence of the disease was 31%. Since the Mediterranean type of the disease is present in the Islamic Republic of Iran and the disease in this region is mostly found in *L.infantum* (Mohebali M, 2001a). This means that VL is an endemic disease in this region, but the current study was therefore conducted to determine the genus and species of VL in owner dogs in Booyerahmad district.

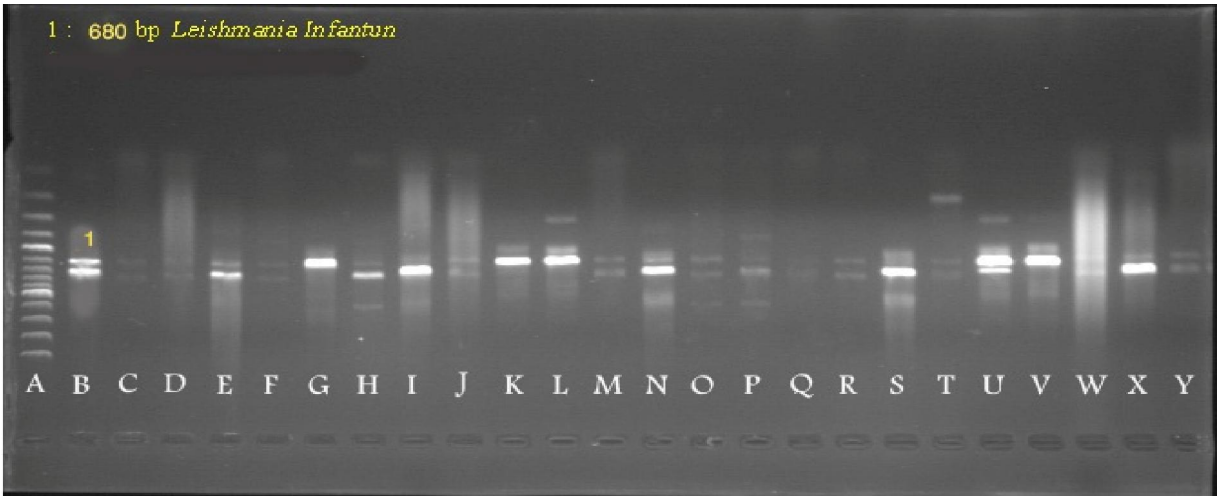

Fig. 1. The results of the electrophoresis of the products of the nested-PCR-based amplification of DNA extracted from the imperssion smears and Tissue sample(spleen and liver). A: 100bp, B: reference strains [L.infantum, 680bp], lanes C-H, J-M, O-Q, R-V,W,Y identified as L.infantum. Lanes I, N, S,and X were negative.

Mohebali et al (2001) declared agent of visceral leishmaniasis, Leishmania infantum species in other parts of Iran at Bushehr and other rejoin(Mohebali M, 2001a. Mohebali M, 2001b). In a study conducted by Moshfe et al (2009) in the city of Meshkin shahr in all samples taken from skin and blood dogs with and without clinical symptoms (71 dogs) were detected Leishmania infantum by polymerase chain reaction (Moshfe,A 2009a. Moshfe, A 2009b). Mohebali et al (1998), the role of rodents as possible reservoirs of visceral leishmaniasis in the city of Ardebil province, Meshkinshahr, 190 rodents of 4 genus and species different were studied. In the microscopic study of the impression smear spleen and liver of rodents in 52 cases (27/3 percent) was leishman amastigoute. One of case Leishmania infantum Leishmania in the golden hamster and one of them Leishmania donovani in the merinos was reported (Mohebali,M 1998). In another study by Mohebali et al (2001), Epidemiology of cutaneous leishmaniasis and visceral, was review and determinate of their reservoir in the city of Bushehr and Dashtestan Dashti, of 1496 samples taken from;9/1 an equal or higher titers 1:800 and 4/3 percent of titres were equal to or greater than 1:3200. In this study investigate, 105 dogs and 5 foxes, 10 jackals and 152 dogs. Finally 3 dogs and 1 jackal dogs leishman amastigoute observed then diagnosis Leishmania infantum by PCR (Mohebali M, 2001 19). In a comprehensive Study by Mohebali et al (2005) condition of Leishmania infantum infection in domestic dogs and wild Canine in Iran between the years 1999- 2003 have been published. In this study serological methods direct agglutination test to determine the amount of pollution and of Molecular, Biochemical Parasitology method for determining genus and species of parasite has been used. Of 1568 serum samples obtained from domestic dogs in different parts of Iran, 222 cases (14.2%) of anti-Leishmania antibody titer equal or higher than 1:320, 10 percent from 30 collar wild canines had Leishmania infantum. 10 out of 11 isolated Leishmania from wild canine and dogs be Leishmania infantum and one case Leishmania Tropicha by biochemical and molecular methods (Mohebali M, 2005). In this area Moshfe and et al studied on seroprevalence rate of infection in dogs and resulted 10% of dogs had positive (Moshfe A, 2012). In some cases, parasites may accidentally be the host of unusual reported or other type of Leishmania parasite is isolated from dogs. Same report of Sarkari et al (2010) *Leishmania infantum* from the cat and contamination from hamster and hamster *Leishmania Infantum* by Mohebali et al (1998) in some areas of non-reservoir animals also are reported(Sarkari B, 2010. Moshfe AA, 2009b).

In conclusion, this study confirmed that domestic dogs are the main reservoir host for VL in this area and they are infected with *Leishmania infantum* as the causative agent of canine VL. Further studies are needed to survey the vectors of the disease in this region.

