Atypical Human Infections by Animal Trypanosomes

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Abstract: The two classical forms of human trypanosomes are sleeping sickness due to Trypanosoma brucei gambiense or T. brucei rhodesiense, and Chagas disease due to T. cruzi. However, a number of atypical human infections caused by other T. species (or sub-species) have been reported, namely due to T. brucei brucei, T. vivax, T. congolense, T. evansi, T. lewisi, and T. lewisi-like. These cases are reviewed here. Some infections were transient in nature, while others required treatments that were successful in most cases, although two cases were fatal. A recent case of infection due to T. evansi was related to a lack of apolipoprotein L-1, but T. lewisi infections were not related to immunosuppression or specific human genetic profiles. Out of 19 patients, eight were confirmed between 1974 and 2010, thanks to improved molecular techniques. However, the number of cases of atypical human trypanosomoses might be underestimated. Thus, improvement, evaluation of new diagnostic tests, and field investigations are required for detection and confirmation of these atypical cases.

Introduction

Trypanosomes are protozoan parasites found worldwide, infecting humans, domestic and wild animals, most often transmitted by blood-sucking insects. The typical pathogenic human trypanosomoses are sleeping sickness, or human African trypanosomosis (HAT) [1], and the Latin American Chagas disease [2]. HAT is a fatal disease occurring in sub-Saharan Africa and transmitted by tsetse flies, caused by two subspecies of trypanosomes: T. brucei gambiense (the chronic form) or T. b. rhodesiense (the acute form) [1], which is derived from the animal parasite T. b. brucei that has acquired the ability to infect humans [1]. Chagas disease, caused by T. cruzi, is transmitted by triatomine bugs but also orally [3], congenitally, and via blood transfusion or organ transplantation [2]. The disease is endemic in Latin America and in most cases is chronic and asymptomatic [2]. In addition to these species, T. rangeli is also a human infective species, although considered nonpathogenic [1].

In contrast to these species or sub-species, most trypanosomes were thought to be infective only to animals, such as T. b. brucei, T. congolense, and T. vivax, the agents of the complex animal trypanosomosis called “nagana” in Africa. T. evansi is responsible for a widely distributed disease called “surra” in domestic and wild animals found in Asia, Africa, South America, and even Europe [4]. T. lewisi is a worldwide non-pathogenic parasite of rats transmitted by fleas [1].

Humans possess an innate protection against most Trypanosoma species [5]. However, 19 cases of atypical human trypanosomoses (a-HT) caused by T. b. brucei [1,6–8], T. vivax [1], T. congolense [9], T. evansi [10–13] and T. lewisi [14–21], which were all considered non-infective to humans, have been reported. In recent years, T. evansi and T. lewisi have emerged as potentially pathogenic for humans. While some of these cases reviewed herein were transient, six required trypanocidal treatments that were mostly successful, although two patients died [11,21]. Out of 15 humans cases recorded between 1974 and 2010, nine have been reported since 2003. Some cases were identified by microscopic observation of trypanosomes only and others using molecular tools, as described hereafter.

With the improvement of diagnostic techniques, in particular molecular assays, it is now easier to identify Trypanosoma species and, hence, investigate a-HT. Furthermore, the lack of awareness and sometimes difficult access to health care services reinforce the hypothesis that the number of cases of a-HT might be underestimated. Therefore, it was decided to review these cases of a-HT, leading to an international collaboration to further evaluate their actual occurrence.

Methods

References for this article were identified through PubMed searches for articles published from 1978 to 2011 using the terms “Trypanosoma”, “human”, “atypical”, “lewisi”, “evansi”, “congolense”, and “Herpetosoma”. Relevant books and articles published


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between 1933 and 2011 were selected through searches in the morphology of T. evansi microscopy reported so far are presented in Table 1, and protein AAI4340.1. RoTat 1.2 variable surface glycoprotein (SRA) CAD90580.1, GI34368410. ApoL1 gene ID 8542, ApoL1 2.1, EU599639.1, FJ011095.1. Human serum associated protein GU252216–GU252221, DQ345394.1, FJ011094.1, EU86119 specific primers), GU252222.1 (human infant case in Thailand), N The detection of a case of a-HT should be based on observation of the parasite by direct microscopy. Evaluating/improving the diagnoses through serological and PCR assays would help in detecting and identifying atypical trypanosomiasis infections in humans. These laboratory research and field activities are needed to evaluate the actual occurrence of atypical cases.

