

Case Report: *Trypanosoma lewisi* or *T. lewisi*-like Infection in a 37-Day-Old Indian Infant

Archana Verma, Samiksha Manchanda, Nirmal Kumar, Archana Sharma, Masha Goel, Partha Sarathi Banerjee, Rajat Garg, Brahma Pal Singh, Fatima Balharbi, Veerle Lejon, Stijn Deborggraeve, Udai Veer Singh Rana, and Jacob Puliye¹*

Department of Paediatrics, St. Stephens Hospital, Delhi, India; Department of Pathology, St. Stephens Hospital, Delhi, India; Division of Parasitology, Indian Veterinary Research Institute, U.P., India; Institute of Tropical Medicine Antwerp, Belgium; National Centre for Disease Control, Delhi, India

Abstract. Trypanosomes were observed in the peripheral blood smear of a 37-day-old Indian infant admitted off feeds, with fever and convulsions. *Trypanosoma (Herpetosoma) lewisi* was identified in the blood. The species identification was confirmed by morphometry, polymerase chain reaction, and sequencing. Human infection with this organism is rare. Only seven cases of this infection have been reported previously in humans. The cases reported are reviewed to develop a composite picture of this disease.

INTRODUCTION

Trypanosomes are flagellated protozoan parasites infecting a wide range of animals and man. Human infection with *Trypanosoma brucei* (*T.b.*) *gambiense* or *T.b. rhodesiense* causes African sleeping sickness (African trypanosomiasis) and *Trypanosoma cruzi* causes Chagas disease (American trypanosomiasis). These infections have not been reported from the Indian subcontinent.

Other species of trypanosomes are known to affect animals in different parts of the world but human infection with them is rare. *Trypanosoma lewisi* is an infection of rats and there have been seven previous reports of human infection with the organism. Here, we report a case of a *T. lewisi* infection in a 37-day-old child from Bagpat, Uttar Pradesh in India. This infant is arguably the youngest case to be reported. The symptoms and treatment of the infant are discussed and the literature is reviewed. We also discuss the morphological identification of the parasite and its confirmation by molecular analysis.

CASE REPORT

A 37-day-old infant, resident of Bagpat, Uttar Pradesh, India, with no history of travel outside Uttar Pradesh was admitted at St. Stephens Hospital, Delhi, India, in August 2010, with pyrexia (going up to 39°C), poor feeding (anorexia), and lethargy for 1 day. On the day before admission, the child got up from his sleep in the afternoon, screaming. His mother went into the room and noticed three red spots on the leg. The area became indurated and inflamed, about 2 cm around the marks. The induration subsided in a couple of hours. The sting marks remained to form a scab (Figure 1). At that time his mother attributed the crying and sting marks to wasps. The child was cuddled and was consolable.

The next day he developed fever, seemed listless, and went off feeds. He then had several episodes of generalized tonic seizures for which he was brought to the hospital. Initially lorazepam at 0.1 mg/kg/dose, and later phenobarbitone (20 mg/kg/dose over 20 minutes, and repeated at 10 mg/kg over 10 minutes) followed by phenytoin (20 mg/kg/dose over 20 minutes) were administered in succession before the seizures were controlled.

On examination, the infant had no hepatosplenomegaly or lymphadenopathy. Investigations on admission were essentially normal (Table 1). The infant had hemoglobin 10.6 gm/dL, white blood cell counts 7,400/mm³, and platelet counts 102,000/mm³. Blood smear showed trypomastigote forms of *Trypanosoma* with the distinct subterminal kinetoplast, nucleus located toward the anterior half, and a flagellum arising from near the kinetoplast forming an undulating membrane before emerging free from the anterior end (Figure 2). Wet smear preparation showed many motile trypanosomes. Biochemical tests were unremarkable. Cerebrospinal fluid (CSF) was partially traumatic and showed normal cell counts and biochemistry but trypanosomes were seen in the specimen. The CSF examination was repeated after 24 hours. This sample was not contaminated by blood and did not show the parasite.

