Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

SHORT COMMUNICATION

Dissemination of Canine Visceral Leishmaniasis to Different Organs of Jackals Experimentally Infected with *Leishmania donovani*

Hind Mahdi Jarallah

Marine Science Center, University of Basrah, Basrah-Iraq
*Corresponding author: jarallah9@yahoo.com

ARTICLE HISTORY (13-530)

Received: November 16, 2013
Revised: May 11, 2014
Accepted: May 30, 2014

Key words:
Canine visceral leishmaniasis
Jackals
Leishmania donovani
Pattren of dissemination

A B S T R A C T

The canine is regarded as a reservoir host for human visceral leishmaniasis in the Mediterranean regions. Ten golden jackals were captured from villages that belong to Basrah province, south of Iraq. The animals were inoculated experimentally with human case *Leishmania donovani* promastigotes, and then the jackals were diagnosed parasitological and serological by two serological tests rk39 and DAT. The different diagnosis and dissemination of the parasites to internal organs were followed up to 16 weeks. *Leishmania* parasites were found in visceral organs: liver, spleen, popliteal lymph node, kidney and lung, both serological tests for the detection of specific antileishmanial antibodies showed positive results in the diagnosis of canine visceral leishmaniasis in Jackals experimentally infected with *Leishmania donovani*. This study confirmed that the golden jackals may play an important role in the transmission of leishmaniasis.

To Cite This Article: Jarallah HM, 2015. Dissemination of canine visceral leishmaniasis to different organs of jackals experimentally infected with *Leishmania donovani*. Pak Vet J, 35(1): 98-100.

INTRODUCTION

Canine visceral leishmaniasis (CVL) is regarded as both an important veterinary problem and a problem concerning human health as the dog is the main reservoir of *Leishmania infantum* and *L. chagasi* an increase in both VL and CVL cases has been reported in most Mediterranean countries in recent years (Toz *et al*., 2005). The dog is the major reservoir of *L. infantum* in the Middle East and the Mediterranean regions and *L. chagasi* South America. The disease pattern in dogs and human is similar (Abranches *et al*.,1991). In natural conditions, the infected sand fly vector deposits a few hundred promastigotes into the dermis of the host, while in the experimental infectious are induced by the injection intradermally or intravenously of millions of promastigotes grown in axenic cultures *in vitro* (Jarallah and Mehdi, 2011). The aim of this work is to determine the dissemination pattern of *L. donovani* strain isolated from a human case of visceral leishmaniasis in internal organs and development of metastatic disease in golden jackals.

MATERIALS AND METHODS

Leishmania donovani (MHOM/IQ/1982/BCR1/AA3) is provided from the *Leishmania* unit at Medical Research Center, Al-Nahrian University, Iraq. The Nicolle –Nove-MacNeal (NNN) diphasic medium was used for *in vitro* maintenance and to the preparation of the antigen of DAT technique.

Golden jackals were captured from villages that belong to Basrah province, Iraq. Out of 10 animals, 8 were used for inoculation and 2 kept as a control, promastigotes of *L. donovani* were harvested from diphasic medium. The parasites were adjusted to the required concentration 2 x10^9 /ml. each animal inoculated intraperitoneal two times in one week.

The two inoculated animals randomly selected were examined and studied at 4, 8, 12 and 16 weeks post infection. After examining signs of leishmanial infection, if any, animals were given anesthesia with ether.

Blood/serum was collected from each animal and stored at 20ºC until used. The sera were tested for the detection of antibodies against *Leishmania* parasite by Dipstick rk39 test and direct agglutination test (DAT) with local prepared antigen following the procedures described earlier (Jarallah, 2009) with a titer of 1:800 as cut-off point. After blood/serum collection, animals were dissected, the internal organs (liver, spleen, popliteal lymph node, kidneys and lungs) were collected and made tissue impression smears. Impression smears were fixed in methanol and stained with Giemsa stain and examined under oil immersion of light microscope to determine the

amastigote form. Internal organs were subjected to aspiration of material for culture aseptically on diphasic medium; the development of promastigote form was checked microscopically at weekly intervals.

