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Study on Seroprevalence of Canine Visceral Leishmaniasis (CVL) in Ownership Dogs of Sarab, East Azerbaijan, Province, Northwest of Iran with Indirect Immuno Fluorescence Antibody Test (IFAT) and its Health Importance in 2008-2009

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Abstract: This study was made to investigate the seroprevalence of Zoonotic Visceral Leishmaniasis (ZVL) among the ownership dogs of villages in Sarab 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 384 ownership dogs for recognizing seroprevalence of visceral Leishmaniasis with Indirect Immuno Fluorescence Antibody Test (IFAT). The serum was conjugated with anti *leishmania infantom* IgG antibodies and examined with fluorescent microscope. The seropervalence values among male and female animal were 9.2 and 6.5, respectively. Sero-Prevalence Rate (SPR) of CVL among ownership dogs in Sarab was 8.5% however, considering the cut off degree (1:80) from 384 dog 33 dogs of them was positive serum on the basis of anti *L. infantom* IgG antibodies. In this study, the antibody titer in male dogs was more than that of female dogs, however, it was not statistically significant ($p = 0.023$). The highest infection was found in dogs of jaldabakhan village 29.6% (8) and the lowest infection was found in khaki 3.3% (1). In this study only in Asbfroshan and Jaldabakhan areas 2 dogs had antibody 1:40, which was a negative serum antibody. There was statistically a significant difference between male and female infection to Visceral Leishmaniasis ($p = 0.962$). Considering the different animal reservoirs and various geographical animal conditions in this area and regarding the zooonotic parasite, controlling the animal reservoirs seems unavoidable and fighting against the vector sand fly and controlling plans are necessary.

Key words: Seroprevalence, visceral leishmaniasis, IFAT, dog, antibodies, Sarab

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 *et al*., 2004). It is transmitted to dogs and human beings via *Phlebotomus* sand fly and *Lutzoumyia*. Dogs are considered as the main domestic hosts and wolf and foxes as the main wilds hosts. Visceral Leishmaniasis was described for the first time in 1908 by Nicolle and Comet (Mohebali, 1996). From then on different reports from different parts of the world were published about that (Mohebali *et al*., 1998). So far at least four endemic foci of this disease from some areas of Ardabil, East Azerbaijan (Fallah *et al*., 2006), Fars, Boushehr and recently from Qom Provinces (Fakhar *et al*., 2004) have been investigated and approved. Every year sporadic cases of Leishmaniasis are reported from other parts of Iran (Mohebali *et al*., 2001a, b). In Ardabil and East Azarbaijan Provinces, the removed Leishmania from animal reservoir is recognized

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to be *L. infantum* lon 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 *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 (Mohebali *et al*., 2001a). An exact method for diagnosing this disease in dogs suspicious to the infection is biopsy tissue) spleen, bone marrow, liver, lymphatic glands) and watching the amastigot form, or culturing the biopsy samples in nutritional environments and watching the promastigote forms of parasite (Rossi, 2004). 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 seropervalence of visceral Leishmaniasis in stray dogs of Sarab by IFAT and probable role of dogs in the transmission of this disease to human being and understanding its health importance.

MATERIALS AND METHODS

The method of this study was descriptive cross section and the sampling method was multi stage cluster random sampling (Moshfe *et al*., 2008). Out of 167 village in Sarab district, 30 villages (cluster) were selected randomly. For this study, on a previous accommodations with Veterinary organization of East Azerbaijan and the office of biological environment, 384 ownership dogs from 30 village of different areas of Sarab were collected randomly methods. Next, the blood samples were taken from the dogs. Five mililitre 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 (exfoliative dermatitis, ulcerations, alopecia, thinness, largeness and complexities of the nails, lymphadenopathy, cachexia cataracts, size of the abdomen, ocular lesions 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 384 dogs investigated, 306 dogs (79.7%) were male and 78 dogs (20.3%) were female. 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 whit 1:20 tenderness. *L. infantum* promastigote were prepared from parasitology department of health faculty of Tabriz Medical University (Harith *et al*., 1986). In this study, anti dog IgG was conjugated with florescence Isothiocyanate (Sigma®, F4012). Titer of this conjugate was 1:50 Anti-leishmania specific antibodies, using the cut-off value of 1:80 and above were detected in male and female domestic dogs. Finally, the samples were investigated with a fluorescence microscope (Olympus, B×50, Japan, 2004).

Data analysis: Chi-square and fisher exact tests were used to compare seropervalence values relative to gender. Analyses were conducted using SPSS software version 13.5 whit a probability ($p<0.05$) as statistically significant.