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**Molecular Assays to Confirm Atypical Human Cases of Trypanosomoses**

In 1998 a mixed infection with *T. brucei* spp. and *T. congolense* was detected in the blood of a patient (patient number 6) in Côte d’Ivoire. The latter trypanosome species was confirmed by PCR assays whereas *T. brucei* was only identified at the species level; therefore *T. b. gambiense* cannot be ruled out. The patient was successfully treated using pentamidine [9]. In 2005 in Ghana, a patient suspected of having HAT (patient number 5) who recovered without treatment was later confirmed to be infected with *T. b. brucei* by molecular analysis of archived blood slides [8]. The number of potentially similar cases on the African continent remains difficult to evaluate. Conventional diagnosis of HAT is based on microscopic detection of trypanosomes in blood, lymphatic fluid, and/or cerebrospinal fluid (CSF). This technique...
used in medical surveys does not allow discrimination between *T. b. brucei*, *T. b. gambiense*, and *T. b. rhodesiense*, since they are morphologically indistinguishable [1].

In 2004, the first molecularly confirmed case of a-HT caused by *T. evansi* was described in Seoni, India, in a farmer (patient number 9) showing a fluctuating trypanosome parasitaemia associated with febrile episodes over the course of 5 months [13]. The same signs were observed in a Sri Lankan patient in 1999 (patient number 8). Microscopical examination of blood smears showed high numbers of parasites confirmed as *T. evansi* by molecular techniques [23].
Additional examinations did not show central nervous system invasion by the parasites and the patient was successfully treated with suramin [24].

In 2003, trypanosomes were detected in blood and CSF of a Gambian baby presenting a severe clinical status with general oedema although no neurological abnormalities were found (patient number 15). PCR assays with primers flanking trypanosome ribosomal DNA internal transcribed spacer 1 (ITS1) resulted in amplicon of ~623 bp that corresponded to the expected size for T. lewisi and allied species (T. lewisi-like) [19], which are closely related species of the subgenus Herpetosoma; the amplified ITS sequence differed by just one nucleotide from T. lewisi [25]. The patient was successfully treated with melarsoprol [18]. Two other babies were reportedly infected with T. lewisi or T. lewisi-like. In 2003 in Thailand, trypanosomes were observed in blood from a 43-day-old infant (patient number 16) displaying fever, anaemia, cough, and anorexia. PCR (ITS1) confirmed the T. lewisi-like infection. The patient was treated with gentamicin [19]. In Bagpat India, 2010, a 37-day-old infant (patient number 19) with fever, anaemia, and leathargy showed T. lewisi on blood smears. Analysis of the DNA sequence (ITS1) amplified by PCR with this infant’s blood confirmed the species identification. The patient was treated using pentamidine for 10 days, and on the seventh day peripheral blood smear did not show any parasites [20]. In 2007, in Pune, India, an adult (patient number 18) was infected by T. lewisi detected by microscopy of blood smears and confirmed by PCR assay (not detailed in the publication). However, treatment with suramin had to be interrupted because of renal complications leading to the patient’s death [21]. This indicates prudence is required when managing a-HT. Toxicity of the drugs and parasite-related pathogenicity must be assessed.

Immunity of Humans to African Animal Trypanosomes

The natural immunity of humans to the livestock pathogen T. b. brucei, but not to the morphologically indistinguishable human pathogens T. b. gambiense and T. b. rhodesiense, is due to the selective killing of the parasite by normal human serum [NHS]. While the mechanism is still not known in the case of T. b. gambiense, a truncated protein named serum resistance-associated (SRA) appears to be the dominant factor responsible for resistance of T. b. rhodesiense to NHS [26]. Human innate immunity against African animal trypanosomes stems from the trypanolytic activity of the human-specific serum protein called apolipoprotein L-I (apoL-I), which is partially associated with high-density-lipoprotein (HDL) [5,27–29].

However, under certain circumstances, it would appear that T. congolense, T. vivax, and T. evansi can be resistant to human plasma [30–33]. The serum of an Indian patient (Table 1, number 9) infected with T. evansi presented a lack of trypanolytic activity due to frameshift mutations in both apoL-I alleles. Therefore, the lack of efficient apoL-I can explain this human T. evansi infection [34]. More isolates of T. evansi from various regions should be tested to check their sensitivity to NHS, and prevalence of apoL-I mutations should be investigated. Similarly, the trypanolytic activity of NHS from various geographical origins could be evaluated against a reference strain of T. evansi and the prevalence of the apoL-I deficit should be investigated in several human populations, to evaluate the potential risk of T. evansi infection in humans.
Immunity to T. lewisi and T. lewisi-Like Parasites

In contrast to T. b. brucei, little is known about the innate immune response that prevents the establishment of human infection by other trypanosome species. T. lewisi and T. lewisi-like species are in general highly host-restricted to rodents and lagomorphs. T. lewisi is primarily a parasite of Rattus spp. but little is known about the mechanisms involved in the highly host-species restriction of T. lewisi and related species and, consequently, why other animal species including primates are not naturally infected under normal conditions.

