

Study on seroprevalence of visceral Leishmaniasis in stray dogs of Marand (East Azerbaijan) with indirect immunofluorescence antibody test (IFAT) and its health importance in 2007-2008

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Citation

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Abstract

This study was made to investigate the seroprevalence of zoonotic visceral Leishmaniasis (ZVL) among the stray dogs of Marand city and to find the possible relationship between to transmit of parasite from infected dogs to human beings. In this study blood sampling was made from 150 stray dogs for recognizing seroprevalence of visceral Leishmaniasis with indirect immunofluorescence antibody test (IFAT). The serum was conjugated with anti leishmania infantum IgG antibodies and examined with fluorescent microscope. All the investigated dogs were reported to be seronegative, from the point of view of infection to visceral Leishmaniasis. In spite of that fact that four dogs were 1:64 regarding antibody titer but taking into account the cut off degree, all of the dogs were negative. In this study the antibody titer in male dogs was more than that of female dogs, however, it was not statistically significant ($p < .05$). Considering the different animal reservoirs and various geographical animal conditions in this area and regarding the zoonotic parasite, controlling the animal reservoirs seems unavoidable and fighting against the vector sand fly and controlling plans are necessary.

INTRODUCTION

Visceral Leishmaniasis is one of the infected – systemic diseases whose importance can not be ignored from hygienic point of view. Visceral Leishmaniasis is greatly widespread in Middle East and its cause is *Leishmania infantum*. (Handemir and et al, 2004). It is transmitted to dogs and human beings via *Phlebotomus* sand fly and *Lutzomyia*. Dogs are considered as the main domestic hosts and wolf and foxes as the main wild hosts. Visceral Leishmaniasis was described for the first time in 1908 by Nicolle and Comet (moheballi, 1996). From then on, different reports from different parts of the world were published about that. (moheballi and et al, 1996). So far at least four endemic foci of this disease from some areas of Ardabil, East Azarbaijan, Fars, Boushehr and recently from Qom provinces have been investigated and approved. Every year sporadic cases of Leishmaniasis are reported from other parts of Iran (moheballi and et al, 2001). In Ardabil and East Azarbaijan provinces, the removed *Leishmania* from animal reservoir is recognized to be *L. infantum* and 49 parasites after biochemical (Isoenzyme) experiments this parasite is exactly

the same strain which is in a variety of cases removed from the people infected to kala azar in the above-mentioned provinces (Mazlomi and et al, 2000). Therefore, it can be absolutely said that the dogs infected to Leishmaniasis are the most important reservoir of this infection for human beings. In addition to veterinary importance, visceral Leishmaniasis dogs are important from medical and hygienic perspectives. It appears in dogs in acute, chronic and sub clinical forms and in a variety of cases without any clinical symptoms leading to the death of animal (moheballi and et al, 2001). An exact method for diagnosing this disease in dogs suspicious to the infection is biopsy tissue (spleen, brain, bone, liver, lymphatic glands) and watching the amastigote form, or culturing the biopsy samples in nutritional environments and watching the promastigote forms of parasite (WHO, 1993). However, it is not always possible to see these forms. Using a valid serology has a great importance in diagnosing this infection in dogs timely. IFAT method is one of the serological methods for which a high sensitivity and quality is reported. The main purpose of this study is recognizing the seroprevalence of visceral

Leishmaniasis in stray dogs of Marand by IFAT and probable role of dogs in the transmission of this disease to human being and understanding its health importance.

