HUMAN TRYPANOSOMIASIS CAUSED BY TRYPANOSOMA EVANSI IN INDIA: THE FIRST CASE REPORT

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Abstract. We report an Indian farmer who had fluctuating trypanosome parasitemia associated with febrile episodes for five months. Morphologic examination of the parasites indicated the presence of large numbers of trypanosomes belonging to the species Trypanosoma evansi, which is normally a causative agent of animal trypanosomiasis known as surra. Basic clinical and biologic examinations are described, using several assays, including parasitologic, serologic, and molecular biologic tests, all of which confirmed the infecting species as T. evansi. Analysis of cerebrospinal fluid indicated no invasion of the central nervous system (CNS) by trypanosomes. Suramin, a drug used exclusively for treatment of early-stage human African trypanosomiasis with no CNS involvement, effected apparent cure in the patient. This is the first case reported of human infection due to Trypanosoma evansi, which was probably caused by transmission of blood from an infected animal.

INTRODUCTION

Human trypanosomiasis is endemic in Africa and South America. In Africa, the disease, known as human African trypanosomiasis (HAT) or sleeping sickness, is caused by Trypanosoma brucei gambiense (chronic form) or T. b. rhodesiense (acute form), whereas the American trypanosomiasis, known as Chagas’ disease, is caused by T. cruzi. Sleeping sickness and Chagas’ disease are both transmitted by vectors.

Human African trypanosomiasis is transmitted during blood meals (causative trypanosomes belong to the Salivaria group) by infected saliva of tsetse flies, while the American trypanosomiasis is transmitted by infected feces of reduviid bugs (causative trypanosomes belong to the Stercoraria group). In addition to human infectious trypanosomes, a variety of other species cause animal trypanosomiasis with a wide geographic distribution. Nagana is caused by T. b. brucei in Africa and affects cattle; T. congolense and T. vivax infect domestic and small animals; and surra is caused by T. (Trypanozoon) evansi and infects mainly camels, cattle, and buffalos and other wild animals on all continents. Trypanosoma b. brucei and T. congolense are, similar to T. b. gambiense and T. b. rhodesiense, transmitted by tsetse fly bites, while T. evansi is spread by mechanical transmission of infected blood through hematophagous insects such as tabanid flies. Trypanosoma evansi is closely related to other Trypanozoon species, including T. b. gambiense and T. b. rhodesiense, at the genetic level. However, there is no cyclical transformation of T. evansi within the flies and this parasite remains monomorphic throughout its life cycle, while T. brucei subspecies are pleomorphic, presenting a range of forms at different points in the life cycle.

Human infection by animal species of Trypanosoma is usually impossible because of a trypanolytic factor in human serum. However, it has been demonstrated that T. congolense and T. evansi are capable of resisting human plasma in certain circumstances, and at least one report describes a patient in Côte d’Ivoire harboring both T. brucei and T. congolense with associated clinical signs. The degree to which trypanosome species that are not usually associated with human infection might actually infect humans is difficult to ascertain since such infections may be short-lived and pass undiagnosed. For example, three people infected by T. lewisi (a common parasite of rats) in India and Malaysia all recovered without treatment.

An Indian farmer had a fluctuating trypanosome parasitemia associated with febrile episodes for five months. Morphologic examination of the parasites indicated the presence of high numbers of trypanosomes of the species T. evansi, the causative agent of animal trypanosomiasis known as surra. Clinical and biologic examinations are described, including parasitologic, serologic, and molecular biologic tests that all confirmed this unusual infection. The immune status of this patient was also investigated with respect to possible infection with human immunodeficiency virus (HIV). Moreover, indicators of Tangier disease, a rare autosomal recessive genetic disorder, were investigated since it has been proposed, albeit controversially, that trypanosome lytic factors might be absent in persons with this disease.

PATIENT, MATERIALS, AND METHODS

Patient. The patient, a 45-year-old man and cattle farmer from Seoni village in Taluka Shindevali, District of Chandrapur, Nagpur (Maharashtra State) in central India who had never traveled outside his district, was admitted to the Rural Hospital in Shindevali September 20, 2004. He had intermittent fever associated with chills and sweating for 15 days. He then developed signs of sensory deficit, and he was disoriented and agitation with violent behavior. A blood smear examination indicated the presence of unknown flagellates, and he was transferred to the Government Medical College in Nagpur, India.

