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Rk39 Dipstick Test as a Practical Solution for Canine Leishmaniasis Diagnosis among Nomadic Populations

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Abstract: Detection of clinical manifestations of leishmaniasis in dogs is difficult and not practical for screening nomadic dogs. Our aim of study was to check out the sensitivity and specificity of a commercially available immunochromatographic dipstick rk39 test compared to Direct Agglutination Test (DAT) used in canine visceral leishmaniasis diagnosis among nomad dogs. Three hundred and twenty nomad households were studied. Four hundred and fifty eight dogs from these households were examined and 5 CC blood from cephalic vain was taken. Samples were tested both with DAT and Dipstick rk39. Data were analyzed using SPSS 11 statistical package. The mean for age of dogs was 5.3 years. Considering of positive result as titers (>=1:160), 7.4 % of dogs had positive DAT results. There was no statistical difference between DAT result and sex of the dog. Results of dipstick test showed to be positive in 17 male and two female dogs. When the serum titer of antibodies reached to high rates such as 1:640, the sensitivity and specificity of dipstick test reached up to 80% and in serum titers of 1:320 or 1:160, sensitivity of dipstick test was lower than 50%. Dipstick test as a simple and practical test can be used for canine leishmaniasis among mobile populations like Iranian nomads.

Key words: Leishmaniasis, DAT, antibodies, normadic, dipstick test, cephalic vain

INTRODUCTION

Leishmania infantum infections are responsible for Visceral Leishmaniasis (VL) in at least 70 countries. In most endemic areas, it is widely believed that domestic dogs (Canis familiaris) are the principal hosts. Dog ownership within villages has been shown to a significant risk factor for child sero positivity (Gavgani *et al*., 2002).

Detection of clinical manifestations of leishmaniasis in dogs is difficult and some times needs to anesthetize dogs for physical examination and imaging or preclinical exams. The Direct Agglutination Test (DAT) although highly sensitive and specific is very time consuming and has got a relative sophistication (Reithinger *et al*., 2002; Saul and Semiao, 1996; Harith *et al*., 1989). So it can not be a suitable test in case of nomadic or mobile populations. Dipstick rk39 tests have been used as quite sensitive and specific human Kala-azar diagnostic tests (Chappuis *et al*., 2005; Veeken *et al*., 2003; Zijlstra *et al*., 2001; Sundar *et al*., 1998; Jelinek *et al*., 1999; Bern *et al*., 2000).

Ardabil province has got a large nomadic population and along with Bushehr is also the most important endemic area for Kala-Azar in Iran. In this study we have checked the sensitivity and specificity of a commercially available immunochromatographic dipstick rk39 test (Cypress Diagnostic Company, Belgium) compared to direct agglutination test used in canine visceral leishmaniosis diagnosis among nomad dogs.

MATERIALS AND METHODS

In a cross-sectional study based on sampling structure of Nomads' health survey, 320 nomad households including 40 clusters were studied. Four hundred and fifty eight dogs from these households were clinically examined for symptoms of kala-azar and 5 CC blood from cephalic vain was taken.

Freezeed Serum of obtained samples were sent to reference laboratory of the faculty of public health, Tehran University of medical sciences where DAT test was conducted also the *L. infantum* antigens for this study were prepared in the above mentioned center.

DAT (Direct Agglutination Test)was performed as the same method of Harith,that reliability of the test was improved by treating the test sera with 0.2 M 2-mercaptoethanol and incubating them at 37°C (Harith *et al*., 1989).

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Meanwhile, all of the samples immediately after sampling were examined with Dipstick test (rk39: Cypress Diagnostic Company, Belgium), based on Leishmania infantum antigen, for the rapid detection of Leishmania-specific antibodies in canine serum samples and test performance was compared Direct Agglutination Test (DAT).

Dogs Examination for clinical manifestation of visceral leishmaniasis such as cachexis lymphadenopathy, cataract was done and history of anorexia was taken from dogs owners. Ten dogs with owner's agreement were explored for pathologic findings. Test results and collected data were entered into computer and analyzed with SPSS 11 statistical package.

RESULTS

The mean for age of dogs was 5.3 years with a standard deviation of 8 years. Nine dogs with hair loss, 9 with weight loss, 50 with lymphadenopathy, 4 with cataract 7 with dermatologic disorders, 4 with nail disorders, 6with anorexia and 3 with paralysis were recognized. More than half of dogs belonged to Nomads of Parsabad district and nearly a quarter of cases belonged to Bilesavar district.

Considering of positive result as titers (>=1:160), 7.4% of dogs including 28 male dogs and 6 female dogs had positive DAT results. There was no statistical difference between DAT result and sex of the dog (Table 1).

Results of dipstick test showed to be positive in 17 male and two female dogs. Although positive rate was higher among male dogs, there was no statistical difference between Dipstick result and sex of the dog (Table 2).

