Brief Report

# The rK39 immunochromatic dipstick testing: A study for K39 seroprevalence in dogs and human leishmaniasis patients for possible animal reservoir of cutaneous and visceral leishmaniasis in endemic focus of Satluj river valley of Himachal Pradesh (India)

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ABSTRACT

**Background:** The newly recognized endemic focus of leishmaniasis in Satluj river valley of Himachal Pradesh (India) has both localized cutaneous leishmaniasis (LCL) and visceral leishmaniasis (VL) predominantly caused by *Leishmania donovani*. Rapid rK39 immunochromatographic dipstick test detects circulating antibodies to recombinant K39 antigen of *L. donovani-infantum* complex and is highly specific/sensitive in diagnosing symptomatic or asymptomatic infection in humans and dogs. **Methods:** The sera from two VL patients and 13 LCL patients, and 31 dogs were subjected to rK39 immunochromatographic dipstick testing with an aim to identify possible animal reservoir for leishmaniasis in this endemic focus. **Results and Conclusion:** The positive rapid rK39 immunochromatographic dipstick test in 100% VL and 31.8% LCL patients, and 6.5% dogs suggests that both VL and LCL in this focus are apparently being caused by *L. donovani-infantum* and that reservoir infection is perhaps being chiefly maintained in asymptomatic dogs. However, it needs corroborative evidence in the form of *in-vitro* parasite cultivation and/or PCR studies for confirmation. A more elaborate study is recommended.

**Key words:** Canine leishmaniasis, Cutaneous leishmaniasis, Visceral leishmaniasis, Dogs, Himachal Pradesh, rK39 dipstick test

## INTRODUCTION

Visceral leishmaniasis (VL) is caused primarily by *Leishmania donovani* and *Leishmania infantum*, and the primary causative agent of localized cutaneous leishmaniasis (LCL) is *Leishmania tropica*, *L. major* or *L. aethiopica* in the 'old world'. Recently, *L. donovani* and *L. infantum* too have been implicated to cause LCL.[1-7] Visceral leishmaniasis is endemic in Indian states of Bihar and West Bengal and has been reported sporadically from hills of Uttar Pradesh and Himachal Pradesh.[8-11] Cutaneous leishmaniasis has been endemic in western-Thar desert of India, Pakistan, Afghanistan, Iran, and China. *Phlebotomus argentipes* is the only proven vector for VL in the Indian subcontinent, while the identity of any animal reservoir in the region has remained elusive.

*L. donovani* and *L. infantum* are primarily viscerotropic but have been recognized to possess cutaneo-viscerotropic tendency in some endemic foci.[3,6] For instance, *L. donovani* is reported to cause LCL in Kenya, Yemen, Sri Lanka, Syria, and more recently in India,[1-5] and *L. infantum* has been frequently isolated from LCL patients in countries like Spain, Italy, Algeria, Morocco, Tunisia, Malta, Cyprus, Greece, and Pakistan.[6,7] *L. infantum* leishmaniasis is a zoonotic infection in Mediterranean countries having sylvatic transmission cycle. Different vector species, for example, *Phlebotomus longiductus*, *P. wui*, and *P. chinensis* are responsible in different regions for this form of infection.[6] Dogs are the main domestic reservoirs, and in wild, it is wolves and foxes.[12] Early diagnosis in dogs is, thus, important for surveillance and control programs.

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Rapid immunochromatographic dipstick test qualitatively detects anti-*Leishmania* circulating antibodies by leishmanial recombinant antigen K39 (rK39) which is the product of a gene cloned from *L. chagasi* containing a 39-amino-acid repeat conserved among viscertropic *Leishmania* species (*L. donovani*, *L .infantum*, and *L. chagasi*). Although cross reactivity may occur with malaria, enteric fever, disseminated tuberculosis, or with samples from dogs infected with *Neospora caninum*, *Hepatozoon canis*, *Toxoplasma gondii*, or *Barbesia canis*, the rapid rK39 immunochromatic dipstick test is both sensitive (67–100%) and specific (93–100%), and often scores over diagnoses that demonstrate the parasites in spleen or bone marrow by smears and cultures.[12-15] Sundar *et al*.[16] also found 100% sensitivity and 98% specificity of the test in Indian VL patients. It is cost effective too, and can be performed in field conditions as it requires no specialized equipment or technical expertise. Apart from its use in diagnosis of VL, it can reliably be used for early diagnosis and planning prophylactic treatment of latent infection in asymptomatic carriers, family members, and contacts.[17] Its utility has also been demonstrated in serodiagnosis of both symptomatic and asymptomatic canine leishmaniasis.[12] Thus, it can be particularly useful in mass screening surveys of both humans and animals in targeted control programs.

