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Attempted Transmission of *Trypanosoma evansi* to Rats and Mice by Direct Ingestion of Contaminated Blood and via Engorged Ticks

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Summary. *Trypanosoma evansi* is a blood parasite principally transmitted by mechanical vectors (tabanids and stable flies) in large animals such as livestock. However, in other types of hosts, such as carnivores and rodents, oral transmission may be more important. In this experiment, attempts were made to infect rats and mice by the peroral route using infected blood, and ticks engorged on infected rats, in order to evaluate the potential role of ticks as passive vectors of trypanosomes. A strain of *Trypanosoma evansi* isolated from a cow in Thailand was grown in a rat and blood was collected at the peak of parasitaemia. In the first experiment, 5 rats and 5 mice were fed respectively with 1 ml and 0.5 ml of blood containing 10^7 *Trypanosoma evansi*/ml. In the second experiment, adult ticks belonging to the species *Rhipicephalus sanguineus*, which had fed on parasitaemic rats, were given as food to 3 healthy rats. For both experiments, the presence of parasites in the blood of the rats and mice was checked daily for 10 days, then every 2 days for the following 20 days. Within an average of 4.5 days post blood ingestion (from 4 to 5), 80% ($CI_{95\%}$ 29–99) of the rats exhibited parasites by direct microscopic examination of the blood. Similarly, with an average of 4.7 days post ingestion (from 4 to 6), 60% ($CI_{95\%}$ 15–95) of the mice exhibited blood parasites. After tick ingestion, no parasites were found in the blood of the rats fed with infected engorged ticks. Consequently, in this experiment, as in others, rats and mice appeared to be receptive by the oral route, but the possible role of ticks as a passive vector could not be demonstrated. Other models could be explored, involving the cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*), to investigate the link from large to small animals.

Key words: *Trypanosoma evansi*, peroral transmission, rats, mice, ticks, *Rhipicephalus sanguineus.*

INTRODUCTION

Trypanosoma evansi is a blood parasite causing a disease called "surra" which is the most widespread trypanosomosis in the world, mainly affecting camels, horses, buffaloes, cattle and carnivores such as dogs (Hoare 1972). In herbivores, *T. evansi* is usually transmitted by biting flies, such as tabanids or stable flies, but, in South America it can also be transmitted by vampire bats (*Desmodus rotundus*) that can be considered as biological vectors (Hoare 1965, Desquesnes 2004). Vampire bats initially catch the infection by ingesting blood from contaminated animals such as horses, by

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trans-mucosal penetration of the parasite (mucosa of mouth, oesophagus and/or stomach) (Hoare 1965). The initial infection of vampire bats by the oral route when ingesting contaminated blood from the host is the best example of the oral transmission of *T. evansi*. However, this is not the only known oral contamination. It has also been reported that dogs, and more generally carnivores, can contract the disease by eating the meat of animals infected with *Trypanosoma brucei* (Moloo *et al*. 1973), as well as with *T. evansi* (Curasson 1943, Bhaskararao *et al*. 1995, Desquesnes 2004). Under natural conditions, *T. evansi* is found in large rodents in Latin America (Franke *et al*. 1994, Arias *et al*. 1997), but also in small rodents in Asia (Jittapalapong *et al*. 2008). Among rodents, oral contamination seems to be more probable than transmission by biting flies, as such flies feeding on large herbivores are not attracted by small rodents, which are most often nocturnal. Experimentally, oral transmission has been achieved with success in dogs and mice by allowing them to feed on infected meat and blood (Raina *et al*. 1985). In an attempt to understand how rodents can become infected and possibly act as a reservoir for the parasite in Thailand, our study was undertaken to investigate the oral route of contamination in rodents with a *T. evansi* strain from Thailand, using infected blood and experimentally infected ticks.

MATERIALS AND METHODS

The first experiment set out to demonstrate that crude *T. evansi*-infected blood was infective to rats and mice by the peroral route. The second experiment set out to demonstrate that ticks having fed on *T. evansi* parasitaemic rats could be passive vectors of the infection when eaten by rodents.

Production of *T. evansi*-infected rat blood

A strain of *T. evansi* was isolated on a farm in Bangkok, from cattle exhibiting high parasitaemia (10^7 trypanosomes/ml). After one passage in a Wistar rat, the infected blood was supplemented with 15% PSG-glycerol (1:1) and cryopreserved in liquid nitrogen pending the experiment. Four days before the beginning of the first experiment, a Wistar rat was inoculated with this strain by intraperitoneal injection of 0.5 ml of the cryopreserved parasites. On the day of the experiment, when a peak of parasitaemia (10^7 trypanosomes/ml) occurred, the rat was anaesthetized and the blood was collected on ice in citrate tubes prior to blood-feeding of the rodents.