Specimens of blood on filter paper and blood thin smears were sent to two laboratories for confirmation of trypanosomiasis infection (Division of Parasitology, Indian Veterinary Research Institute (IVRI), Izatnagar, U.P. and Institute of Tropical Medicine, Antwerp, Belgium). On the basis of detailed morphologic characters as described previously and micrometry (average length 30.8 µm and width 1.9 µm) the eukaryote was tentatively identified as a member of the subgenus *Herpetosoma*, resembling *Trypanosoma lewisi*.

At IVRI, Izatnagar, DNA was extracted from 200 µL of the blood sample using the GENE AID Genomic DNA Mini Kit (Taipei, Taiwan). Polymerase chain reaction (PCR) was performed for amplification of the internal transcribed spacer 1 (ITS1) region, which is flanked by the 18S and 5.8S ribosomal genes, and this yielded an amplicon of 623 bp. This corresponds to the size of this region for *T. lewisi*.¹ Furthermore, the organism was experimentally inoculated intraperitoneally in laboratory-bred Swiss mice. The mice were negative for trypanosome up to the 28th day post-inoculation. *Trypanosoma lewisi* is host specific and fails to develop in mice.¹ A follow-up investigation was conducted at Bagpat by the National Center for Disease Control (formerly NICD), Delhi. Ten rats (*Rattus rattus*) were trapped from the surroundings of the patient's house and blood was examined both microscopically and by PCR at IVRI. Two of the 10 rats were also found to be infected with the same hemoflagellate.

At the Institute of Tropical Medicine Antwerp (Belgium), the World Health Organization (WHO) reference laboratory, DNA was extracted from the blood on filter paper using the QIAamp DNA micro kit (Qiagen, Valencia, CA). To check if DNA was successfully extracted from the blood specimens,

* Address correspondence to Jacob Puliye, St. Stephens Hospital, Tis Hazari, Delhi, India 110054. E-mail: puliyej@gmail.com



FIGURE 1. Sting marks seen on child's leg.

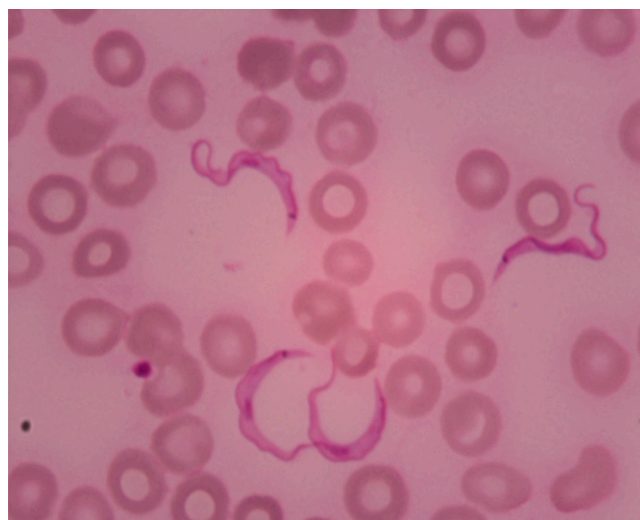


FIGURE 2. Magnification 100×.

a control PCR targeting the human beta-globin gene was performed. To identify the trypanosome, PCRs specific for the Trypanosomatidae,² *Trypanozoon*,³ *T. evansi*,⁴ and *T. lewisi*¹ were performed. The PCR for *T. evansi* and *Trypanozoon* were negative but it was positive for in the Trypanosomatidae. The length of the amplified ITS1 DNA sequence corresponded with that of *T. lewisi* described by Desquesnes and colleagues.¹ The ITS1 DNA sequence differentiates *T. lewisi* (amplicon of 623 bp) from *T. brucei* and *T. evansi* (amplicon of 520 bp). The ITS1 PCR product was sequenced at the University of Antwerp, Belgium. The DNA sequence was analyzed with the Bioedit software (Ibis Therapeutics, Carlsbad, CA) and aligned with the *T. lewisi* DNA sequence reported by Desquesnes and others using Multalin⁵ (see Box 1).

Sequencing results. The upper ends of the ITS1 PCR product could not be sequenced because sequencing was done directly on the PCR product. Sequencing similarity of 90% was observed.