RESULTS

The IFAT test is highly sensitive and specialized and is used as a quality test in diagnosing leishmania (Semiao *et al*., 1996). 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 (Mancianti *et al*., 1995). In this study, only in Asbfroshan and Jaldabakhan areas 2 dogs had antibody 1:40, which was a negative antibody. The sero-pervalence values among male and female animal were 9.2 and 6.5, respectively. The Sero-Prevalence Rate (SPR) of CVL among ownership dogs in Sarab was 8.5% (Table 1). However, considering the cut off degree (1:80) from 384 dogs, 33 dog of them was positive serum on the basis of anti *L. infantom* IgG antibodies (Table 2). There was statistically a significant difference between male and female infection to Visceral Leishmaniasis ($p = 0.962$). The highest infection was found in dogs of Jaldabakhan village 29.6% (8) and the lowest infection was found in Khaki 3.3% (1) (Table 3).

Table 1: Seropervalence of CVL infection by gender in Sarab district (2008-2009)

Gender	No. of dogs (%)	IFA* test positive	
		No.	Seroervalence (%)
Male	306 (79.7)	28	9.2
Female	78 (20.3)	5	6.5
Total	384	33	8.5

*Immuno Fluorescence Antibody

Table 2: Distribution of titers of anti leishmania antibodies in dogs with IFA test

Titer of Ab	No. of dogs (%)	Result
-	349 (90.9)	(-)
1: 40	2 (0.5)	(-)
1:160	2 (0.5)	(+)
1:320	5 (1.4)	(+)
1:640	11 (2.9)	(+)
1:1280	9 (2.3)	(+)
1: 2560	4 (1.5)	(+)
Total	384 (100)	

Table 3: Distribution of CVL infection different villages of Sarab district

Villages	No. of dog (%)	IFA test positive	
		No.	Seropervalence (%)
Ardeha	40 (10.4)	5	12.5
Jahizdan	15 (3.9)	3	20.0
Bahraman	16 (4.1)	4	25.0
Arzang	31 (8.1)	2	6.4
Baraghosh	22 (5.7)	5	22.7
Asbfroshan	70 (18.2)	-	-
Jaldabakhan	27 (7.03)	8	29.6
Khaki	30 (7.8)	1	3.3
Asfestan	28 (7.2)	5	17.8
Total	279 (72.6)	33	11.8

DISCUSSION

World Health Organization (WHO) considered the infection of *L.infantom* 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 (Gavgani *et al*., 2002; Garnham, 1971). This disease exists in >30 countries as an epidemic one. Dogs and wild canine (fox and jackals) are the main reservoirs of visceral Leishmaniasis in Iran (Mohebali *et al*., 2001b). 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 *et al*., 1998; Giraudoux *et al*., 2008).

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 (Edrissian *et al*., 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 and Daneshvar, 1994). In the study of 1994 in Qort 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. 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. 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. In other study on infection of dogs in Meshkin-Shar from Ardabil province with DAR methods the sero-pervalence rate was estimated to be 17.4% (Moshfe *et al*., 2008).

In a study on the dogs of Meshkin Shahr city, only 13.6% 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 (54.5%) had clinical symptoms (Bokaei *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.

CONCLUSION

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 (Tesh, 1995; Mohebali *et al*., 2001a, b; Palatnik-de-Sousa *et al*., 2001).

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REFERENCES

Bokaei, S., I. Mobedi, Gh.H. Edrissian and A. Nadim, 1998. Seroepidemiological study of canine visceral leishemaniasis in Meshkin shahr, North West of Iran, Arch. Irs. Razi, pp: 48-49, 41-49. http://journals.tums.ac.ir/full_text.aspx?org_id=59&culture_var=en&journal_id=17&issue_id=1358&manuscript_id=11859&segment=en.

Edrissian, G.H., A.R. Ahanchin and A.M. Harachahi, 1993. Seroepidemiological studies of visceral leishmaniasis and search for animal reservoirs in province Southern Iran. Iranian J. Med. Sci., 18: 99-105. http://www.iranmedex.com/english/articles.asp?issueID=13499.

Fallah, E., M. Farshchian, A. Mazlomi, J. Majidi, A. Kusha, A. Mardi and M.N. Poorzareh, 2006. Study on the prevalence of visceral leishmaniasis in rodents of Azarshahr district (New focus), North West of Iran. Arch. Razi Inst., 61 (7): 27-33. http://www.sid.ir/En/VEWSSID/J_pdf/83520060103.pdf.

Fakhar, M., M. Mohebali and M. Barani, 2004. Introduction of an endemic focus of kala-azar in Ghom province and seroepidemiological survey on visceral leishmaniasis in human and animal Reservoirs (dogs) in this area. Armaghane-danesh J., 33: 43-52. http://www.sid.ir/fa/VEWSSID/J_pdf/76013870406.pdf.

Garnham, P.C.C., 1971. The leishmania with special reverence to the role of animal reservoirs, Bull., WHO, 44: 471-489. PMID: 5316250. PMCID: PMC2427834. http://whqlibdoc.who.int/bulletin/1971/Vol44/Vol44-No4/bulletin_1971_44(4)_477-489.pdf.