Table 1: Distribution of DAT titers among nomad dogs

Dog	DAT Titers							
sex	Negative	Suspected	1:160	1:360	1:640	1:1280	1:2560	Total
Male	281	15	6	14	2	2	4	324
Female	125	3	2	2	0	0	2	134
Cum	406	18	8	16	2	2	6	458

Table 2: Distribution of dipstick test in both sexes

Negative	Positive dipstick	Sex
307	17	Male
132	2	Female
439	19	Total

Chi-sq = 3.36 and p = 0.06

Table 3: Sensitivity, specificity and positive predictive value of dipstick test based on DAT

Positive predictive value (%)	Specifity dipstick (%)	Sensitivity dipstick (%)	Positive DAT titers
75	77	35.2	1:160≥
62.5	77	38.4	1:320≥
50	80.9	80	1:640≥

Using DAT test as a golden standard, sensitivity, specifity and predictive values of Dipstick test was calculated (Table 3). As showed in Table 3, when the serum titer of antibodies reaches to high rates such as 1:640, the sensitivity of dipstick test reaches to 80% and in serum titers of 1:320 or 1:160 ,sensitivity of dipstick test is below than 50%.

DISCUSSION

Direct Agglutination Test (DAT) was first described by Allain and Kagan (1975) and the method was then adapted by El Harith *et al.* (1986; 1988). The DAT is very accurate under laboratory conditions with high specificity when control groups were composed of healthy persons from endemic areas (Schaefer *et al.*, 1995; Boelaert *et al.*, 1999a). In field conditions, the DAT is very sensitive, but low specificity values were reported in series of clinical suspect patients, with a range depending on the reference test used, between 58 and 72% (Zijlstra *et al.*, 1991).

Recently, serological testing against a recombinant antigen derived from a 39-amino acid repeat in Leishmania chagasi (rK39) was developed. It is very accurate when used in an ELISA format (Singh *et al.*, 1995; Badaro *et al.*, 1996). It was later developed as a dipstick format and has been use in human and canine poulations showing different rates of sensitivity and specificity.

According to previous studies (Saul and Semiao, 1996; Harith *et al.*, 1989; Edrissian *et al.*, 1996; Boelaert *et al.*, 1999) the performance of the DAT for detection of L. infantum infection in humans and dogs was excellent. So we used DAT as the gold standard and compared the dipstick's results with DAT. In our study comparing this dipstick test with DAT, when the serum titer of antibodies reached to high rates such as 1:640, the sensitivity and specificity of dipstick test reached up to 80% and in serum titers of 1:320 or 1:160, sensitivity of dipstick test was lower than 50%.

Previous studies have used rk39 dipstick more for human and less for canine leishmaniasis. Zijlstra *et al.* (2001) found a sensitivity of only 67% in Sudan. Chappuis *et al.* (2005) found the DUAL IT L/M rK39 antigen-based dipstick from Dia-Med AG, Switzerland, to be highly sensitive (97%) and specific (97%) for the diagnosis of VL in northeastern Uganda. In this study the Kalazar Detect dipstick was also highly specific (99%) but substantially less sensitive (82%). The FGT was less sensitive (66%) and specific (90%) than the rK39 dipsticks.

Chappuis also evaluated the Direct Agglutination Test (DAT) and an rK39-antigen-based dipstick test, compared to parasitological diagnosis in a human population in Nepal. The rK39 dipstick in this study

showed a sensitivity of 97% and a specificity of 71%. The DAT was up to 99% sensitive with a low cut-off titre (1:400) but its specificity did not exceed 82% even with a high cut-off titre (1:51 200).

Edrissian *et al.* (2003) also found the rapid "Dipstick rK39" test to be sufficiently sensitive and quite specific in diagnosis of visceral leishmaniasis in humans as well as in dogs.

Ozensoy (1998), studding 24 confirmed VL cases from western Turkey, found the rK39 ELISA to be more sensitive than a combination of cultivation and microscopy of bone marrow aspirates. The specificity of rK39 for leishmaniasis was demonstrated by its lack of cross-reactivity with sera from other human diseases in the same sites. He stated that use of the rK39 ELISA as a sensitive tool makes it possible to demonstrate coendemicity of canine and human VL, as expected in the case of IVL.

In another study on a canine population, serum from all patent dogs showed high antibody titers to rK39 in Enzyme-Linked Immunosorbent Assays (ELISA) and reacted by western blotting with several antigens, 12 to 120KDa, including gp63 and gp70. In the case of asymptomatic dogs, antibody titers to crude antigen were low and only a few antigens were identified by western blotting (Rhalem *et al.*, 1999).

Scalone *et al.* (2002) compared a standardized ELISA using a purified recombinant antigen (rK39) specific to VL to the Immunofluorescent Antibody Test (IFAT) as the standard. Antibodies reacting with rK39 were more common in asymptomatic canine infections than reported for subclinical human VL. The rK39 ELISA is a relatively simple and rapid assay for assessing the infection status of dogs and is an alternative to IFAT, especially when screening large numbers of samples.

CONCLUSION

Based on our findings and previous studies, because of simple and time saving advantages of rk39 dipstick test we suggest it to be used for screening purposes among dogs in nomadic populations that other tests are not practically suitable.

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