

## SHORT REPORT: HUMAN TRYPANOSOMIASIS CAUSED BY *TRYPANOSOMA EVANSI* IN A VILLAGE IN INDIA: PRELIMINARY SEROLOGIC SURVEY OF THE LOCAL POPULATION

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**Abstract.** After discovery of the first recorded case of human infection with *Trypanosoma evansi*, serologic screening of 1,806 persons from the village of origin of the patient in India was performed using the card agglutination test for trypanosomiasis and *T. evansi*. A total of 410 (22.7%) people were positive by whole blood, but only 81 were confirmed positive by serum. However, no trypanosomes were detected in the blood of 60 people who were positive at a high serum dilution. The results probably indicate frequent exposure of the human population to *T. evansi* in the study area, which suggests frequent vector transmission of parasites to humans. Although *T. evansi* is not infective for humans, a follow-up of seropositive persons is required to observe the evolution of human infection with this parasite.

In 2004, the first case of human infection with *Trypanosoma evansi* was found in the central part of India (Seoni village, Taluka Sindevahi, district of Chandrapur, Maharashtra State<sup>1</sup>). *Trypanosoma evansi* is not known to be pathogenic to humans, but is the cause of a common animal disease called surra.<sup>2</sup> Although investigations are ongoing, the reason for such an unusual infection in humans remains unknown. We currently speculate that the patient, a cattle farmer, was probably contaminated through a wound on his finger when manipulating blood of infected cattle. However, possible transmission by an insect vector, such as horse or stable flies (*Tabanidae* or *Stomoxys*) cannot be excluded.

On the basis of this case, the Directorate of General Health Services (DGHS) of Maharashtra State in Mumbai decided to conduct a serologic survey of individuals in Seoni village. Special attention was paid to the exposed population living or working in contact with cattle (mainly cows). The survey was conducted by the DGHS with the assistance of the World Health Organization and the Institut de Recherche pour le Développement (France). A protocol was used similar to that for detection of human African trypanosomiasis (HAT). This protocol included serologic screening and microscopic search for trypanosomes in blood.<sup>3</sup> The purpose was to investigate possible occurrence of *T. evansi* in the human population within the village of the first patient.

Seoni village is 120 km east of Nagpur in central India. Most people in the village are farmers and raise cattle. Of a total population of approximately 3,000 people, 1,806 were screened in May 2005, including 26 veterinarians and assistants. After registration and recording of name, age, sex, activity, location, and family, a code was given to each person.

We used the card agglutination test for trypanosomiasis (CATT), CATT/*T. evansi*, (Institute of Tropical Medicine [ITMA], Antwerp, Belgium), which detects antibodies in serum or plasma of infected animals.<sup>4</sup> Although ITMA recom-

mended using plasma, we used whole blood for mass screening for practical and ethical reasons because blood could be collected into a hematocrit tube by fingerprick, but serum required venipuncture. The antigen for CATT/*T. evansi* is made using cloned bloodstream form trypanosomes of RoTat 1.2, a predominant variable antigen type of *T. evansi*. The test is performed on a plastic card with 10 reaction zones. The reconstituted antigen suspension is mixed with whole blood (equal volume: one drop) and rotated for 5 minutes at 60–70 rpm on a card test rotator. Agglutination indicates a positive result. If the CATT/*T. evansi* result was positive using whole blood, a quantitative method was performed using serum and serial dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32. Twenty-five microliters of serum or dilution was spread and mixed with 45  $\mu$ L of CATT reagent on a reaction zone and rotated using the same conditions mentioned above. All CATT/*T. evansi* using serum and serial dilutions were repeated and confirmed in laboratory conditions.

According to the protocol recommended by ITMA for diagnosis of surra, if the CATT/*T. evansi* result was positive with a 1:4 serum dilution, trypanosomes were detected using the mini anion exchange centrifugation technique (mAECT<sup>5</sup>) (Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo) and 300  $\mu$ L of whole blood. This chromatography system for field use concentrates parasites in a collecting tube. After centrifugation at 1,500 rpm for 10 minutes, the bottom of the collecting tube was examined microscopically using an appropriate reading chamber (magnification  $\times$  100). Palpation to detect swollen lymph nodes was done according to HAT procedures.<sup>3</sup>

The CATT/*T. evansi* results are summarized in Table 1. Of 1,806 people screened using this test, 410 (22.7%) were positive using whole blood but only 81 were confirmed positive using serum (4.5%). Of these 81 people, 17 (21%) were CATT positive and 4 (5%) CATT negative at a serum dilution of 1:2, 45 (55.5%) were positive at a serum dilution of 1:4, 10 (12.3%) were positive at a serum dilution of 1:8, and 5 (6.2%) were positive at a serum dilution of 1:16. Of the 26 veterinarians and assistants, 7 (27%) were CATT positive at a serum dilution of 1:4. The mAECT result was negative for 60 persons who were CATT positive at serum dilutions  $\geq$  1:4.

