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ORIGINAL ARTICLE

EVALUATION OF RAPID "DIPSTICK RK39" TEST IN DIAGNOSIS AND SEROLOGICAL SURVEY OF VISCERAL LEISHMANIASIS IN HUMANS AND DOGS IN IRAN

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Background and Objective – **Visceral leishmaniasis is endemic in northwest and southern Iran and is found sporadically in other parts of the country. It manifests as the Mediterranean type of kala-azar, which mainly affects children. Early diagnosis and prompt treatment of the disease could prevent its high mortality. We evaluated the rapid "Dipstick rK39" test in diagnosis and serological survey of the disease in human as well as in dogs, and compared it either with the direct agglutination test (DAT) or enzyme-linked immunosorbent assay (ELISA).**

Methods – **"Dipstick rK39" kits were used for diagnosis in suspected and confirmed kala-azar patients and for case finding among children in the endemic area of Meshkin-Shahr, Ardebil Province, in northwest Iran. The kits were also used to diagnose visceral leishmaniasis in dogs experimentally infected with *Leishmania infantum* as well as in dogs with clinical symptoms of infection in endemic areas. Results were evaluated based either on results with DAT, ELISA or, in a few cases, microscopic diagnosis.**

Results – **The rapid "Dipstick rK39" test was sufficiently sensitive and quite specific in diagnosis of visceral leishmaniasis in human as well as in dog.**

Conclusion – **The "Dipstick rK39" test is rapid and noninvasive. It does not require much expertise or elaborate equipment and it can be used for diagnosis of visceral leishmaniasis in remote endemic areas.**

Keywords "Dipstick rK39" dog human Iran visceral leishmaniasis

Introduction

Visceral leishmaniasis (VL) is an endemic disease in some areas of northwest and southern Iran. In other parts of the country, the disease occurs sporadically. The causative agent is *Leishmania infantum* and dog is the main animal reservoir.[1]

Microscopic examination, mostly based on bone marrow aspirate, and specific serological tests such as indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) and direct agglutination test (DAT) have been used on a large scale for diagnosis and sero-epidemiological studies of VL.[1] Although demonstration of the parasite is conclusive for diagnosis of VL, but it is an invasive tool, is not sensitive, and its performance is not practical in most VL endemic areas.[1]

The sensitivity of microscopic diagnosis, evaluated in VL patients from endemic areas of northwest Iran in whom the diagnosis was based on clinical features plus positive IFAT or DAT, was only 69%.[2] DAT, when used in patients clinically suspected of having VL, is relatively

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Table 1. Comparison of "Dipstick rK39" and DAT in diagnosis of VL infection.

Studied individuals	No. tested	rK39 positive	% Positive	DAT positive*	% Positive
Suspected VL patients	88	46	52.27	56	63.63
Confirmed VL patients	52	52	100.00	48	92.30
Children from endemic area	220	12	5.45	27	12.27

* Positive titers: 1: 3200 – 1: 102400.

specific and sensitive enough for diagnosis. However, both DAT and IFAT remain positive for a long period in treated kala-azar patients.[1]

We evaluated the rapid "Dipstick rK39" test in diagnosis and serological survey of VL in human and dog, compared to DAT or ELISA, in this study.

Materials and Methods

The rapid immunochromatography test kit "Dipstick rK39" (In Bios International Inc., USA), received from the World Health Organization, was compared with DAT in 88 clinically suspect VL cases and 52 confirmed VL patients from hospitals in different provinces of Iran including Ardebil, East Azerbaijan and Tehran. The two tests were also used in 220 children (less than 12 years old) in a serological survey in the VL endemic area of Meshkin-Shahr, Northwest Iran. The clinically suspected VL patients were mostly children from endemic areas and confirmed VL patients were those who had clinical features of the disease and were serologically positive or, in a few cases, parasitologically positive. The DAT antigen was made at the Protozoology Unit, School of Public Health, Tehran University of Medical Sciences, according to the method described by Harith et al.[3]

In a preliminary experiment, 15 dogs inoculated with *L. infantum* were tested with rK39 and ELISA at 1, 2.5 and 3.5 months after inoculation. Four noninoculated dogs were used as controls.