On the day of admission to the Government Medical College (September 26, 2004), a blood smear stained with Giemsa showed numerous parasites with morphology typical of monomorphic, slender, flagellated, dividing, trypomastigote-form trypanosomes (with a parasitemia of approximately 8 × 10^6 trypanosomes/mL).
Blood was collected by venipuncture using heparinized Vacutainer® (Becton Dickinson, Franklin Lakes, NJ) tubes, serum was collected into dry Vacutainer tubes®, and CSF was collected into a 15-mL sterile tube by lumbar puncture. Blood and serum were collected 72 days and 108 days after initial hospital admission. The CSF was collected 108 days after initial hospital admission. Additional blood, serum, and CSF samples were collected after September 2004 by the Department of Microbiology at the Government Medical College. Blood samples were collected 9, 39, and 55 days after initial hospital admission and stored at −80°C. These latter samples were compared by polymerase chain reaction (PCR)–based methods to blood samples collected 72 days and 108 days after initial hospital admission. Serologic analysis of serum was performed, and blood and CSF samples were mixed with an equal volume of guanidine lysis buffer (5.5 M guanidium thiocyanate, 0.18 M EDTA, Gibson WC, unpublished data) before being shipped to Montpellier, France for subsequent DNA analysis.

Rodent inoculation. One milliliter of patient blood was inoculated by intraperitoneal injection into two Wistar rats 55 days after initial hospital admission in an attempt to isolate and propagate the trypanosomes.

Clinical and laboratory analysis. To confirm the previous identification of the parasite based on the trypanosome morphology, additional tests were performed 72 days and 108 days after initial hospital admission. The following laboratory investigations were conducted in the Department of Microbiology at the Government Medical College. Classic biochemical quantitative analysis of serum was performed with emphasis on lipid levels, which are a possible indication of Tangier disease. The card agglutination test for trypanosomiasis (CATT)/T. evansi,® (Institute of Tropical Medicine, Antwerp, Belgium) was conducted. This is a highly specific direct agglutination test that uses the Rotat 1.2 antigen.® It was performed using whole blood and serum (diluted 1:2–1:128) that were collected 72 and 108 days after initial hospital admission. The mini-anion centrifugation technique10 (Institute of Tropical Medicine), a chromatographic-based system that concentrates and purifies trypanosomes, was conducted using 300 μL of heparinized blood collected 108 days after initial hospital admission, and 24 hours after the first, second, and last treatments with suramin. A direct latex IgM agglutination test11 with CSF, which has been shown to effectively demonstrate dysfunction in the blood-brain barrier, was conducted using CSF dilutions from 1:2 to 1:128. A positive result was reactivity at a dilution of 1:12.12 Approximately 1.5 mL of CSF was centrifuged in a sealed Pasteur pipette13 at 3,000 rpm for 10 minutes. The sediment in the pipette was examined by bright-field microscopy at a magnification of ×400 for the presence of trypanosomes. Lymphocytes in the CSF14 were counted in a Thomas chamber because their presence is frequently an indicator of parasite invasion.

The following laboratory tests were conducted at the Institut de Recherche pour le Développement in Montpellier, France using samples collected 9, 39, 55, 72, and 108 days after initial hospital admission. Polymerase chain reaction–based methods were performed to confirm the diagnosis of infection with T. evansi. The DNA was extracted from the mixed solution of blood and guanidine lysis buffer using a standard phenol/chloroform/isoamyl alcohol–based method. The DNA templates (diluted 1:100 and 1:1,000) were stored at 4°C until used. Such dilutions were required to avoid inhibition due either to guanidine lysis buffer or a high concentration of DNA. Three independent PCR assays were performed using trypanosome DNA from blood. Parasites were tested for their relatedness to the subgenus Trypanozoon using a seminested PCR method based on internal transcribed spacer (ITS) of ribosomal DNA using primer ITS1/2, which was derived from KIN1/2 primers.® Relatedness to the T. brucei group was established using a single PCR of the 177-basepair repeats characteristic of this group.® A T. evansi-specific amplification was conducted using a 994-basepair mitochondrial kinetoplast minicircle template with the primer TEV 1/2.17 Two reference strains were used as controls: T. b. gambiense Bat 61® and T. evansi CIRDES (kindly provided by Dr. Marc Desquesnes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement/Centre Internationale de Recherche-Développement sur l’Elevage en Zone Subhumide, Bobo Dioulasso, Burkina Faso).

Three tests were conducted to determine whether the patient was infected with HIV. These tests were carried out at the Institut de Recherche pour le Développement Unit 145, HIV/AIDS et Maladies Associées, Collaborative Center (Montpellier, France). The tests used were the Determine HIV 1/2 assay (Abbott Laboratories, Rungis, France), specific enzyme-linked immunosorbent assay (ELISA) procedures,® and the NNO-LIA HIV 1/2 Score test (Innogenetics N.V., Ghent, Belgium). These tests were conducted to corroborate the results of the ELISA used with patient blood in India.