RESULTS

There are no clinical signs such as skin infection, hair down and ocular-nose secretion that appeared on infected animals in this study. Abnormal nails and abdominal enlarged were observed in 62.5 and 37.5% animals at 12th and 15th weeks post infection, respectively. By smear study and Dipstick rk39 test detected *Leishmania donovani* at 12 and 16 weeks post infection in jackals whereas culture evaluation detected infection as early as 8 week while with DAT test infection was detected at 4th week of infection (Table 1). Parasites were not detected by smear examination in kidneys and lungs at any experimental days while in spleen, liver and popliteal lymph nodes parasites were detected from all, 4th week, 8th week and 12th week, respectively in various intensities (Table 2). Culture examination showed presence of parasites in all experimental days in spleen, showed presence of parasites from 4th week while (liver and popliteal lymph nodes), kidneys and lungs showed at 8th week, 12th and 16th week, respectively (Table 2).

Table 1: Methods used for the diagnosis of *Leishmania donovani* in experimentally infected jackals

Weeks post infection	Diagnosis			
	Parasitology		Serology	
	Smear	Culture	Dipstick rk39 test	DAT test
4	-	N	N	P
8	-	P	N	P
12	+	P	P	P
16	+	P	P	P

N: Negative; P: Positive

Table 2: Detection of parasites in visceral organs by microscopic examination

Organs	Method	Weeks post infection			
		4	8	12	16
Liver	S	-	+	+	++
	C	N	P	P	P
Spleen	S	+	++	++	+++
	C	P	P	P	P
Popliteal lymph node	S	-	-	+	++
	C	N	P	P	P
Kidneys	S	-	-	-	-
	C	N	N	P	P
Lungs	S	-	-	-	-
	C	N	N	N	P

S: Smears; C: Culture; N: Negative; P: Positive; -:No *Leishmania* amastigotes seen after 10 minutes search; +: *Leishmania* amastigotes scare; ++: *Leishmania* amastigotes numerous; +++: *Leishmania* amastigotes very numerous

DISCUSSION

The skin is considered the most important tissue reservoir of parasites in healthy and sick *Leishmania* infected dog (Solano-Gallego *et al*., 2001). Different species cause by clinically distinct diseases and the severity of the disease caused by any given parasite can vary markedly between individual hosts (Jarallah, 2011). Animals models are expected to mimic the pathological features and immunological responses observed in humans when exposed to a variety of *Leishmania* spp. with different pathogenic characteristics. Surveillance of the canine reservoir is highly important to help control of VL in human (Jarallah, 2009). In the present study the performance of Direct Agglutination Test (DAT) assay is evaluated, dipstick rk39 test and parasitological detection for diagnosis of Canine Visceral Leishmaniasis (CVL) of jackals experimentally were infected with *L. donovani* strain a human cases of visceral leishmaniasis. The results showed that the standard DAT was highly sensitive for the detection of the anti-leishmanial antibodies from other diagnostic methods. According to previous studies (Jarallah, 2009) the performance of the DAT for detection of *L. infantum* infection in human and dogs was excellent. Parasitological confirmation might be the best standard for diagnosing VL, but not for diagnosing infection with *L. donovani* complex in a community at risk (Jarallah and Aabadi, 2012). During the parasitological diagnosis, the various types of inflammatory cells were monitored. A relationship was found between the several of inflammatory cells in the smears and the number of amastigotes (Jarallah and Awad, 2006). The leishman bodies, amastigotes cannot survive outside the macrophage because of the human immune system (Dabiri *et al*., 2001).

