

# Seroprevalence Survey of Visceral Leishmaniasis among Children up to 12 Years old and Domestic Dogs in Rural Areas of Dehloran District, Ilam Province of West Part of Iran, 2014

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## Abstract

**Background:** Visceral leishmaniasis (VL), caused by *Leishmania infantum* (*L. infantum*), is a life-threatening vector-borne parasitic disease is distributed in some parts of the world. The disease is endemic in some parts of Iran. This study was aimed to determine the seroprevalence of VL among children and domestic dogs (as a reservoir of the parasite) in Dehloran, west of Iran.

**Materials and Methods:** This cross-sectional study was carried out in Dehloran County. The blood samples of 872 children up to 12 years old and 52 dogs were collected from 10 villages of Dehloran using randomly-clustered sampling method. Sera were separated from all peripheral blood samples and tested by direct agglutination test (DAT). Anti-*Leishmania infantum* antibodies at titers of  $\geq 1:800$  and  $\geq 1:80$  were considered as *Leishmania infantum* infection in human and dog, respectively.

**Results:** In general, among 872 human samples, 1.03% of samples had anti-*Leishmania* antibody with 1:1600 titers and 1.26% had 1:800 titers. In addition, from 52 dog samples, 21.15% of dogs had a titer of  $\geq 1:320$  and 25% had 1:80 and 1:160 titers.

**Conclusion:** Our findings indicate that the seropositive dogs in the studied areas are considerable and *L. infantum* may be circulated between human and domestic dog in the studied area. Further study of isolation and molecular identification of *Leishmania* spp. is recommended.

**Keywords:** Visceral Leishmaniasis, Direct Agglutination Test, Human, Dog, Iran

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## Introduction

Visceral Leishmaniasis (VL) or kala-azar is an insect-

transmitted infection caused by *Leishmania infantum-donovani* complex. Leishmaniasis, which is observed throughout the world, occurs in three major forms;

cutaneous, visceral and mucocutaneous. Visceral leishmaniasis is considered as the fatal form of the infection; however most of the dead cases are the non-cured ones<sup>1,2</sup>. Leishmaniasis is endemic in more than 90 countries across the world including Iran<sup>3</sup>. VL is known as the second most life-threatening parasitic disease in the world, after Malaria. It is estimated that about 500000 new cases occur in the world annually and 59000 deaths report each year<sup>4,5</sup>. Kala-azar is commonly found in middle-East countries where *Leishmania infantum*, domestic dogs (*Canis familiaris*) and sand mosquito (*Phlebotomus sp.*) are known as the etiological agent, reservoir host and vector in this areas, respectively<sup>6</sup>.

There are two forms of cutaneous and visceral leishmaniasis in Iran, in which 20000 new cases of cutaneous form and 100-300 new cases of VL report annually<sup>7-10</sup>. VL is counted as an endemic disease in various parts of Iran such as Ardebil<sup>11</sup>, Fars<sup>12</sup>, Golestan<sup>13</sup>, Bushehr<sup>14</sup>, Kerman<sup>15</sup>, Qom<sup>16,17</sup> and North Khorasan<sup>18</sup> and; also it has been reported from other provinces sporadically<sup>19,20</sup>. VL is detected mostly in infants with the general signs of fever, hepatosplenomegaly, paleness, lymphadenopathy and blood cell reduction. These signs usually followed by inside hemorrhage or secondary infection and lead the person to his death<sup>8</sup>. Pentavalent antimony ingredients have been widely used for treatment of all forms of leishmaniasis. Pentostam<sup>®</sup> and Glucantim<sup>®</sup> were introduced as the first-line treatment in 1940. In recent years, many drugs, by injection or orally, including Paramomycin, Ketoconazole or Allopurinol, have been recommended as an adjunct<sup>21-23</sup>.