Treatment. Treatment was initiated 109 days after initial hospital admission using suramin (Germanin; Bayer, Leverkusen, Germany). This was requested by the Department of Public Health, Government of Maharashtra State, India, and provided by the World Health Organization. Suramin is used as treatment for the first stage of HAT caused by T. b. rhodesiense,® and its efficacy against T. evansi infection (surra) in cattle has previously been established.® The suramin solution was administrated by slow intravenous infusion over 90 minutes (1 g of suramin IV on days 1, 8, 15, 22, and 29). Due to the risk of a possible severe anaphylactic reaction, a sensitivity test was conducted before starting treatment by injecting intravenously 0.1 g of suramin over a period of three minutes. Suramin was always administered in Intensive Care Unit with cardio-pulmonary monitoring in the presence of trained senior physicians and anesthetists with due emergency precautions. Before starting treatment, all other parasitic infections were ruled out and the nutritional status of the patient was improved by giving intravenous infusions of multivitamin injections and oral hematinics.

RESULTS

The cattle farmer came to the presented to the Government Medical College complaining of an intermittent fever that progressed to sensory deficit. He had intermittent fever, chills, and sweating for 15 days, followed by sensory deficit for seven more days before transfer to the hospital. On the day of initial admission, a blood smear stained with Giemsa showed numerous parasites with morphology typical of monomorphic, slender, flagellated, dividing, trypomastigote form trypanosomes. The fever disappeared and the general state of
the patient improved. Trypanosomes were not detected in the peripheral blood smear from 1 to 10 days after the initial hospital admission. A blood smear prepared during an additional febrile peak showed numerous trypanosomes (Figure 1). The parasitemia disappeared within 24 hours and reappeared for approximately 24 hours, 17 days after initial hospital admission, concomitantly with a new febrile peak. The patient again developed fever 54 days after initial hospital admission and trypanosomes were again detected in blood smears at this time. No parasite was found in blood, CSF, or bone marrow samples, which were collected following this presentation. The patient was discharged two months after initial admission without treatment, but continued to have intermittent fevers and parasites were again detected when he was examined as an outpatient. This prompted a second admission to the hospital five months after initial parasite detection (the second hospital admission occurred three months after the first admission). Blood smears again showed a high number of trypanosomes (105 days after initial hospital admission).

Morphologic examination of the trypanosomes indicated that the nucleus was in central position with a small kinetoplast visible at the posterior position. A careful examination of these morphologic characteristics indicated that the causative agent was *T. evansi*.†

Having diagnosed a highly unusual *T. evansi* infection, we made the decision to treat the patient with suramin using a regimen frequently used in the treatment of *T. b. rhodesiense* HAT. Results of parasitologic examinations were negative for blood collected 24 hours after the first, the second, and the last infusions of suramin. Analysis of CSF did not detect any trypanosomes, either before or after suramin treatment. Clinical examination showed no neck stiffness or focal neurologic deficit. Vital signs were stable and no icterus or swollen lymph node were detected. The spleen was just palpable. Results of cardiovascular and respiratory system examinations were normal. Abdominal ultrasonography showed a mild splenomegaly, but no intra-abdominal lymphadenopathy. A cerebral scan was normal. Electrocardiogram and two-dimensional Doppler echocardiography were within normal limits. A biopsy of inguinal lymph node was conducted and the examination showed non-specific reactive hyperplasia, but did not show any trypanosomes. A scar on a finger indicated previous healed injury sustained during farming (Figure 1).

Despite systematic examination, no *Plasmodium* parasites were found in any blood smear. Results of ELISAs for HIV and a Widal test were both negative, as were a series of additional tests for HIV conducted in France. The following biochemical results were obtained: total cholesterol = 108 mg/dL (2.81 mmol/L), high density lipoprotein = 38 mg/dL (0.988 mmol/L), low density lipoprotein = 66 mg/dL (1.72 mmol/L), apolipoprotein A1 (APO A1) = 103 mg/dL (normal = 110–205 mg/dL), apolipoprotein B (APO B) = 109 mg/dL (normal = 55–140 mg/dL), and APO A1 to APO B ratio = 1.05 (normal = 0.3–1). The result of the CATT/ *T. evansi* was strongly positive with whole blood and serum diluted up to 1:64. The result of the latex IgM test was positive at a CSF dilution of only 1:2, but clearly negative at a dilution of 1:8 dilution, which is considered as diagnostic for trypanosome invasion of the central nervous system (CNS). White blood cell counts in the CSF (at 0/mm³) were also not indicative of trypanosome invasion. Infected rodents, despite several blood examinations, remained trypanosome negative for 53 days after inoculation.