This endemic focus in Himachal Pradesh appears peculiar wherein LCL exists with sporadic VL, and *L. donovani* is a predominant pathogen for LCL except for few cases due to *L. tropica*.[4,10] The collected sandflies from this endemic focus were mainly *P. longiductus*, while *P. argentipes*, the only proven vector for VL in the Indian subcontinent, has neither been found by us nor anyone in the past.[4,18,19] Additionally, preliminary PCR analysis of isolates from some LCL patients was suggestive of these being *L. infantum* (unpublished data). All these features are epidemiologically similar to those of human leishmaniasis in Mediterranean countries. In such settings, we carried out rapid rK39 immunochromatographic dipstick testing of human and animal sera with an objective to identify possible animal reservoir for leishmaniasis in this new endemic focus.

## METHODS

The study area comprised Rampur and Tapri, the two most active endemic pockets, in the previously delineated endemic area for human leishmaniasis in the region.[18] Venous blood samples of 13 LCL and two VL patients diagnosed by demonstrating amastigotes in tissue smears and bone marrow/splenic aspirates, respectively; and venous blood samples from 14 stray and 17 pet apparently healthy dogs from patients' villages were collected in buffered citrate. These were centrifuged separately for 30 minutes at 3000 rpm and serum was separated with sterile pipettes to be used for rapid rK39 immunochromatographic dipstick test. The dipstick test was performed according to the manufacturers' (*InBios* International, Inc. Seattle, WA 98104) instructions. Briefly, 20 $\mu$l of serum was added to the sample wells of the strip, followed by 100 $\mu$l of buffer solution provided with the test kit. The results were read 10 minutes after the addition of the serum. They were considered positive when two distinct new red or pink lines, one in the test region and another in the control region, appeared. The buffy coat was subjected to culture in modified NNN medium (Novy, MacNeal, and Nicoll's medium containing RPMI 1640 and 10% heat inactivated fetal bovine serum), and to prepare smears which were stained with Geimsa and examined for amastigotes.

## RESULTS AND DISCUSSION

This recently recognized leishmaniasis endemic area is spread across villages in the valley of river Satluj on both banks; between Pooh subdivision of Kinnaur district toward north-east border, Kumarsain subdivision of Shimla district toward south-west, Rampur division (Shimla district) and adjoining villages toward southern side, and Nirmand subdivision of Kullu district on the northern side (924–2900 meters above mean sea level).[18] It has China bordering toward north-east.

Serum samples from two (100%) VL and four (31.8%) LCL patients were positive for rapid rK39 immunochromatographic dipstick test [Figure 1A]. Serum samples from two (6.5%) dogs also displayed light-colored bands in rapid rK39 immunochromatographic dipstick strips [Figure 1B]. One of these dog's owner family had a child afflicted with LCL. The dog died about five months after it was first seen. All the Geimsa stained smears and cultures in modified NNN media prepared from buffy coat samples, both from dogs and patients, were negative.

*L. tropica* is predominantly a cutaneotropic species and known to produce both zoonotic as well as anthroponotic LCL. It has been isolated from VL


**Figure 1: Rapid immunochroamtographic rK39 dipstick test results from serum samples of (A) localized cutaneous leishmaniasis patient. (B) Stray dog The single band (C) represents a negative control, while the double band (A and B) reflects positive result**

patients and even implicated to cause Indian 'Kala Azar', but it remains unestablished that it causes typical VL.[5,20,21] Evidently, it may produce mild visceralization but does not express K39 repeats like *L. infantum*. Thus, the antibody response to rK39 is largely VL (*L. donovani-infantum* complex)-oriented and there is virtually no response in infection with *L. tropica* (cutaneous leishmaniasis).[22] Positive rapid rK39 immunochromatographic dipstick testing in the two (100%) VL and four (31.8%) LCL patients suggest presence of *L. donovani-infantum* infection in this endemic focus. Similarly, positive rapid rK39 immunochromatographic dipstick test in two dogs indicates K39 seroprevalence in both, domestic as well as stray dogs, suggesting that they are perhaps harboring the *L. donovani-infantum* infection and providing a constant reservoir for the infection. However, this needs corroborative evidence from *in-vitro* cultivation of parasite and/or PCR studies.

Despite limitation of small number of samples subjected to rK39 immunochromatographic dipstick testing, the results imply that dogs are apparently the most probable animal reservoirs in transmission cycle of *Leishmania* in this endemic focus. However, this is purely conjectural at the moment and we recommend a more elaborate study to establish complicity of dogs or to identify the animal reservoir of human leishmaniasis in this endemic focus.

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