During the course of infection in the rat, T. lewisi produces two antigenic variants: the first represents the initial reproducing population and the second, the non-reproducing population. The reproductive forms are inhibited by ablastin [35] and the late population by antibody dependent cytotoxicity. The capacity to evade trypanocidal and ablastic antibodies and complement is crucial in the establishment of the infection. In rats infected with T. lewisi the parasitaemia normally resolves within 30 days. Thereafter, the rodents become immune to re-infection and complement does not appear to play a major role in this process [36,37]. However, human resistance mechanisms against infection by this species have not been investigated yet.

Investigations in Rodents

Following the detection of T. lewisi in a sick Thai infant (Table 1, patient number 16), T. lewisi infection in rodents was investigated to identify possible sources of human cases in Thailand. Blood samples from 276 rodents were tested with PCR (ITS1), and the trypanosome species identified by ITS1 sequence analysis. T. lewisi was detected in Rattus spp. (14.3%) and Bandicota spp. (18.0%). The ITS1 sequence from one sample from R. tanezumi showed 96.4% similarity compared to the sequence amplified from the blood of the T. lewisi-infected Thai infant [19,38]. Habitats where rodents were collected suggested that the degree of anthropization may influence the transmission of T. lewisi [39]. The hypothesis that T. lewisi can be transmitted from rats to non-human primates by rodent fleas arose from the finding of T. lewisi infection in captive monkeys infected by fleas, and living in poor conditions in rat-infested cages [37]. This hypothesis is corroborated by the fact that humans infected with T. lewisi also lived in poor dwellings certainly infested by domestic rats [19].

Improving Diagnostic Methods for a-HT

Several techniques such as IFAT, ELISA [40,41], and PCR-based methods [42] have been developed for the detection of trypanosomes in animals, especially for T. evansi. Serological techniques making use of crude antigens proved to have strong inter-specific cross-reactions [43]. They are therefore not consid-
ered as species-specific, although their genus specificity is satisfactory [43] and some improvements are expected for more specific antigens such as VSG Rotat 1.2 T. evansi [44]. Few of these techniques have been applied to a-HT.

In 2005, a serological survey was conducted using the Card Agglutination T. evansi Test for Trypanosomiasis/Trypanosoma evansi (CATT/T. evansi) [45] in the residential area of the patient number 9 (Indian case of T. evansi infection in Seoni [13]). Out of 1,806 individuals tested with CATT/T. evansi, 81 were positive using serum [46]. No trypanosome was detected in the blood of 60 persons positive to the test at a significant serum dilution (minimum 1:4). These results may suggest a frequent human exposure to T. evansi in the study area and possibly a frequent transmission of parasites to humans leading to transient infections in a “normal” immune population. The specificity of the CATT/ T. evansi test has not been previously evaluated for human screening and was used for the first time in this study in Asia. Consequently, this and other diagnostic techniques must be evaluated for screening a-HT in human populations, such as ELISA- T. evansi, and the immune trypanalysis test with T. evansi RoTat 1.2, which is considered to be highly specific for T. evansi infection in livestock but cannot detect some strains of T. evansi when the RoTat 1.2 VSG gene is missing [47]. So far, species-specific identification of Trypanosoma species can only be implemented by PCR with various sets of primers [48], or by sequence analyses of PCR products [37].

More recently, molecular tools were developed for the species-specific identification of T. lewisi by PCR or LAMP techniques [38,49]. These methods are under evaluation in several rodent species and might be useful in humans. Species-specific diagnoses will allow a better assessment of the prevalence of a-HT and a more accurate characterization of cases. They could also be useful to assess the results of potential treatments, with the goal of improving the management of any emerging a-HT infection.

Conclusion

The number of a-HT cases attributable to primarily animal trypanosomiasis is possibly underestimated. An international collaboration could help develop tools and strategies to better detect infection, identify the causative species, and manage new cases. The risk and potential impact related to a-HT cannot be evaluated thoroughly at the present time because diagnostic, clinical, and epidemiological data remain insufficient. Further studies are required to address relevant aspects of a-HT: (i) enhance awareness and detection in suspected areas where a-HT could be prevalent; (ii) real-time and detailed reports of a-HT including the clinical history of the patients; (iii) easy, sensitive, and species-specific methods for identification of the trypanosome; (iv) investigations on potential vectors, reservoirs (wild and domestic animals), and infection pathway. Therefore, it is perfectly justified to establish an international network to evaluate whether a-HT could be an emerging and neglected threat to human health.

References