Primers expected to yield positive results for *T. evansi* DNA on 1:100 diluted blood samples showed that the patient was positive for this parasite in samples taken 39 and 55 days after initial hospital admission. For 1:1,000 diluted templates, two samples gave no amplification product. These were samples collected 9 and 55 days after initial hospital admission. For 1:8 dilution, which is considered as diagnostic for trypanosome invasion of the central nervous system (CNS). White blood cell counts in the CSF (at 0/mm³) were also not indicative of trypanosome invasion. Infected rodents, despite several blood examinations, remained trypanosome negative for 53 days after inoculation.

**Figure 1.** Trypanosomes in a blood smear of the patient stained with Giemsa (original magnification × 1,000). Inset, Scar (arrow) of wound on his right index finger.

**Figure 2.** Products of polymerase chain reaction amplification of the internal transcribed spacer (ITS) of trypanosome-specific ribosomal DNA using primers ITS1/2 and visualized on a 2% agarose gel stained with ethidium bromide. Lane 1, DNA ladder (AXX174 Hae III digest; Eurogentec, Liege, Belgium); lane 2, negative control; lanes 3–6, blood samples collected 9, 39, 55, and 72 days after the initial hospital admission of the patient (templates diluted 1:100); lanes 7–10, blood samples collected 9, 39, 55, and 72 days after the initial hospital admission (templates diluted 1:1,000); lane 11, *Trypanosoma brucei gambiense* reference strain Bat 61; lane 12, *T. evansi* reference strain CIRDES. The arrow indicates the expected band (392 base-pairs) for *Trypanozoon* characterization.
Trypanosoma evansi strain isolated from the patient needs to be effective against T. evansi. Treatment was successful because parasites could not be detected in the blood following treatment, and the general state of health of the patient improved. However, follow-up for at least a year (at months 3, 6, and 12), including blood and CSF serial examinations, is required to ensure no relapse.

This is the first report of a T. evansi infection of humans. Given the unusual nature of this infection, we determined whether the patient was in an immunocompromised state due to HIV infection. Four independent tests indicated no HIV infection. We also tested whether the patient may have had Tangier disease, a rare autosomal recessive genetic disorder. This was done because it has been postulated that the high density lipoprotein deficiencies associated with this disease could include loss of trypanosome lytic factors, such as APO A1, which is believed to be responsible for non-immune-mediated lysis of trypanosomes that do not express specific genes that protect against such lysis. However, the patient had normal levels of APO A1 and no other signs indicative of Tangier disease.

During the initial admission, a scar on one of the patient’s fingers was noted, which indicated a relatively recent injury that was possibly acquired during farming. It is conceivable that this injury occurred near the time of infection, in which case contact with infected animal blood as an occupational hazard associated with cattle farming may have been a route of infection. However, this is purely speculation and the route of infection is not presently known.

Infected laboratory rodents remained trypanosome negative for 53 days after inoculation, which is an unusual result. Although camels are the animal species most susceptible to experimental infection with T. evansi, infection, laboratory rodents are also suitable hosts for this species. The pathogenicity of the T. evansi strain isolated from the patient needs to be investigated. A variety of mammalian hosts are known to be susceptible to T. evansi infection, with many domestic and wild mammals vulnerable to infection. Some experimental infections of primates, such as Macaca rhesus or Orang utan, have been described, with each showing a similar clinical pattern (especially fever), which in the case of M. rhesus leads to death 38 days after inoculation. This indicates that humans and other primates could also be potential hosts. However, no human infection by T. evansi has been reported previously.

Previous reports from India and from Malaysia have identified trypanosomes similar to T. lewisi in peripheral blood of three patients with short febrile episodes. In all three patients, infection was only transient and the patients recovered without treatment. The present case is extraordinary in that these normally non-human infectious parasites survived and proliferated for at least five months in a human. It is now a priority to determine whether other people in the Chanderpur district and beyond harbor T. evansi infections and to learn how this parasite, hitherto believed to be exclusively a pathogen of animals, is transmitted.

The patient was treated with suramin, a drug used in treatment of early stage T. b. rhodesiense HAT and also known to be effective against T. evansi. Treatment was successful because parasites could not be detected in the blood following treatment, and the general state of health of the patient improved. However, follow-up for at least a year (at months 3, 6, and 12), including blood and CSF serial examinations, is required to ensure no relapse.

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