MATERIALS AND METHODS

For this study, on a previous accommodations with Veterinary organization of East Azerbaijan and the office of biological environment, 150 stray dogs from five different areas of Marand (Abarghan 29 dogs, Far Far 24 dogs, Ordakloo 31 dogs, Garahe Tappeh 27 dogs and Koshksarai 39) were collected randomly by catching and entrapping methods. Next, the blood samples were taken from the dogs. 5 ml blood from cephalic or saphenous vein from each dog was taken. It is necessary to be said that before blood sampling all the information about age, sex, color and even the place and clinical examinations from the point of view of the presence of Visceral Leishmaniasis symptoms (dermal damages, loss of hair, thinness, largeness and complexities of the nails, lymphadenopathy, cataracts, size of the abdomen and diarrhea) was recorded on especial forms which were designed for this purpose. After the samples were removed to lab, the serums were separated by experimental techniques, and finally the serums were prepared for indirect immunofluorescence test. From 150 dogs investigated, 54 dogs (%35.76) were 2 years old and 97 dogs (%64.23) were older than 2 years old. There was not any discrimination among dogs on the basis of races. For indirect immuno fluorescence test *L. infantum* promastigote antigen strain (Lon 49) which were cultured in RPMI 1640 environment were used accompanied with %10 serum of cow fetus (Fcs). promastigote were removed from the culture environment and were washed 8 times in PBS. Parasites were focused on a micro slide with Acetone and the dogs serums were started to test by IFAT with 1:8 tenderness. *L. infantum* promastigote were prepared from parasitology department of health faculty of Tabriz Medical University (Harith and et al, 1986). In this study, anti dog IgG was conjugated with fluorescence isothiocyanate (Sigma®, F4012). Titer of this conjugate was 1:32. Abranches and Arc key 1984 was used for the interpretation (Abranches and et al, 1984). According to this key, serum titer 1:128 was considered as positive. Finally, the samples were investigated with an Olympus microscope which was made in Japan (2004).

RESULTS

The IFAT test is highly sensitive and specialized and is used as a quality test in diagnosing leishmania. The interpretation

of the test is very important and that's why those people and animals that have antibody against leishmania are considered as leishmania patients. This can be considered as a risk factor. In this study only in Abarghan and Far far areas 3 and 1 dogs respectively had antibody 1:64 which was a positive antibody. However, considering the cut off degree from 151 dogs all of them were negative serum on the basis of anti *L. infantum* IgG antibodies. There was not statistically a significant difference between male and female infection to Visceral Leishmaniasis ($P < 0.05$).

DISCUSSION

World Health Organization (WHO) considered the infection of *L. infantum* as one of the most important and common deceases. The Mediterranean kind of Visceral Leishmaniasis is an important disease transmittable from animals to human beings (WHO, 1993). This disease exists in more than 30 countries as an epidemic one. Dogs and wild canine (fox and jackals) are the main reservoirs of visceral Leishmaniasis in Iran (Mohebali, 2001). Dogs are considered as an important resource of infection in endemic areas of Visceral Leishmaniasis in Iran. Because, first, the population of dogs in Iran is high and second, the level of infection in dogs are higher and more importantly, the parasite concentrates easily in blood or under the skin of the dogs and becomes more accessible to *Phlebotomus* sand fly (Mohebali and et al, 1996).

A study made in Meshkin Shahr city, from 303 examined dog's %14 and %20 of dogs with DAT and IFA methods respectively, were positive. In another study in Firooz Aabad, Jahrom and Gir the level of infection of the examined dogs with DAT and IFA were reported to be %41.6 and %29.1, respectively (Edrissiyan and Ahanchin, 1993). In the other study on the infection of dogs of Baft in Kerman province, with IFA and ELISA methods, the level of infection of dogs was estimated to be 18% and %14.5, respectively (Sharifi, 1996). In the study of 1994 in Qoort Tappeh village of Meshkin Shahr city, from 164 examined dogs with DAT and ELISA methods there were %12.2 and %16.4 positive serums, respectively (Mohebali and et al, 1994). In the other studies by the same group in 2000 in Parikhan village of Meshkin Shahr city from 344 investigated dogs with DAT and ELISA tests there were %4.9 and %9.8 positive serums, respectively (Mohebali and et al, 1994). In another study by the same group in 2000 in Dashti city from 105 investigated dogs with DAT and ELISA tests there were %53.8 and %1.9 positive serums,

respectively (Mohebali and et al, 1994).

In a study on the dogs of Meshkin Shahr city, only %6.13 of dogs serums had positive clinical signs and on the basis of the results of this study, from 22 dogs whose antibody titer anti Leishmania with DAT method reached 1:2048, only 12 dogs(%5.54) had clinical symptoms(Bokaei and et al,1998). This issue has a great importance regarding epidemiology and the transmission of Visceral Leishmaniasis to human beings, because the dogs without any clinical symptoms similar to the dogs with clinical symptoms have the ability to transmit Visceral Leishmaniasis to human beings. Therefore, in order to control Visceral Leishmaniasis in epidemic areas it is suggested that all the stray dogs be killed and the guard ones be monitored with a serological test and be killed in the cases of positive results by the above-mentioned test . Finally, the infected people should be cured accompanying with controlling the vector mosquitoes provided that there is no damage to biological environment and no cause of a hygienic danger to human beings and Planning controlling programs is a proper step in the prevention of Visceral Leishmaniasis.

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