Human trypanosomiasis is rare in India and the anti-*Trypanosoma* medication for this child (Pentamidine and Suramine) had to be sent from WHO, Geneva; this took 5 days. Pending availability of definitive treatment, the infant was started empirically on Liposomal Amphotericin B along

with Ceftriaxone as given in infants with clinical signs and symptoms of sepsis. Amphotericin B is an antifungal medication used also in *Leishmania* infections, which is endemic in India. The infant became asymptomatic after 3 days. The serial blood reports are shown in Table 1. Injection of Pentamidine was started on Day 5 of admission with cardiac monitoring. Blood sugar and blood pressure were also monitored. Ceftriaxone and Phenytoin were stopped on Day 10 of admission. Pentamidine was continued for a total of 10 days. The numbers of trypanosomes progressively decreased. On the seventh day of admission, peripheral smear did not show the parasite.

The child was discharged on 17/08/2010. At the followup examination on 4/10/10, the child was asymptomatic and showed no parasites on peripheral blood smear and had normal blood counts. His high-density lipoprotein (HDL) levels were estimated at this time and found to be normal.

DISCUSSION

We describe here a case of *T. lewisi* infection in a 37-day-old infant. There are only seven previously reported cases of *T. lewisi*-like infection in humans (Table 2). Infants seem to be

TABLE 1
Investigation reports

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Hemoglobin (g/dL)	9.7	7.7	9.8	7.9	8.0	8.6	5.8	7.4	9.2	8.2	9.1	8.0	8.6
Total leukocyte counts ($\times 10^3/\mu\text{L}$)	8.7	8.0	9.4	5.9	6.2	8.3	6.7	6.8	11.7	7.5	7.8	8.5	8.9
Platelet counts ($\times 10^3/\mu\text{L}$)	27	41	51	68	317	151	542	467	416	591	477	601	570
Peripheral smear remark	Tryp seen	Tryp seen	Tryp seen	Tryp seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen	Tryp not seen
Blood urea nitrogen (mg/dL)	17.4			3.9		12.7	6.7	4.5	5.2	5.2	6.1	6.3	8.9
Creatinine (mg/dL)	0.3			0.3		0.1	0.3	0.2	0.3	0.3	0.2	0.3	0.3
Sodium (mmol/L)	132			145		133	133	134	145	146	143	146	134
Potassium (mmol/L)	4.6			4.91		5.6	4.6	5.0	6.31	5.7	5.68	5.53	5.1
Calcium (mg/dL)	9.9							9.1		10.1		9.8	

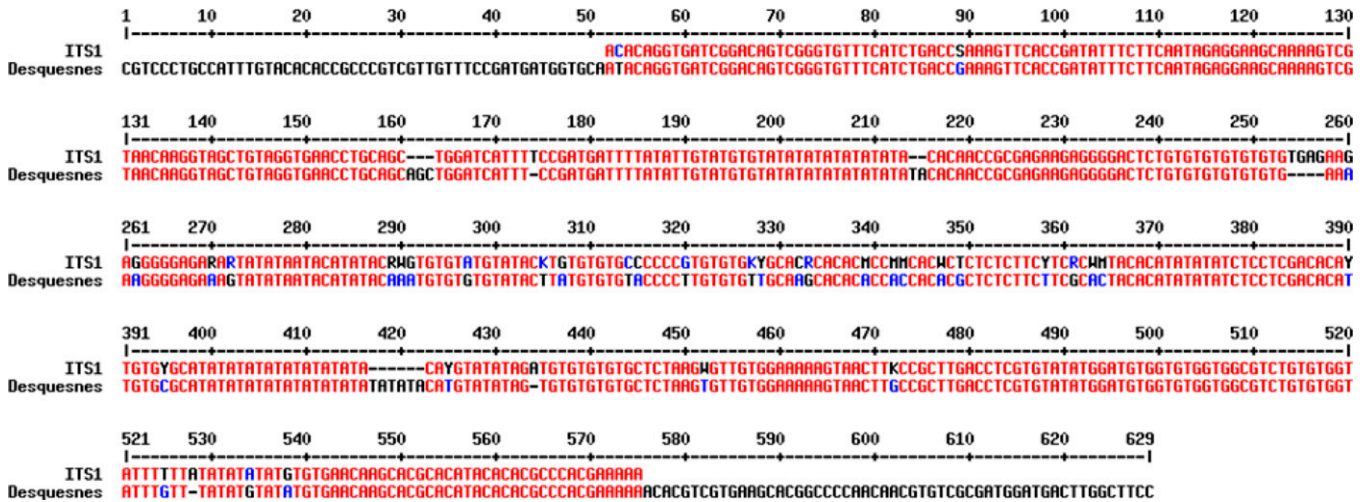
Cerebrospinal fluid (CSF) analysis: Day 1 – Trypanosomes seen in blood stained CSF.