Diagnosis of VL is usually carried out using serological or molecular tests; which serological tests are considered as a sensitive and non-invasive examination that is suitable for field studies<sup>24</sup>. DAT is one of the most common serological methods, which has been widely used in epidemiological surveys; since it is a simple, inexpensive and reliable test<sup>25-27</sup>. There is little information concerning the prevalence of VL in Dehloran; however, there have been reported a number of VL cases in recent decades. The present study undertakes an epidemiological investigation taking into account human and canine to determine the prevalence of VL among human and dogs (as a

reservoir of the parasite) in Dehloran, west of Iran.

## Methods

**Geographical location of Dehloran district:** Dehloran city is located in the south part of Ilam in the west of Iran (Fig1). It has 63671 people population (40182 urban, 2072 rural and 2757 nomad) and 48 villages. Having 6229 square kilometer area, Dehloran forms one-third of the total area of Ilam. The height of the city is about 230 meters above the sea level and has the longest common border with Iraq compared to other parts of the province. The region has an arid to semi-arid weather with a very hot summer (mean temperature of 50°C), a mild winter and a poor annual rainfall. The present study was carried out during March 2014 to Feb 2015 (whole of the Iranian year 1393) in 10 selected villages of Dehloran.

**Sampling:** This cross-sectional study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences and was done in rural areas of Dehloran. In order to observe the dispersion of villages, first of all, all the villages were numbered, and then they were divided into two east and west half based on their location in the map. Ten villages were randomly selected based on the number and population of villages in two divided parts. Selected villages in this study circle marked on the map (Figure 1).

The numbers of human samples were calculated using the sample-size estimation formula based on the prevalence rate of 3.5%<sup>28</sup>, the confidence limit of 95% and the accuracy of 1.3%. Proportionally, the size of reservoir sample was calculated as 872 samples from under 12-years old children and 52 samples from dogs with no age limitation. Samples were taken in two terms, firstly, 41 sample were taken from the selected villages, secondly, 11 sample were collected from dogs belonged to those who had been detected as seropositive. In addition, seropositive children were followed up after 6 months and, surely, their dogs.

Two milliliter (ml) blood was taken from each target children. For dogs, 4 ml blood was taken after calming down the dogs by 0.2 ml Asperomazine injection. Blood samples were swiftly transferred to the main laboratory of Dehloran hospital. Sera were

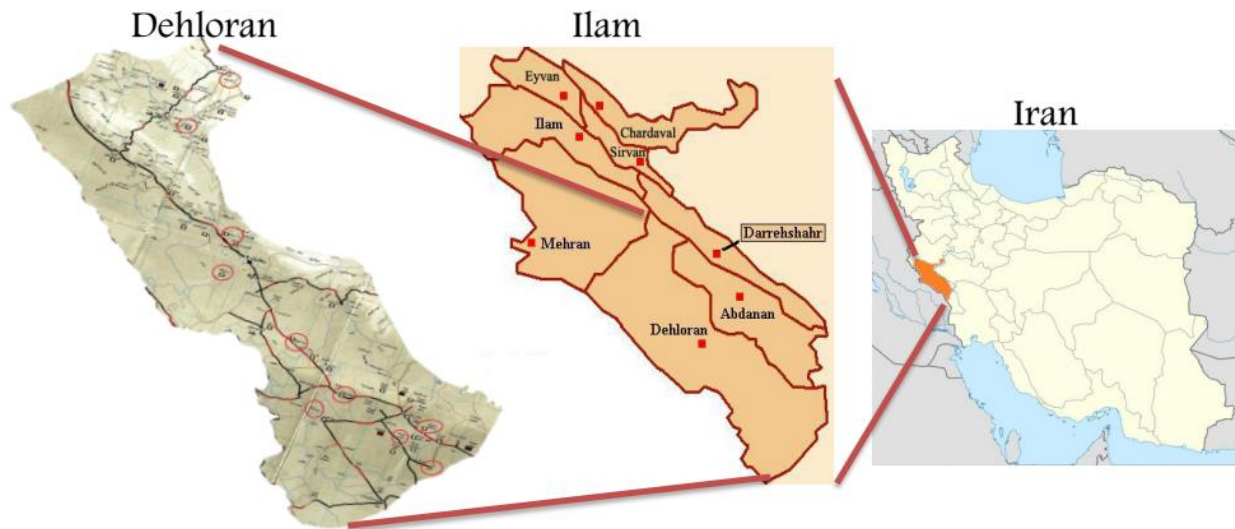


Figure 1. Geographical locations where this study was carried out.

