

SEROPREVALENCE OF VISCERAL LEISHMANIASIS IN CHILDREN UNDER 10 YEARS OLD IN 8 RURAL AREA IN BOYER AHMAD DISTRICT 2015, AFTER ELIMINATION OF INFECTED DOGS

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Published online: 01 January 2018

ABSTRACT

Background: *Leishmania infantum* is the main cause of kala-azar in Iran. This study aimed to determine the prevalence of visceral leishmaniasis in children under 10 years old, who live in infested villages of Boyer-Ahmad district, south of Iran, to evaluate the current status of the disease after reservoir host (owner ship dogs) elimination.

Material and methods: In previous studies, villages infected with humans and animal reservoirs were identified. The infected dogs were destroyed. Now, 127 serum samples of children from 8 rural area of Boyer-Ahmad district was taken and tested by DAT. They were also investigated to determine the signs and symptoms of the disease.

Results: All 127 serum samples were negative for anti leishmania antibodies ($\leq 1:800$ titers). 64 and 63 individuals were male and female respectively. The age of 112 cases was 5-10 years old and 15 cases (8.11%) have 1-4 years old. Nobody showed symptoms of the disease.

Conclusion: To control the disease, the important factor is reservoir elimination. Previously infected dogs were killed at 2010 in the district. The current results may be due to destruction of dogs. Therefore, according to success of this work, this method is recommended for disease control in other region.

Keywords: *Leishmania infantum*, visceral leishmaniasis, Iran, DAT, Elimination

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doi: <http://dx.doi.org/10.4314/jfas.v10i1.31>



INTRODUCTION

The *Leishmania infantum* protozoa is transmitted to humans by a sand flies bite from the animal reservoir (canids), causing kala-Azar that affects most of the spleen, liver and bone marrow. This parasite causes infections and illness in more than 95% of children under the age of 10 years and, if left untreated, causes the death of the patient(1,2,3).

This disease has been reported from all provinces of the country and, according to previous studies in Boyer Ahmed, visceral leishmaniasis (kala-azar) in children has been reported and dogs have been identified as the main reservoirs of the disease(4-8).

In the study of seroepidemiology of the disease in Boyer Ahmed, which was done by Sarkariand et al in 2005-2006, a total of 1,628 primary school children were reported with a serum infection rate of 3.1%.The bone marrow sample from 6 kala-azar patients admitted to pediatric ward was examined by PCR method and the parasitic agent was determined in these patients with *Leishmania infantum*(7).After this study, a study was conducted by Moshfe and et al. to investigate the contamination status in the dogs of the owner of Boyer Ahmed district in 2010 and contamination rate was reported in 170 dogs, 10%.In the recent study, eight villages were identified as polluted villages of Boyer Ahmad district. The names of these villages are Dilgon, EshkaftSeyah, Puleh, DarreSehe, Zirana, Jahangir Abad, Allah Abad and Ganjgun(9).Since it is recommended to control the disease of visceral leishmaniasis in humans, identification and elimination or treatment of infected dogs with *Leishmania infantum*, In another study in 2010, all known infected dogs in the previous study were eliminated in all villages, and the type of parasite was detected by sampling of these animals by PCR method(10, 11).Now, after five years of intervention in these eight villages, this study was conducted to evaluate the prevalence of infectious *Leishmania infantum* in children under 10 years of age in these villages in Boyer Ahmed district in 2015 and the results of this study were compared with the previous results.

MATERIALS AND METHODS

This study is a cross-sectional descriptive study.The sample was all children under the age of 10 living in high risk villages.After obtaining consent from parents, personal information and questions about the research variables and clinical symptoms of children were recorded in the questionnaire. From each child, 2-5 ml of blood was taken and after separating the serum, kept in a freezer at- 20 ° C.Sera were transferred to the Laboratory of Leishmaniasis of the School of Public Health and the Institute of Health Research of Tehran University of Medical Sciences in order to conduct direct agglutination test (DAT).In this serologic test, the titres are

less than and equal to 1: 800 negative; the headline 1: 1,600 suspect and the titer 1: 3200 and above is positive(12-14). SPSS software and chi-square test were used to analyze the variables.

RESULTS

A total of 127 children were enrolled in the study, 64 males and 63 females who were 50.4% and 49.6%, respectively. The largest number of children from Puleh village from Ludab district was 26 (20%). From the age group, 112 (88%) were from the age group of 5-10 years old and 15 (11.8%) were from the age range of 1-4 years. The parents of 35 children (27.6%) were livestock farmers and 92 (72.4%) were non-dairy farmers, and the same 35 children from the livestock families were in contact with the dog at their place of residence; 19 of them with a dog and the remaining 16 were associated with 2 or more dogs. Children aged 5-10 years were more likely to be exposed to dogs, with 27% of those 35.77 children (66.6%) mentioned the history of mosquito bites, and 58.3% of these cases are related to children aged 5-10 years. No child had a history of kala-azar. Also, none of them had a history of chronic fever and chills, and had no splenomegaly and hepatomegaly in examination. Four children (3.1%) had anemia, of which 3 were boys and one girl. The four children were from Dilgon, Allah Abad and Ganjgun villages. Two children (1.6%) also suffered from weight loss in Allah Abad and Ganjgun villages. In serological study of serum samples, none of these 127 specimens had positive antibody titers against *Leishmania infantum*. Therefore, all samples are reported negative.