Day 2 – No trypanosomes seen in CSF. No biochemical abnormality.

Tryp – *Trypanosoma*.

Box 1

Alignment sequence of the internal transcribed spacer 1 (ITS1) polymerase chain reaction (PCR) product from the child's blood and *T. lewisi* ITS1 product, Desquesnes and others.¹



Obtained sequence internal transcribed spacer 1 (ITS1) polymerase chain reaction (PCR) product sample ACACAGGTGATCGGACAGTCGGGTGTTTCATCTGACCSAAAGTTCACCGATATTTCTTCAATA GAGGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCCGAT GATTTTATATTTGTATGTGTATATATATATATATATACACAACCGCGAGAAGAGGGGACTCTGTGT GTGTGTGTGTGAGAAGAGGGGGAGARARTATATAATACATATACRWGTGTGTATGTATACT GTGTGTGCCCCCGTGTGTGKYGCACRCACACMCCMMCACWCTCTCTCTCTCYTCRCWMTACA CATATATATCTCTCGACACAYTGTGYGCATATATATATATATATATATACAYGTATATAGATGT GTGTGTGCTCTAAGWGTGTGTGAAAAAGTAACCTKCCGCTTGACCTCGTGTATATGGATGTGG TGTGGTGGCGTCTGTGTGGTATTTTTTATATATATATGTGTGAACAAGCACGCACATACACACG CCCACGAAAAA

more vulnerable to this infection. Five of the eight infections so far have been in infants. This might be attributed to immunologically naive status of this group of patients.⁶ Our infant is arguably the youngest case to be reported until now.

The first case of human trypanosomiasis reported from India was a case of *T. evansi* in a herdsman of village Seoni in Chandrapur district of Maharashtra in 2004.⁷⁻⁹ *Trypanosoma evansi* is also the most commonly occurring hemoflagellate of domestic animals in India. *Trypanosoma evansi* can be

cultured easily in mice within 3 to 5 days. Failure of development in mice is a good indicator that the organism, in all probability, is not *T. evansi*. *Trypanosoma evansi* can also be cultured in rats, but it takes more time to develop. *Trypanosoma lewisi* is more host specific and fails to develop in mice but often can be cultured in rats. Parasitemia is intermittent and rate of development depends upon the strain of rats used.

It was postulated earlier that a trypanolytic factor in human plasma prevents infection with zoonotic trypanosomes. Lack

TABLE 2
Blood smear showing Trypomastigotes*

Author year (reference)	Place	Age of patient	Presenting complaints	Method of identification	Treatment given	Outcome
Johnson, 1933 ¹²	Malaysia	4 months	Anorexia, lassitude, fever	Morphology in blood	None	Recovered
Shrivastava and others 1974 ¹³	Madhya Pradesh, India	35 years	Fever, lassitude	Morphology in blood Immune fluorescence antibody test Formol-gel test	None described	Recovered
Shrivastava and others 1974 ¹³	Madhya Pradesh, India	40 year	Fever, lassitude	Morphology in blood Immune fluorescence antibody test Formol-gel test	None described	Recovered
Howie and others, 2003 ¹⁵	Gambia	2 months	Fever, generalized edema	Morphology in blood and CSF PCR analysis	Melarsol- Prol	Recovered
Kaur and others, 2006 ¹⁴	Bombay India	2 months	Fever	Morphology in blood	None described	Recovered
Sarataphan and others, 2007 ¹¹	Thailand	45 days	Fever, cough, anorexia, depression	Morphology on blood smear dissimilar to <i>T. lewisi</i> . ITS1 sequence analysis and amplicon size similar to <i>T. lewisi</i> -like Herpetosoma	Injection gentamycin	Recovered
Banerjee and others, 2008 ⁶	Pune, Maharashtra	55 years	Intermittent fever, anorexia, pedal oedema, lethargy, splenomegaly, and hepatomegaly	Morphology similar to <i>T. lewisi</i> . ITS1 amplicon size similar to <i>T. lewisi</i>	Injection suramin	Died