Giraudoux, P., F. Raoul, D. Pleydell and P.S. Craig, 2008. Multidisciplinary studies, systems approaches and parasite eco-epidemiology. Something old, something new. Parasite Rev., 15 (3): 469-476. PMID: 18814725.

Gavgani, A.S., H. Mohite, G.H. Edrissian, M. Mohebal and C.R. Davies, 2002. Domestic dog ownership in Iran is a risk factor for human infection with *Leishmania infantum*. Am. J. Trop. Med. Hyg., 67 (5): 511-515. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2553493http://www.ajtmh.org/cgi/reprint/67/5/511.

Handemir, E., T. Once and K. Kamburgil, 2004. Seroprevalence of visceral Leishmaniasis in stray Dogs in Istanbul. TPD, 28 (3): 123-125. http://www.tparazitolderg.org/pdf/pdf_TPD_149.pdf. http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=20043182731.

Harith, A.E., A.H.J. Kolk and P.A. Kager *et al.*, 1986. A simple and economical direct agglutination test for serodiagnosis and seroepidemiological studies of visceral leishmaniasis. Trans. R. Soc. Trop. Med. Hyg., 580: 583-587. PMID: 3101241.

Mohebali, M., Y. Hamzavi, E. Fallah and Z. Zareii, 2001a. Study of canine visceral leishmaniasis in some parts of Islamic republic of Iran and its health importance, 56 (3): 55-59. http://209.85.229.132/search?q=cache:a_0-bOkbCgwJ:tbzmed.ofis.ir/default.aspx%3Fat_one_look%26member%3D150+"study+of+canine+visceral+leishmaniasis+in+some+parts+of+Islamic+republic+of+Iran+and+its+health+importance"&cd=1&hl=en&ct=clnk.

Mohebali, M., K. Bahmanrokh and A. Mosavi Far, 2001b. Parasitological and histological of canine visceral leishmaniasis in some dogs from meshkin Shahr Province. Pajhohesh and Sazandeghi, 37 (10): 122-125.

Mohebali, M., 1996. Protistozological disease common between Human and animals. Nadi Pub, Iran, pp: 31-81.

Mohebali, M., E. Fallah and H. Hajjarran, 1998. Vaccine trial against Canine Visceral leishemaniasis in the Islamic Repubic of Iran. E. Mediterr. Health J., 4 (2): 234-238. http://www.emro.who.int/publications/EMHJ/0402/05.htm.

Moshfe, A., M. Mohebali, G.H. Edrissian, Z. Zarei, B. Akhoundi, B. Kazemi, Sh. Amshidi and M. Mahmoodi, 2008. Seroepidemiological study on canine visceral leishmaniasis n Meshkin-Shahr District, Ardabil Province, Northwest of Iran during 2006-2007. Iranian J. Parasitol., 3 (3): 1-10. http://journals.tums.ac.ir/ upload_files/pdf/11859.pdf

Mazlomi, A.S., D. Evans, C. Davis and M. Mohebali, 2000. Species and stairs identification of leishemania parasites in VL endemic focus of North West, Iran. Acta Parasitol., 45 (3): 157.

Mancianti, F., M.L. Falcone, C. Giannell and A. Poli, 1995. Comparison between and enzyme-linked immunosorbent assay using a detergent-soluble *Leishmania infantum* antigen and indirect immunofluorescence for the diagnosis of canine leishmaniosis. Vet. Parasitol., 59: 13-21. PMID: 7571333.

Palatnik-de-Sousa, C.B., W.R. Dos Santos, J.C. Franca_Silva, R.T. Dacosta, A.B. Reis Palatnik, W. Mayrink and O. Genaro, 2001. Impact of canine control on the epidemiology of canine and human visceral leishmaniasis in Brazil. Am. J. Trop. Med. Hyg., 65: 510. PMID: 11716106.

Rossi, P., 2004. Diagnostic kits in parasitology which controls. Parassitologia Rev. Italian, 46 (1-2): 145-149. PMID: 15305705.

Semiao Santos, S.J., P. Abranches, S. Pereira, S. Games, J.P. Fernandes and J.C.M. Vetter, 1996. Reliability of serological methods for defection of Leishmaniasis in Portuguese Domestic and wild reservoirs, Mem Inst Oswald cruz. Rio De Joneiro, 91 (6): 747-750. PMID: 9283658.

Sharifi, I. and H. Daneshvar, 1994. The prevalence of visceral leishmaniasis in suspected canine reservoirs in Southern Iran? Iranian. Med. Sci., 21 (3, 4): 130-134. http://www.iranmedex.com/english/articles.asp?issueID=13493.

Tesh, R., 1995. Control of zoonotic visceral leishmaniasis is it time to change strategies. Am. J. Trop. Med. Hyg., 57: 92-287. PMID: 7694973.