separated from blood samples by centrifuging in 3000rpm for 5 minutes and kept in  $-20^{\circ}\text{C}$  until used.

**DAT:** Basically, in DAT, promastigotes, as antigen, are mixing with different dilutions (serial dilution) of sera and in the presence of anti-leishmania antibody agglutination would occur after 18 hours.

The antigen preparation step was done in protozoology unit of Tehran University of medical sciences. Antigen was prepared by cultivating the parasite in RPMI1640 medium containing 10% fetal bovine serum, Tripsination of *L. infantum* promastigotes, fixation by 2% formalin and staining with 0.02% Coomassie-blue. After the fixation of a volume of promastigotes (50million per milliliter) by formalin, it was covered with aluminum foil and kept in  $4^{\circ}\text{C}$  until used.

The human sera were diluted by a dilute solution of 0.78% 2-Mercaptoethanol (2ME) and the dog sera were also diluted by 1.56% 2ME solution in V-shape 96-well micro titer plates. The diluted dog sera were incubated in  $37^{\circ}\text{C}$  for 1 hour.

Firstly, in order to sample screening, all the samples were analyzed in 1:800 dilutions and if positive, the test was continued up to 1:102400 dilutions. For human samples, the anti-leishmania antibody titers at  $\geq 1:3200$  considered as positive, at titers  $\leq 1:800$  negative and at 1:1600 categorized as suspicious sample. In addition, for dog samples the titers at

$\geq 1:320$ ,  $\leq 1:80$  and 1:160 considered as positive, negative and suspicious respectively.

**Ethical Consideration:** This study reviewed and confirmed by the Ethics Committees of Shahid Beheshti University of Medical Sciences, Iran. As well as it was reviewed and confirmed by the Tehran University of Medical Sciences as a joint research project.

**Statistical analyses:** Chi-square test was used to compare seroprevalence values relative to gender, age, and contact with the dog. Analyses were conducted using SPSS software version 16 with a probability (p) value of  $<0.05$  as statistically significant.

## Results

**Result of human samples:** In the present study, 872 samples were analyzed by DAT which the minimum and maximum age of children was 1.5 months and 12 years old respectively. Generally, 20 persons were seropositive; 11 samples with titer 1:800 and 9 samples had 1:1600. Among the sero-positive samples, 12 cases (1.37%) belonged to the 9-12 years old age group (Table 1). In addition, the majority of sero-positive samples were from 3 villages of Nasr (6 cases), Esteghlal (6 cases), and Farrokhabad (6 cases) (Table 4). It is worth mentioning that rising titer were

**Table 1:** Seroprevalence *L. infantum* infection by age group in Dehloran district, West of Iran (2014).

Age (years)	No. of sample	Antibody titer				Total of seroprevalence	
		1:800		1:1600		No. of samples	%
		No. of samples	%	No. of samples	%		
≤4	219	0	0	1	0/45	1	0/45
5-8	278	3	1/07	4	1/43	7	2/51
9-12	375	6	1/6	6	1/6	12	3/2
Total	872	9	1/03	11	1/26	20	2/29

No statistically significant differences between human *Leishmania* infection and age groups (≤ 12 year ages) were observed

**Table 2:** Seroprevalence *L. infantum* infection by age group dogs of rural areas in Dehloran district, West of Iran (2014).