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Corresponding Author:
Dr. Abdolali Moshfe
Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
E-mail: amoshfea@yahoo.com

References

1. Desjeux P. Worldwide increasing risk factors for leishmaniasis. Med Microbiol Immunol 2001; 190(1-2): 77-9.
2. Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 2004; 27: 305–18.
3. Fakhar M, Mohebali M, Barani M. Identification of endemic focus of Kala _ azar and seroepidemiological study of Viseral Leishmania infection in Human and Canine in Qom province, Iran. Quartely Journal of Yasuj University of Medical Science(Armaghane-danesh) 2004; 33: 43-51.
4. Gavgani AS, Mohite H, Edrissian GH, Mohebali M, Davies CR. Domestic dog ownership in Iran is a risk factor for human infection with Leishmania infantum. Am J Trop Med Hyg 2002; 67(5): 511-5.
5. Harith A, Salappendel RJ, Reiter I, Knapen F, Korte P, Huigen E, et al. Application of a direct agglutination test for detection of specific antileishmania antibodies in the canine reservoir. J. Clin. Microbiol 1998; 27: 2252–7.
6. Khalili M, Nourollahi-fard SR. Detection and genotyping of cutaneous leishmaniasis species in the southeast of Iran: restriction enzyme analysis (RFLP). Tehran University Medical Journal 2009; 67(3):168-72.
7. Moemenbellah-Fard MD, Kalantari M, Rassi Y, Javadian E. The PCR-based detection of Leishmania major infections in Meriones libycus (Rodentia:Muridae)from southern Iran. Annals of Tropical Medicine &Parasitology 2003; 97(8): 811-6.
8. Mohebali M, Poormohammadi B, Kanani A, Edrissian GhH, Anvari S. Rodents-Gerbillidae-Cricetidae-another animal host of visceral leishmaniasis in Meshkin-Shahr district IR of Iran. East Mediterr Health J 1998; 4: 376-78.
9. Mohebali M, Hamzavi Y, Edrissian GhH, Frouzani AR. Seroepidemiological studies of visceral leishmaniasis in Bushehr province South I R of Iran. East Mediterr Health J 2001a; 7: 912–7.
10. Mohebali M, Motazedian MH, Parsa F, Hajjaran H. Identification of Leishmania species from different parts of Iran using a randomamplified polymorphic DNA in human, animal reservoirs and vectors. Med J Islamic Rep Iran 2001b; 15: 243–6.
11. Mohebali M, Hamzavi Y, Fallah E, Zarei Z. Study of canine visceral leishmaniasis in some parts of Iran and its health importance. Tehran Univ Vet Fac J 2001c; 56: 55–9.
12. Mohebali M, Hajjaran H, Hamzavi Y, Mobedi I, Arshi Sh, et al. Epidemiological aspects of canine visceral leishmaniosis in the Islamic Republic of Iran. Vet Parasitol 2005; 129: 243–51.
13. Mohebali M, Edrissian GhH, Nadim A, Hajjaran H, Akhoundi B, Hooshmand B, et al. Application of direct agglutination test (DAT) for the diagnosis and seroepidemiological studies of visceral leishmaniasis in Iran. Iran J Parasitol 2006;1:15–25.
14. Mohebali M, Edrissian GH, Shirzadi MR, Akhoundi B, Hajjaran H, Zarei Z, et al. An observational study on the current distribution of visceral leishmaniasis in different geographical zones of Iran and implication to health policy. Travel Medicine and Infectious Disease 2011; 9: 67-74.
15. Moshfe A, Mohebali M, Edrissian GhH, Zarei Z, Akhoundi B, Kazemi B, et al. Seroepidemiological study on canine visceral leishmaniasis in Meshkin-Shahr district, Ardabil province, northwest of Iran during 2006e2007. Iranian J Parasitol 2008; 3(3): 1-10.
16. Moshfe A, Zarei Z, Akhoundi B, Edrissian Gh. Comparsion between Serology and PCR methods for the diagnosis of viseral leishmaniasis quarterly. Journal of YasujUniversity of Medical Science(Armaghane-danesh) 2009a;14(2): 31-43.
17. Moshfe AA, Mohebali M, Edrissian GH, Zarei Z, Akhoundi B, Kazemi B, et al. Canine visceral leishmaniasis: Asymptomatic infected dogs as a source of L. infantum infection. Acta Tropica 2009b; 112: 101–5.
18. Moshfe A, M Mohebali, E Afshoun, A Mousavizadeh, Z Zarei, H Abidi, B Akhoundi, V Barati, S Joukar. Canine Visceral Leishmaniasis in Boyer Ahmad District, Kohgiluyeh & Boyer Ahmad Province, Southwest of Iran. Iranian J Parasitol: Vol. 7, No.4, 2012, pp. 75-81.
19. Motazedian MH, Noyes HA. Leishmania and Sauroleishmania: the use of random amplified polymorphic DNA for the identification of parasites from vertebrates and invertebrates. Exp Parasitol 1996; 83(1): 15-154.
20. Sarkari B, Pedram N, Mohebali M, Moshfe AA, Zargar MA, Akhoundi B, et al. Seroepidemiological study of visceral leishmaniasis in booyerahmad district, south-west Islamic Republic of Iran. Eastern Mediterranean Health Journal EMHJ 2010; 16: 2010.
21. World Health Organization. 2007. http://www.who.int/ctd/chagas/disease.htm