ELISA was based on an antidog immunoglobulin conjugated with alkaline phosphatase and an antigen prepared from intact promastigotes of *L. infantum* made at the Protozoology Unit according to the method of Mohammed et al.[4]

Results

The specificity of rK39 was evaluated in 38 non-VL serum samples, collected from six cutaneous leishmaniasis, six viral hepatitis, six brucellosis, and 20 malaria patients. All results were negative. Among eight VL patients for whom parasitological diagnosis was available, only four had a positive bone marrow aspirate.

The results of rK39 and DA tests, in suspected and confirmed VL patients as well as in children of the endemic area, are summarized and compared in Table 1. The specificity of rK39 was evaluated by applying the test examination of 38 non-VL serum samples, collected from six cutaneous leishmaniasis, six viral hepatitis, six brucellosis, and 20 malaria patients. All results were negative.

Among eight VL patients for whom parasitological diagnosis was available, only four had a positive bone marrow aspirate. The results of "Dipstick rK39" and ELISA in the 15 experimentally infected dogs with *L. infantum* are summarized in Table 2.

In the 4 control dogs "Dipstick rK39" and ELISA tests, during the above mentioned periods, remained negative. The spleens and livers of nine of the inoculated dogs were examined parasitologically, six months after inoculation. Amastigotes were found in eight rK39 and ELISA-positive dogs, while no parasite was detected in one rK39 and ELISA-positive dog, although the dog had splenomegaly. In five clinically suspected VL dogs from endemic areas, rK39, ELISA and microscopic diagnosis were all positive. In the two studies on human and dog, about 3% of the Dipstick kits did not work properly, i.e. after addition of buffer solution (phosphate buffered saline, pH 7.2) the serum or blood samples did not move upward to the positivity and control lines on the nitrocellulose strips, even after 15 minutes.

Discussion

Cloning of a *Leishmania chagasi* antigen gene, preparation of recombinant protein rK39 and its successful application in detection of leishmanial antibody by ELISA in VL patients were reported by Burns et al in 1993.[5] In India, the rK39 antigen, impregnated on nitrocellulose paper, was used as a Dipstick in rapid field diagnosis of VL with good sensitivity and specificity.[6] The "rK39 Dipstick" test and DAT were evaluated in bone marrow parasitologically-positive cases of VL and control

Table 2. Comparison of rK39 and ELISA in the 15 experimentally infected dogs with *L. infantum*.

Months after inoculation	No. of rK39 positive	% Positive	No. of ELISA positive	% Positive
1	8	53.3	2	13.3
2.5	10	66.6	13	86.6
3.5	14	93.3	13	86.6

patients from Nepal. With the dipstick test, both sensitivity and specificity were 100%. With DAT, the sensitivity was 100% and specificity was 93%.[7] In another comparative evaluation performed in Nepal, DAT showed slightly better results than those with rK39 in the confirmatory diagnosis of VL cases.[8] The rK39 strip test and DAT were also used in diagnosis of VL in parasitologically-positive and apparently cured patients in Sudan. Both tests showed limited specificity and sensitivity, and the Dipstick test remained positive to a lesser extent after treatment. Nevertheless, the Dipstick test was reported as ideal for use in the field.[9] The specificity of the "rK39 Dipstick' for differential diagnosis of VL from Chagas disease, malaria, schistosomiasis and toxoplasmosis in Venezuela was 100%.[10]

In the present study, the specificity of the "rK39 Dipstick" test in nonvisceral leishmaniasis infections was also 100%. The DAT seropositivity rate in clinically suspected VL patients and children from VL endemic areas was higher than that with rK39 (Table 1). This study confirmed the report of Sundar et al in India that the "rK39 Dipstick" test usually detects active VL infection,[6] but DAT also shows residual *Leishmania* antibodies in treated patients.[1]

In conclusion, this study showed that the rapid "Dipstick rK39" test, compared to DAT and ELISA, is simple, practical, sufficiently sensitive and highly specific in the diagnosis of active VL in human as well as in dog. The test can be performed with a few drops of blood, serum or plasma in field conditions.

Further application and evaluation of the "rK39 Dipstick" test in serodiagnosis and serological survey of VL in kala-azar endemic areas is recommended.

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