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Zoonotic trypanosomes in South East Asia: Attempts to control *Trypanosoma lewisi* using veterinary drugs

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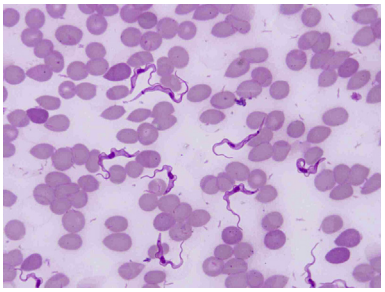
HIGHLIGHTS

- There is an increasing number of clinical reports of *Trypanosoma lewisi* infections in humans.
- The 4 veterinary trypanocidal drugs used were unable to cure *T. lewisi* infected rats.
- In rats infected by *T. lewisi* and *Trypanosoma evansi*, melarsomine hydrochloride cured *T. evansi* only.
- No veterinary trypanocidal drug proved to be efficient against *T. lewisi* in rats.
- Further investigations are needed to identify efficient drugs for *T. lewisi* control in humans.

GRAPHICAL ABSTRACT



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ABSTRACT

A growing number of atypical human infections due to the livestock parasite *Trypanosoma evansi*, or to the rat parasite *Trypanosoma lewisi*, are reported in humans in Asia. In some cases, clinical evolutions request treatments, however, so far, there were very few attempts to control *T. lewisi* using trypanocidal drugs. In a study published elsewhere, the efficacy of human trypanocides is evaluated in laboratory rats, and it concludes that none of them is able to cure rats experimentally infected with *T. lewisi*. Control of *T. lewisi* in rat would be a step for identification of drugs against this parasite. In the present study, 4 veterinary drugs: diminazene aceturate, isometamidium chloride, melarsomine hydrochloride and quinapyramine sulfate and chloride, were evaluated at low and high doses, in intra-muscular injections to normal rats experimentally infected with a stock of *T. lewisi* from Thailand. None of these treatments being efficient, a trial was also made using melarsomine hydrochloride in *T. evansi* infected rats and in mixed *T. lewisi* and *T. evansi* infected rats, in order to demonstrate the efficacy of the drugs under the present protocol. *T. evansi* was cleared from the rat's blood the day after the treatment, while, *T. lewisi* remained unaffected until the end of the experiment. These observations clearly demonstrated the efficacy of melarsomine hydrochloride against *T. evansi* and its inefficacy against *T. lewisi*. In conclusion none of the veterinary drugs was efficient against this stock of *T. lewisi*. Other protocols using higher

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doses or other drugs and *T. lewisi* stocks should be investigated in further studies. The control of *T. lewisi* infection in Wistar rats, using veterinary trypanocidal drugs, remains so far unsuccessful.

1. Introduction

Trypanosomes are flagellate parasitic protozoa of the family *Trypanosomatidae* (genus *Trypanosoma*) found in vertebrates. In mammals, they are mostly transmitted by biting insects acting as vectors. Several *Trypanosoma* species or sub-species are found both in humans and animals, they are zoonotic pathogens, while others are pathogenic to animals only, such as *Trypanosoma congolense*, *Trypanosoma brucei brucei*, *Trypanosoma evansi* and *Trypanosoma vivax*. Others are considered as non-pathogenic, such as *Trypanosoma theileri* (*Megatrypanum*) found in bovines and antelopes, or *Trypanosoma lewisi* and *T. lewisi*-like species (*Herpetosoma*), widely distributed in rodents (Hoare, 1972). Depending on the part of the digestive tract of the vector where the parasitic cycle is implemented, the *Trypanosoma* genus is subdivided into 2 sections: Salivaria, developing into the salivary glands, thus mostly transmitted by inoculation, to which belong most of the pathogenic trypanosomes, and, Stercoraria, developing in the posterior part, thus mostly transmitted by contamination, to which belong most of the non-pathogenic trypanosomes, at the exception of *Trypanosoma cruzi* a high zoonotic pathogen.

Typical human trypanosomoses are the sleeping sickness due to *T. b. rhodesiense* or *T. b. gambiense*, transmitted by tsetse flies in Africa, and Chagas disease due to *T. cruzi*, transmitted by triatomine bugs in Latin America. Atypical human trypanosomes are due to other *Trypanosoma* species, usually found specifically in animals, such as *T. evansi* or *T. lewisi* (Truc et al. 2013).