The results of this studying have demonstrated that the golden jackals are suitable animals for studding experimentally canine visceral leishmaniasis. *L. donovani* in this animal model produced disseminated infection in internal visceral organs. The parasite had the ability to disseminate to visceral organs firstly to spleen at 4 weeks post infection, liver and popliteal lymph node at 8 weeks post infection and kidney at the 12 weeks post infection. The results obtained in this study simulate the observation of other investigation, which demonstrated the dissemination of the *L. major* parasites that occurs in Balb/c mice (Jarallah, 2003). On the other hand, Youssef *et al*. (1996) reported that the invasion of the lung by *L. tropica* strain was observed at 8 weeks post infection, but the invasion of parasite for the lung only observed at 16th weeks post infection. No amastigotes found in tissue impression smears for kidney and lung in spite of the fact that there are positive growths that occurred in cultured kidney and lung of jackals in NNN media. The standard diagnosis of VL is parasites (amastigotes) identification in tissue smears with aspirate materials of liver, spleen, lymph node and bone marrow (Murray *et al*., 2005). There are no clinical signs such as skin infection, hair down and ocular- nose secretion that appeared on infected animals in this study, while the abnormal nails and abdominal enlargement appeared in this study. In recent study, it is shown that it is possible to detect sick dogs with normal skin, but harboring parasites as well (Solano-Gallego *et al*., 2004). The symptomatic dogs probably play an important role in the transmission of leishmaniasis. The presence of *Leishmania* parasite in dogs without clinical signs enhances the importance of asymptomatic dogs in the epidemiology of VL. The present study describes a remarkable pathological picture of jackals experimentally infected with *L. donovani*. Our results demonstrated that both serological tests DAT and rk39 dipstick a test that gives positive results for the diagnosis of canine visceral leishmaniasis, the parasite disseminated from the site of inoculation to visceral

organs; liver, spleen, popliteal lymph node, kidney and lung. The number of inflammatory cells is correlated with the number of amastigotes.

Conclusion: This study confirmed that the golden jackals may play an important role in the transmission of CVL, the experimentally infected of these animals *with L. donovani* promastigotes can produce a pattern of metastatic disease, able to survive and replicate in visceral organs. Further studies in serodiagnosis and serological survey of CVL in VL endemic areas are recommended on canine population.

REFERENCES

Abranches P, G Santos-Gomes, N Rachamim, L Campino, LF Schnur and CL Jaffer, 1991. An experimental model for canine visceral leishmaniasis. Parasite Immunol, 13: 537-550.

Dabiri S, SS Meymandi and MA Nadji, 2001. Description of parasite-harbouring cells in localized lymphadenitis in dry type cutaneous leishmaniasis. Acta Trop, 79: 129-133.

Jarallah HM, 2003. Effect of some plant extract and antibiotics with histopathological study on *Leishmania major* strain. MSc Thesis Edu Coll Univ Basrah, Iraq.

Jarallah HM and AH Awad, 2006. Cellular study of the patterns of skin lesion of BALB/c mice infected experimentally with *Leishmania major*. J Basrah Res Sci 32: 105-110.

Jarallah HM, 2009. Epidemiological and immunological study on visceral leishmaniasis in marshlands villages South of Iraq. PhD Thesis Coll Edu Univ Basrah, Iraq.

Jarallah HM and HI Aabadi, 2012. Use of the recombinant rk39 antigen detection for diagnosis of visceral leishmaniasis in Maysan children, Iraq: with blood parameters. J Environ Bio Sci, 26: 15-21.

Jarallah HM and DS Mehdi, 2011. Antileishmanial activity of alcoholic extract of (*Trigonella foenum-graecum*) seeds against ulceration of cutaneous leishmaniasis *in vivo.* J Kuffa Bio, 3: 275-279.

Jarallah HM, 2011. Experimental study of the pattern infection with cutaneous leishmaniasis between males and females of mice. J Thiqar Sci, 2: 41-46.

Murray HW, JD Berman, CR Davies and NG Saravia, 2005. Advances in leishmaniasis. Lancet, 366: 1561-1577.

Solano-Gallego L, H Fernandez-Bellon, P Morell, D Fondevila, J Alberola, A Ramis and L Ferrer, 2004. Histological and immmunohistochemical study of clinically normal skin of *Leishmania infantum* infected dogs. J Comp Pathol, 130: 7-12.

Toz SO, H Ertabaklar and Y Ozbel, 2005. Seroprevalence of canine visceral leishmaniasis in Kusadasi, Turkey. Turk J Vet Anim Sci, 29: 23-26.

Youssef MYM, MM Eissa, IF AbouEl-Naga and SH El Gowhary, 1996. Dissemination of Leishmania to organs of mice experimentally infected with *Leishmania tropicato*. J Egypt Soc Parasitol, 26: 719-731.