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TABLE 1

Results of CATT/*Trypanosoma evansi* for 1,806 persons tested using whole blood (WB), serum(s), and serial dilution of serum (1:2, 1:4, 1:8, 1:16, and 1:32).\*

No. tested	WB (-)	WB (+)	S (-)	S (+)	1:2 (+)	1:4 (+)	1:8 (+)	1:16 (+)	1:32 (+)
1,806	1,396	410	6	81	17	45	10	5	0

\* CATT = card agglutination test for trypanosomiasis.

Although four persons had swollen lymph nodes, no trypanosomes were detected by microscopic examination of lymphatic fluid.

The number of CATT/*T. evansi*-positive persons at serum dilutions  $\geq$  1:4 (60 people or 3.9% of the entire population, including 7 veterinarians or assistants) may indicate frequent exposure to *T. evansi* in the study area. Furthermore, 15 people were CATT positive at serum dilutions  $\geq$  1:8. Levels of specific antibodies against *T. evansi* in these 15 people were high. A CATT was performed for the first patient,<sup>1</sup> who was positive at a serum dilution of 1:64, but this result was obtained when the patient had approximately  $10^6$  trypanosomes/mL before treatment. Twenty four hours or three months after initial treatment, the CATT result was positive at plasma dilutions of 1:16 and 1:4, respectively. After six months, the CATT result was positive only for a plasma dilution of 1:2. This indicates a reduction of seropositivity likely caused by lysis of trypanosomes.<sup>6</sup>

In human exposure to *T. evansi*, serologic results seem to be proportional to parasitemia. We have not observed a similar situation for HAT, where serologic results may be low when parasitemia is high and *vice versa*. In the present study, the mAECT result was negative for 60 people who were CATT positive at serum dilutions  $\geq$  1:4. However, we have not conducted mass screening for *T. evansi*, and to our knowledge, such a survey has not been conducted.

Two hypotheses could explain the negative results of parasitologic tests. First, *T. evansi* is supposedly a non-human infective trypanosome. More specifically, the trypanolytic factor in human serum would normally be expected to prevent infection with *T. evansi*, and has recently been identified as apolipoprotein L1.<sup>7</sup> Second, according to previous observations for the first human case, parasitemia was only detectable during febrile episodes associated with a high parasitemia in blood,<sup>1</sup> which is easy to detect by microscopy. Therefore, a follow-up of seropositive persons is required to observe the evolution of human infection with this parasite. It will also be necessary to monitor whether febrile episodes occur in any of these 60 persons with positive CATT results at serum dilutions  $\geq$  1:4 and repeat parasitologic tests if fever is observed.

The fluctuating parasitemia observed in the original patient may essential for further case detection. Although we can use polymerase chain reaction-based methods to detect parasite DNA, we do not know why the first patient was infected. Therefore, we cannot exclude the occurrence of other human cases. This study has demonstrated the high proportion of people who have been exposed to *T. evansi* in this part of India. Such a study should be repeated in another village where *T. evansi* is endemic in livestock. For the area under study, epidemiologic surveillance is required to follow-up the evolution of this infection and the emergence of a new zoonotic disease.

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## REFERENCES

- Joshi PP, Shegokar V, Powar S, Herder S, Katti R, Salkar S, Dani V, Bhargava A, Jannin J, Truc P, 2005. Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. *Am J Trop Med Hyg* 73: 491-495.
- Hoare CA, 1972. *The Trypanosomes of Mammals. A Zoological Monograph*. Oxford, United Kingdom: Blackwell Scientific Publications.
- World Health Organization, 1998. Control and surveillance of African trypanosomiasis. Report of a WHO expert committee. *World Health Organ Tech Rep Ser* 881: 1-114.
- Pathak KM, Singh Y, van Meirvenne N, Kapoor M, 1997. Evaluation of various diagnostic techniques for *Trypanosoma evansi* infections in naturally infected camels. *Vet Parasitol* 69: 49-54.
- Lumsden WH, Kimber CD, Evans DA, Doig SJ, 1979. *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low parasitemias; adaptation for field use. *Trans R Soc Trop Med Hyg* 73: 312-317.
- Joshi PP, Chaudhari A, Shegokar VR, Powar RM, Dani VS, Somalwar AM, Jannin J, Truc P, 2006. Treatment and follow up of the first case of human trypanosomiasis caused by *Trypanosoma evansi* in India. *Trans R Soc Trop Med Hyg* 100: 989-991.
- Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan D, Lins L, van den Abbeele J, Pays A, Tebabi P, Xong H, Jacquet A, Moguilevsky N, Dieu M, Kane JP, de Baetselier P, Brasseur R, Pays E, 2003. Apolipoprotein L-1 is the trypanosome lytic factor of human serum. *Nature* 422: 83-87.