T. evansi is a livestock parasite derived from *T. brucei* lineage (Carnes et al. 2015); it is mainly mechanically transmitted by biting flies such as tabanids and stomoxes (Desquesnes et al. 2013). Several human cases have been reported, and the contamination is thought to occur by peroral or transcutaneous route, such as suggested in a case described in India (Powar et al. 2006). Control of *T. evansi* infection can be made using the same trypanocidal drugs used against sleeping sickness, since these parasites are very closely related (Joshi et al. 2006).

T. lewisi is a cosmopolitan parasite of rats, cyclically transmitted by flea, through the flea faeces. Rats are probably infected when rooming their fur or eating fleas. Eight *T. lewisi* infections were fully reported in humans (all in Asia, at the exception of one case in The Gambia (Truc et al. 2013); however more cases may remain undetected. In cases such as the one described in Thailand (Sarataphan et al. 2007), the clinical signs may justify the need for a curative treatment. In India, a patient infected by *T. lewisi* received suramine as treatment, however, the treatment had to be interrupted because of renal complications leading to the patient's death (Doke and Kar, 2011). The need to identify efficient trypanocides against *T. lewisi* appears then clearly. In a work recently published (Dethoua et al. 2013) fexinidazole was shown to be the best candidate amongst human trypanocidal drugs to cure cyclophosphamide-treated rats experimentally infected by *T. lewisi*. However, in a work published elsewhere, human drugs evaluated in experimentally infected rats proved to be inefficient against a Thai stock of *T. lewisi* (Desquesnes et al. submitted). Thus, it appears important and urgent to identify trypanocidal drugs able to control *T. lewisi*. Some veterinary drugs may be used in humans, such as diminazen aceturate, which has been extensively used in endemic countries against the early stages of the sleeping sickness (Bacchi, 2009).

The present work aimed at pre-identifying drugs for further mechanism study and/or potential use in humans for the control of *T. lewisi* infections. The four main veterinary drugs known for their significant activity against livestock pathogenic trypanosomes were evaluated. To determine the efficacy against a local stock of *T. lewisi* in experimentally infected Wistar rats, drugs were used as recommended by manufacturers, in single day administration protocols, using rodent adapted normal dose and double dose (low and high doses).

To validate the protocol with a known susceptible parasite, melarsomine hydrochloride, the most efficient trypanocide against *T. evansi*, was used to control *T. evansi* in single and mixed infections (*T. lewisi* and *T. evansi*) in Wistar rats.

2. Material and methods

Female Swiss mice (ICR) and Wistar rats were kept and used in our animal housing facility for more than 2 weeks before the experiments started. Animal care and experiments followed the "Ethical Principles and Guidelines for the Use of Animals" edited by the National Research Council of Thailand (1999) and agreed by the Ethical committee of the Faculty of Veterinary Medicine, Kasetsart University, Bangkok.

2.1. Stocks and production of parasites

A unique stock of *T. lewisi* (R6465) recently isolated from a wild rat (*Rattus tanezumi*) trapped in Kanchanaburi Province, Thailand (Pumhom et al. 2014) was used in this study, similarly to a previous study carried out on human trypanocidal drugs. This isolate was expanded in a laboratory rat and characterised by microscopic observation and PCR using pan-specific Trypanosomatid primers, TRYP1 (Desquesnes et al. 2002) and species-specific primers for *T. lewisi*, LEW1 (Desquesnes et al. 2011). It was kept in liquid nitrogen and grown once in a Wistar rat, by intraperitoneal (IP) injection of 150 µl of glycerol-cryopreserved parasites. Rats were followed-up daily by microscopic observation (magnification × 400 in dark ground conditions) of a drop of blood collected from the tip of the tail, and placed between slide and cover slide. When parasitaemia reached 15 trypanosomes/field (an estimated parasitaemia of 60 million trypanosomes/ml of blood), the animal was anaesthetized with a combination of tiletamine and zolazepan (Zoletil 1001 Virbak, Glattbrugg, Swiss), the blood was collected on 3.2% citrate buffer and suspended into 1% glucose-Phosphate saline buffer (PSG1%), to a concentration of 10^6 parasites/ml, prior to inoculation to experimental rats.

For *T. evansi* protocol and mixed infection protocol, a unique stock of *T. evansi*, isolated in a mouse from a horse in Ratchaburi Province, Thailand, in 2011 (TeHRCH11) was used. This isolate was expanded in a laboratory rat and characterised by microscopic observation and PCR using pan-specific Trypanosomatid primers, TRYP1 (Desquesnes et al. 2002), sub-genus specific primers for Trypanozoon, TBR (Masiga et al. 1992) and pMUTEC F/R (Wuyts et al. 1994), and species-specific primers for *T. evansi*, TEPAN (Panyim et al. 1993) and TE2249/50 (Artama et al. 1992). It was kept