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

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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 entered 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 microscopy (average length 30.8 µm and width 1.9 µm) the eukaryote was tentatively identified as a member of the genus *Herpetosoma*, resembling *Trypanosoma lewisi*.

At IVRI, Izatnagar, DNA was extracted from 200 µL of the blood sample using the GENEAID 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*. 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* host specific and fails to develop in mice. 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,
a control PCR targeting the human beta-globin gene was performed. To identify the trypanosome, PCRs specific for the Trypanosomatidae, Trypanozoon, T. evansi, and T. lewisi 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. 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 Multalin (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>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 15</th>
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</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.7</td>
<td>7.7</td>
<td>9.8</td>
<td>7.9</td>
<td>8.0</td>
<td>8.6</td>
<td>5.8</td>
<td>7.4</td>
<td>9.2</td>
<td>8.2</td>
<td>9.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Total leukocyte counts (×10³/µL)</td>
<td>8.7</td>
<td>8.0</td>
<td>9.4</td>
<td>5.9</td>
<td>6.2</td>
<td>8.3</td>
<td>6.7</td>
<td>6.8</td>
<td>11.7</td>
<td>7.5</td>
<td>7.8</td>
<td>8.5</td>
</tr>
<tr>
<td>Platelet counts (×10³/µL)</td>
<td>27</td>
<td>41</td>
<td>51</td>
<td>68</td>
<td>317</td>
<td>151</td>
<td>542</td>
<td>467</td>
<td>416</td>
<td>591</td>
<td>477</td>
<td>601</td>
</tr>
<tr>
<td>Peripheral smear remark</td>
<td>Tryp seen</td>
<td>Tryp seen</td>
<td>Tryp seen</td>
<td>Tryp not seen</td>
<td>Tryp not seen</td>
<td>Tryp not seen</td>
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<td>Tryp not seen</td>
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<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>17.4</td>
<td>3.9</td>
<td>12.7</td>
<td>6.7</td>
<td>4.5</td>
<td>5.2</td>
<td>5.2</td>
<td>6.1</td>
<td>6.3</td>
<td>8.9</td>
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<td>8.9</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Sodium (mmol/L)</td>
<td>132</td>
<td>145</td>
<td>133</td>
<td>133</td>
<td>134</td>
<td>145</td>
<td>146</td>
<td>143</td>
<td>146</td>
<td>134</td>
<td>146</td>
<td>134</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.6</td>
<td>4.91</td>
<td>5.6</td>
<td>4.6</td>
<td>5.0</td>
<td>6.31</td>
<td>5.7</td>
<td>5.68</td>
<td>5.53</td>
<td>5.1</td>
<td>5.53</td>
<td>5.1</td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>9.9</td>
<td>9.1</td>
<td>10.1</td>
<td>9.1</td>
<td>10.1</td>
<td>9.8</td>
<td>9.8</td>
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<td>9.8</td>
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</tr>
</tbody>
</table>

Cerebrospinal fluid (CSF) analysis: Day 1 – Trypanosomes seen in blood stained CSF. Day 2 – No trypanosomes seen in CSF. No biochemical abnormality. Tryp – Trypanosoma.
Table 2

<table>
<thead>
<tr>
<th>Author year (reference)</th>
<th>Place</th>
<th>Age of patient</th>
<th>Presenting complaints</th>
<th>Method of identification</th>
<th>Treatment given</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson, 1933&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Malaysia Madhya Pradesh, India</td>
<td>4 months 35 years</td>
<td>Anorexia, lassitude, fever Fever, lassitude</td>
<td>Morphology in blood Morphology in blood Immune fluorescence antibody test Formol-gel test</td>
<td>None None described</td>
<td>Recovered Recovered</td>
</tr>
<tr>
<td>Shrivastava and others 1974&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Madhya Pradesh, India</td>
<td>40 year</td>
<td>Fever, lassitude</td>
<td>Morphology in blood Immune fluorescence antibody test Formol-gel test</td>
<td>None None described</td>
<td>Recovered</td>
</tr>
<tr>
<td>Howie and others, 2003&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Gambia</td>
<td>2 months</td>
<td>Fever, generalized edema</td>
<td>Morphology in blood and CSF PCR analysis</td>
<td>Melarso- Prol</td>
<td>Recovered</td>
</tr>
<tr>
<td>Kaur and others, 2006&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Bombay India</td>
<td>2 months 45 days</td>
<td>Fever, cough, anorexia, depression</td>
<td>Morphology in blood Morphology on blood smear dissimilar to &lt;i&gt;T. lewisi&lt;/i&gt; ITS1 sequence analysis and amplicon size similar to &lt;i&gt;T. lewisi&lt;/i&gt;-like Herpetosoma ITS1 amplicon size similar to &lt;i&gt;T. lewisi&lt;/i&gt;</td>
<td>None None described Injection gentamycin</td>
<td>Recovered</td>
</tr>
<tr>
<td>Saratapan and others, 2007&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Thailand</td>
<td>55 years</td>
<td>Intermittent fever, anorexia, pedal oedema, lethargy, splenomegaly, and hepatomegaly</td>
<td></td>
<td>Infection suramin</td>
<td>Died</td>
</tr>
<tr>
<td>Banerjee and others, 2008&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Pune, Maharashtra</td>
<td>65 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup> 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.\textsuperscript{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 \textit{T. lewisi} in two cases so far. In one infant from Thailand, ITS1 PCR identified \textit{T. lewisi} infection but the morphology of the organism was different,\textsuperscript{11} and this suggests that the identification based on ITS1 PCR is perhaps not indicated.

It appears to be a self-limiting infection in humans. However, a 55 year old, with the infection, died in spite of receiving Suramine.\textsuperscript{5} One case reported use of Gentamycin injections for treatment.\textsuperscript{11} The Gambian infant was treated with Melarsoprol.\textsuperscript{11} 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 \textit{T. lewisi} is detected in the blood film. Aggressive anti-trypanosomal treatment is perhaps not indicated.

In rats, \textit{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 fleas bites transmitting infection to our child.

Received January 1, 2011. Accepted for publication April 30, 2011.

Acknowledgment: We acknowledge WHO (Dr. P. Simarro, World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, Geneva, Switzerland) for technical assistance. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

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