* CSF = cerebrospinal fluid; PCR = polymerase chain reaction; ITS1 = internal transcribed spacer.

of this trypanolytic factor is seen in persons with low HDL levels and this can make a person vulnerable to trypanosomiasis.¹⁰ Our infant had normal HDL levels.

Fever and lassitude were the chief presenting complaints in all the cases reported previously. Four of the seven cases reported previously were identified only by morphology. The ITS1 PCR has been used for identification of human infection with *T. lewisi* in two cases so far. In one infant from Thailand, ITS1 PCR identified *T. lewisi* infection but the morphology of the organism was different,¹¹ and this suggests that the identification based on ITS1 PCR is not specific. The ITS1 PCR tallied with the morphology in our case. We further confirmed species by DNA sequencing.

In four cases no specific treatment was administered¹²⁻¹⁴; these cases recovered clinically and showed disappearance of trypanosomes from blood films. Follow-up did not show any relapses. However, a 55 year old, with the infection, died in spite of receiving Suramine.⁶ One case reported use of Gentamycin injections for treatment.¹¹ The Gambian infant was treated with Melarsoprol.¹⁵ Our infant responded symptomatically within 3 days of admission, while being administered Liposomal Amphotericin B and Ceftriaxone. The parasitemia however persisted albeit in reduced numbers up to Day 7. Pentamidine was started on Day 5, but in retrospect, it is difficult to say that it was needed for clearing the parasites. Specific treatment with anti-trypanosomal drugs (Melarsoprol and Suramine) was given in only two of the seven previously reported cases. It appears to be a self-limiting infection in humans. However, given the present evidence it will be prudent to prescribe antibiotics in an infant with clinical evidence of sepsis, even if *T. lewisi* is detected in the blood film. Aggressive anti-trypanosomal treatment is perhaps not indicated.

In rats, *T. lewisi* is an infection transmitted by the excreta of fleas through contamination of rat food or ingestion of fleas by the rats. The route of transmission to humans is unclear. Our child had bite marks over the left leg. We cannot say with certainty if these were flea bites transmitting infection to our child.

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Authors' addresses: Archana Verma, Samiksha Manchanda, and Nirmal Kumar, Department of Pediatrics, St. Stephens Hospital, Delhi, India, E-mails: dr_archanaverma@yahoo.com, manchanda_sami@yahoo.com, and nsk9_2000@yahoo.com. Archana Sharma and Masha Goel, Department of Pathology, St. Stephens Hospital, Delhi, India, E-mails: drarchna_sharma@yahoo.com and Mashagoel86@gmail.com. Partha Sarathi Banerjee, Rajat Garg, and Brahma Pal Singh, Division of Parasitology, Indian Veterinary Research Institute, U.P., India, E-mails: banerjeeeparth62@gmail.com, rajatgarg_2000@yahoo.com, and bpsingh_para@yahoo.co.in. Fatima Balharbi, Veerle

Lejon, and Stijn Deborggraeve, Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium, E-mails: fbalharbi@itg.be, vlejon@itg.be, and sdeborggraeve@itg.be. Udai Veer Singh Rana, National Centre for Disease Control, Delhi, India, E-mail: druvrsana@gmail.com. Jacob Puliyel, St. Stephens Hospital, Delhi, India, E-mail: puliyel@gmail.com.

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