Age	No. of samples	Antibody titer						Seroprevalence
		1/80	1/160	1/320	1/640	1/2560	1/5120	
≤3	14	4	1	1	0	0	0	6
4-7	21	2	1	2	2	0	0	7
≥7	17	4	1	1	2	2	1	11
Total	52	10	3	4	4	2	1	22

No statistically significant differences between dog *Leishmania* infection and age groups were observed

**Table 3:** Seroprevalence *L. infantum* infection by gender in children up to 12 years old and dogs in Dehloran district, West of Iran (2014).

Gender	Seroprevalence (Children)		Total of samples (Children)		Seroprevalence (Dogs)		Total of samples (Dogs)	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
Male	7	1/68	415	47/59	14	26/92	30	57/69
Female	13	2/84	457	52/41	10	19/23	22	42/31
Total	20	2/29	872	100	24	46/15	52	100

No statistically significant differences between Human and canine *Leishmania* infection and gender were found

seen in none of the sero-positive samples after following up. The seropositive individuals included 7 males and 13 females, which none of them had the experience of traveling to endemic areas (Table 3).

**Results of dog samples:** Generally, 52 samples were examined by DAT that the minimum and maximum age of animals were 3 months and 15 years old respectively. Lastly, 24 samples were detected as seropositive that most of them were from the 3 villages of Nasr (6 cases), Esteghlal (5 cases), and Farrokhabad (8 cases); just the same as the human results (Table 4). Moreover, the maximum rate of infection was seen in ≥7 year old age group (Table 2). It should be noted that in two villages (Hazermil and Bardi) neither human seropositive was seen (except one of 1:800 in Hazermil) nor dog seropositive (Table 4). Analyzing the result of seropositive dogs which belonged to the seropositive human revealed that the dogs belonged to those with 1:1600 titers showed a

higher titration compared to those with 1:800 titers (Table 5).

## Discussion

Visceral leishmaniasis is the most malignant form of leishmaniasis that is considered as a deadly parasitic infection in endemic parts of the world<sup>4,6</sup>. More than 90% of VL cases are observed in Bangladesh, India, Brazil, Ethiopia, South Sudan, and Sudan which is estimated that about 310 million people are in danger of infection in those counties and 20000 deaths occur annually<sup>29</sup>.

VL cases have been reported from most parts of Iran sporadically, whereas some areas like Ardebil<sup>11</sup>, Fars<sup>12</sup>, Golestan<sup>13</sup>, Bushehr<sup>14</sup>, Kerman<sup>15</sup>, Qom<sup>16,17</sup> and North Khorasan<sup>18</sup> have been known as endemic areas to the disease.

Since there was no published data regarding the

**Table 4:** Comparing the results of human and dog seroprevalence rate in each village in Dehloran district, West of Iran (2014).

Village	Result of human		Result of dogs		seroprevalence of human (%)	seroprevalence of dogs (%)
	Total of samples	Prevalence	Total of samples	Prevalence		
Hazermil	109	1	5	0	0/91	0
Bardi	99	0	4	0	0	0
Esteghlal	137	6	8	5	4/37	62/5
Nasr	119	6	9	6	5/04	66/66
Kheybar	55	1	5	3	1/81	60
Barah Bijja	44	0	2	1	0	50
Farrokh Abad	163	6	13	8	3/68	61/53
Eyne Khosh	130	0	6	1	0	16/66
Total	872	20	52	24	2/29	46/15

No statistically significant differences between Human and canine *Leishmania* infection and Village were found

**Table 5:** Comparing the results of human and dog seroprevalence In terms of people with dogs.

Row	Results of people with dog (DAT)	Results of their dogs (DAT)
1	1/1600	1/320
2	1/1600	1/80
3	1/1600	1/2560
4	1/1600	1/80
5	1/800	1/80
6	1/800	1/80
7	1/800	1/160
8	1/800	1/80
9	1/800	No titer
10	1/1600	1/2560
11	1/800	1/80

prevalence of VL in Dehloran, the present study was carried out to clarifying the situation of VL in both human and reservoirs. Direct agglutination test was used in the present study as it is known as a valid, inexpensive, executable in field studies and with high sensitivity and specificity<sup>30</sup>.

