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Short Communication

Leishmania tropica: Another Etiological Agent of Canine Visceral Leishmaniasis in Iran.

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Abstract

Background: Dogs have been previously reported to be reservoirs of *Leishmania infantum* as the etiological agent of human visceral leishmaniasis in Iran. We report a case of canine visceral leishmaniasis (VL) caused by *L. tropica* from the north- west of Iran where human visceral leishmaniasis is endemic. **Methods:** The canine VL was initially screened by dipstick rK39 and direct agglutination test, then the dog was dissected and obtained samples were examined by parasitological (direct exam, cultivation) and molecular techniques (RAPD-PCR and RFLP-PCR). **Results:** *Leishmania* parasites were found in spleen and liver of the dog. The serological tests for the detection of specific anti-*leishmania* antibodies showed positive results. **Conclusion:** *L. tropica* as another agent of canine VL was determined.

Keywords: *Canine visceral leishmaniasis, Molecular methods,* L.tropica*, Iran.*

Introduction

Leishmania infantum is the main etiological agent of canine visceral leishmaniasis (CVL) around the Mediterranean Basin and also in Iran (1). The dog has also been demonstrated to carry *L. infantum*, principal causative agent of human visceral leishmaniasis in Iran (2). In 1993, *L. tropica* as principal causative agent of visceral leishmaniasis was isolated from veterans of operation desert storm (3, 4). Recently, there are some reports that *L. tropica* is isolated from both viscera of humans and dogs in some parts of Middle East (2, 5, 6). In this report, a case of CVL caused by *L. tropica* in Iran is described.

Materials and Methods

An ownership male dog with specific signs of CVL was observed in Takli village from Pars abad (Moghan Plane) near the Azerbaijan country in the north west of Iran where human visceral leishmaniasis (HVL) is endemic. Physical examination was carried out and blood sample was taken from the dog. The blood was centrifuged at 800×g for 5-10 min and serum was separated and tested for anti-*Leishmania* antibodies using Dipstick rK39 (7) and direct agglutination test (8, 9). The infected dog was dissected and a few smears were prepared from its spleen and liver. All prepared smears were fixed by methanol, stained by Giemsa stain 10% and examined microscopically for the presence of amastigotes. Biopsy specimens were collected aseptically from spleen and liver and then cultured into biphasic (NNN) and monophasic culture media (Schneider's and RPMI 1640 media). The inoculation cultures were incubated at 21 ºC and promastigotes were seen one-week post cultivation. Some *Leishmania* promastigotes which had been isolated from spleen and liver of the dog following mass production in RPMI1640 (Gibco), were analyzed by RAPD-PCR technique and compared the results with

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standard species of *L. infantum* (MCAN/IR/96/ LON49), *L. tropica* (MHOM/IR/99/YAZ1) and *L. major* (MRHO/IR/75/ER) using 4 primers including AB1-07 (5' GGT GAC GCA G), A4 (5' AAT CGG GCT G), primer327 (5'ATA CGG CGT C) and primer329 (5' GCG AAC CTC C) in the School of Public Health, Tehran University of Medical Sciences (10-12). Also, the isolate was confirmed as *L. tropica* by RFLP analysis of PCR amplified from the sample using specific restriction enzyme discrimination under supervision of Prof. K.P. Chang from the University of Chicago, USA.

Results

We found an ownership male dog, approximately 8 yr old that was shown active CVL with typical signs including alopecia, cachexia, noise hyperkeratosis, mild splenomegaly and with no cutaneous lesions meanwhile, we found an old scar of CL on the face of the dog's owner. Anti-*Leishmania* antibodies were detected at a titer of 1:20480 using the cut-off value of 1:320 and above by DAT and also the result of Dipstick rk39 was positive. Hypertrophy of the liver, spleen with a severe infiltration by mononuclear cells and hyperplasia of macrophages with a great number of amastigotes in their cytoplasm were observed after necropsy. The species of this isolate was identified as *L. tropica.* Profile of the *Leishmania* isolate, showed high similarities to profile of *L. tropica* in human CL by RAPD-PCR (Fig. 1, bands: 4 and 5) and confirmed by RFLP-PCR method with nagt-gene fragment by K.P.Chang from the University of Chicago, USA.

Discussion

The canidae family especially domestic dogs are the most important source of *L. infantum* infection for humans (1). One of the *Leishmania* sp. isolated from a domestic dog in northwestern Iran near the Azerbaijan country was identified as *L. tropica*. This *Leishmania* species as a cause of visceral leishmaniasis in humans and dogs was described recently (2-6).

In Iran, *L. tropica* is the most important agent of urban cutaneous leishmaniasis and VL is known to be caused by *L. infantum* (13, 14). A molecular-epidemiology survey which was carried out for the isolation and characterization of *Leishmania* spp., isolated from CVL in an endemic focus of visceral leishmaniasis in northwest of Iran from 2002 to 2004 where infantile cases of VL caused by *L. infantum*, were identified (2). Based on the previous experiments (11, 12), we used RAPD-PCR method for characterization of *Leishmania* isolate. Since RAPD-PCR does not require previous knowledge of primer sequence and only randomly decamer with 60-70% G/C content is sufficient. We could use different primers with different genomic DNA samples or both. The profile obtained by RAPD could discriminate among *Leishmania* species.

Since the results of RAPD is highly dependent on parameters that may vary from one laboratory to another thus, the isolate was confirmed by PCR-RFLP method with nagt-gene fragment by Prof. K. P.Chang from the University of Chicago, USA.

This is a report for the isolation of *L. tropica* from viscera of a domestic dog with typical signs of CVL in Iran. This finding raises the question of possible consequences on the epidemiology of *L. tropica* in the region, even if the significance of this single case is uncertain. It should be mentioned that in the last five years, cases of HVL have been reported from pars-abad near the Azerbaijan country. Active dog surveys in the areas are necessary to detect the same cases of the disease.

These findings should help for more understanding of epidemiological aspects of leishmaniasis in northwest of Iran.

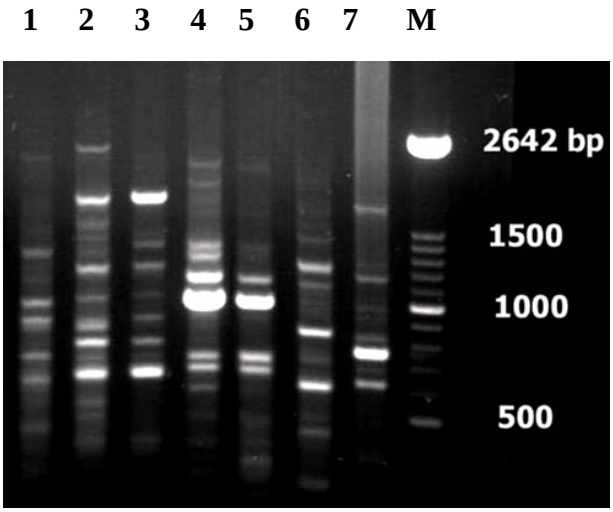

Fig. 1: Random amplified polymorphic DNA (RAPD) profiles obtained from the *Leishmania* stocks and isolates with the AB1-07 primer. Lanes 1,3,6,7 are *L. infantum* and lane 4 *L. tropica* (dog). Reference stocks lanes 2 and 5 are: *L. infantum* and *L.tropica* respectively. M: 100 bp size marker (XIV) (Roche).

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