DISCUSSION

In the present study, the serological results of all samples were negative. However, as visceral leishmaniasis was endemic in Kohgiluyeh and Boyer Ahmad region, in 2005 and 2006, Sarkari et al. performed a seroepidemiological study on the children of this region using DAT method, and the incidence of visceral leishmaniasis in the province was 3.1%.

This study concluded that the incidence of visceral leishmaniasis in the Boyer Ahmad region was higher in the mentioned years than in other regions of Iran (7,15). Due to this level of infection in children in the region, Moshfe et al., In 2010, a study was conducted to determine the rate of infection of dogs in the Boyer Ahmad region as the main reservoir of this parasite, estimated at 10%. According to this study, the Boyer Ahmad region was considered as the focal of endemic visceral leishmaniasis, and the villages of Dilgon, EshkaftSeyah, Puleh, DarreSehe, Zirana, Jahangir Abad, Allah Abad and Ganjgun were introduced as polluted

villages(9).According to health network reports, infected dogs were eliminated during health measures to control the disease and prevent human infection with leishmaniasis (10).

In this study, a seroepidemiologic study was conducted on children under the age of 10 years to study the current status of the disease in the identified villages of Boyer Ahmad region and to evaluate the effect of remediation of the reservoirs in the incidence of disease.127 samples were taken from the population studied and collected along with other information such as parenting, contact with the dog in the living environment, number of dogs associated with the environment, history of anemia and previous infection with kala-azar, samples were sent to Tehran University of Medical Science for The DAT test. Fortunately, all of these 127 specimens were negative for anti-leishmania antibodies. This desirable result could be due to the destruction of parasite reservoirs in the year 2010 following the study of Moshfe et al. (10,11). With the negative result of serology and the absence of splenomegaly, hepatomegaly, chronic fever and chills, and leukopenia, the presence of the disease in the studied children is completely ruled out. Parents of 35 children (27.6%) were livestock farmer and the same 35 children were in contact with dogs; due to the presence of dogs as a domestic animal in rural area, trainees should be systematically monitored for clinical signs of disease, and serological tests in regular filaments is performed every few years to identify asymptomatic infected animals. The control criteria should target the reservoirs, identify the affected dogs as quickly as possible and stop the transmission to the mosquitoes and carriers, reducing their number and preventing contact with humans. There is no consensus between killing and treating infected dogs.

The World Health Organization recommended the killing of infected dogs with *L.infantum*, but at the same time it was recognized that doing so is difficult in countries where people have a high level of protection from animals (2).

Due to the fact that it is an endemic country for visceral leishmaniasis, Brazil is regularly monitoring plans for prophylactic or epidemiological control of the disease, including treating sick people, controlling insect vectors, and destroying infected seropositive dogs follow up. In the CB Palatnik report, the eradication of the disease was announced in Minas Gerais in 2001, with the three methods mentioned, while mentioning that eliminating positive serum dogs resulted in a definite reduction in the incidence of dog leishmaniasis as well as man(16). In Brazil, in a interventional part of the killing of positive serum dogs during a 5-year period, it was compared with another without killing. The results indicated an immediate decrease in the incidence of disease in dogs (serum changes from 35% to 5% in one year) and, similarly, a decrease in prevalence (from 35% to 8% in two years); But as a result of the killing of dogs,

both rates increased again (incidence up to 14% and prevalence of 13% in two years). On the other hand, in a region where dogs were killed, a clear decrease in human leishmaniasis (from 12 to 2 per thousand) in a long period was observed, which ended several years after the five-year period (17). The results of this study are in accordance with the current survey. Therefore, it is recommended that the rate of serum contamination of dogs in the area be investigated again. In a pilot program on the island of Alba, a combination of killing and treating dogs reduced the incidence of disease in dogs from 11.6% to 4.6% in two years (18); this study also suggests the value of eliminating the disease reservoirs.

According to the previous study in these areas and the prevalence of 3.1% of the cases of visceral leishmaniasis in children, the negative results of all samples in this study indicate a better and more favorable situation in the region. Regarding the measures taken during the previous study and this study, it can be argued that the principles of prevention and control of visceral leishmaniasis have been effective in destroying parasite contaminated reservoirs in the studied areas.

ACKNOWLEDGEMENTS

Some parts of this study received financial support from Yasuj University of Medical Sciences. We thank Dr. M. Mohebbali and Dr. B. akhondifar helping us in laboratory techniques and providing DAT materials, and the staff of the District Health Centers in Boyer-Ahmad district.

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How to cite this article:

Moshfe A, Farhadipour F, Manzouri L, Jamshidi A, Mohammadi R. Seroprevalence of visceral leishmaniasis in children under 10 years old in 8 rural area in boyer ahmad district 2015, after elimination of infected dogs. *J. Fundam. Appl. Sci.*, 2018, 10(1), 436-442.