We focused on children less than 12 years old because previous studies have indicated that approximately 99% of seropositive cases in endemic areas seen in this age group<sup>8</sup>.

Our finding showed that among 872 (2.29%) human samples were seropositive in general, including 1.26% with 1:800 titers and 1.03% with 1:1600 titers. In addition, no positive cases were observed in  $\geq$  year old age group which could be due to their lesser contact with infected mosquitoes because of their special way of wearing or the longevity of the latency

period of VL<sup>30</sup>.

Our results showed a VL seroprevalence rate of 42.3% among 52 dog samples which 11 cases (21.15%) of them had  $\geq$ 1:320 titers.

The majority of cases, both human and dog, were in three villages (Nasr, Esteghlal, and Farrokhabad) which they had a large population of stray dogs and poor sanitation situation. Moreover, no positive cases were found in two villages (Hazermil and Bardi). Hazermil is very close to Dehloran city, and it seems it causes changes in the life style of people, rural culture and reducing the number of stray dogs; consequently it diminishes the probability of infection. Likewise, Bardi is located at a higher height from sea level that it might not be suitable for mosquitoes.

In contrast, most of positive cases were observed in Moosian district where previous surveys have revealed the presence of sand fly in this area<sup>31,32</sup>.

Our finding indicated that 7cases (0.8%) of seropositive were male and 13 cases (1.49%) were female, but no significant correlation was seen between sex and rate of infection. Our results seem compatible with those who find no statistical correlation between gender of cases and the rate of infection in Lorestan<sup>30</sup>, North Khorasan<sup>18</sup> and Kerman<sup>33</sup>.

In addition, Edrisian claimed that despite of the higher rate of infection in males, asymptomatic positive rate in females is significantly higher, which is seemed compatible with the results of our study<sup>19</sup>. In contrary to our results, some studies from other parts of Iran such as Qom<sup>17</sup>, Ardebil<sup>34</sup> and

Meshkinshahr<sup>35</sup> showed a significant difference in prevalence rate between males and females.

Our findings were also similar with those who examined 402 human sample by DAT in Mazandaran which they reported 8 cases (2%) as suspicious (1:1600 titers) and no positive case<sup>36</sup>.

The present study indicated a prevalence rate of 21.15% in dogs which was relatively similar with those who reported 16.3% infection among rural dogs in Ahvaz<sup>37</sup>. Moreover, similar results were observed in Qom and Kerman<sup>17,33</sup>.

Mohebbali *et al*, in a retro perspective study in northwest of Iran during a period of ten years (2002-2012) showed that the prevalence rate of VL in dogs was about 16.9-19.6% which is relatively near to our results<sup>8</sup>.

The present study revealed that most of the seropositive dogs (11.53%) were in the  $\geq 7$  years old age group which was clearly similar with those who reported the same results in Tehran<sup>38</sup>, Alborz<sup>39</sup> and Meshkinshahr<sup>11</sup>.

Regarding the gender of dogs, our finding indicated that anti-leishmania antibody was found in 26.92% of male dogs and 19.23% of females; but no positive statistical correlation was observed between two genders. In this context, the same results have been reported in many studies<sup>11,17,38,40,41</sup>.

In general, the differences in seroprevalence reported in various parts of Iran, could be due to the factors like climate condition, humidity and temperature, style of living, number of dogs and self-protection against mosquitoes.

## Conclusion

Our findings indicate that the seropositive dogs in the studied areas are considerable and *L. infantum* may be circulated between human and domestic dog in the studied area. Further study for isolation and molecular identification of *Leishmania* spp. is recommended.

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