Production of ticks engorged with *T. evansi*-infected blood

Five batches of thirty adult *Rhipicephalus sanguineus* ticks obtained from the breeding unit at Kasetsart University, Bangkok, were dropped onto the backs of 5 Wistar rats, inside a covering body cloth to protect the ticks until fully engorged; the rats were inoculated with 10^7 *T. evansi* 2 days after the adult ticks were dropped onto their backs (5 days before the adult ticks were expected to be engorged).

Feeding rats and mice with contaminated blood (first experiment)

Ten Wistar rats (*Rattus norvegicus*) and ten ICR mice (*Mus musculus*), from a breeding unit free of trypanosomes, were used for the experiment. The rats and the mice were randomly divided into four experimental groups: groups A and B contained five rats each and groups C and D contained 5 mice each. Three days before the beginning of the experiment, all the animals were checked for trypanosomes by direct microscopic observation of a drop of blood between slides and cover-slides under dark ground conditions (magnification × 400) and by the haematocrit centrifuge technique (Woo 1969). All the animals tested negative.

On Day 0 of the experiment, rats from group A received twice 0.5 ml of infected blood as food by the oral route, at 10-minute intervals. The blood was dropped into their mouths using a sterilized 1 ml syringe without the needle. Similarly, mice from group C received twice 0.25 ml of infected blood, at 10-minute intervals. Once all the meals were given, the mobility of the parasites in the citrate tube was checked to make sure the parasites were still alive. Groups B and D did not receive anything and were used as control groups. Each group was bred separately under healthy conditions, and food and water were given *ad libitum*.

Feeding rats with contaminated ticks (second experiment)

For three days (from day 7 to day 9 after adult ticks were dropped onto the rats), engorged ticks were detached by hand from the rats and given as food to three healthy Wistar rats in a cage temporarily without bedding in order to ensure that the ticks were ingested. Parasitaemia in the infected rats was checked for daily from day 7 to day 9. On day 8, two engorged ticks were dissected and the presence of living trypanosomes in their gut was evaluated by microscopic observation of the gut content under dark ground conditions (magnification × 400) between slides and cover-slides.

Follow-up of the animals

Post-ingestion, the presence of parasites in the blood of the rats and mice was checked for daily on the first 10 days, then every 2 days for the next 20 days. This was done by direct microscopic observation of a drop of blood between slides and cover-slides under dark ground conditions (magnification × 400), up to the appearance of more than 20 trypanosomes per field. At that stage, stained blood smears were prepared for morphological observation of the parasites. When the animals were still negative on day 30 post-ingestion, the presence of parasites in their blood was assessed by the haematocrit centrifuge technique (Woo 1969). At the end of the experiment, healthy animals were kept in healthy conditions while infected animals were euthanized. The presence of any oral lesions was assessed during post-mortem examinations. Although group sizes were small, we tried to highlight any statistical differences in the infection success rates using the two-sided Fisher's exact test computed with the free software R (R Development Core Team 2008).

Table 1. Experimental infection success rates among rats and mice by oral contamination with infected blood or infected ticks.

Source of infection	Host	Sample size	Number of infected	Infection success rate ($CI_{95\%}$)
Infected blood	Rats	5	4	80% (29–99)
Infected blood	Mice	5	3	60% (15–95)
Infected blood	Rats + mice	10	7	70% (35–93)
Infected ticks	Rats	3	0	0% (0–71)

RESULTS

For the first experiment, although some blood was not fully ingested, especially in the mice where it sometimes poured out the side of the mouth, most of the blood administered was ingested by the experimental animals. In group A, two rats displayed parasites in their blood four days post-ingestion (p-i), and two others five days p-i. Seven days p-i, the four rats exhibited parasitaemia exceeding 10^6 trypanosomes/ml. At that stage, stained blood smear observations confirmed the identity of the parasites, as they exhibited the typical morphology and morphometry of the subgenus Trypanozoon, as described by Hoare (1972). The last rat remaining negative over the thirty days of observation, the infection success rate in rats could be evaluated at 80% ($CI_{95\%}$ 29–99). In group C, parasites were observed four days p-i in two mice, and six days p-i in another. Eight days p-i, the three mice exhibited parasitaemia exceeding 10^6 trypanosomes/ml. The other two mice remained negative over the thirty days of observation, leading to an infection success rate in mice of 60% ($CI_{95\%}$ 15–95). At thirty days p-i, the blood of the rat and the 2 mice remaining negative were proved to be negative with the haematocrit centrifuge technique. In control groups B and D, parasites were never observed up to the end of the experiment. For any of the infected animals, no obvious oral lesions could be observed during post-mortem examinations. The global infection success rate in rodent while ingesting infected blood could thus be evaluated at 70% ($CI_{95\%}$ 35–93).

For the second experiment, 5 engorged ticks were collected daily for three days from each of the five experimentally infected rats. At the time, these rats displayed parasitaemia exceeding 10^5/ml. The dissection of two engorged infected ticks carried out on day 8 proved that parasites were present in the gut, alive but in low concentrations (around one living parasite per field at a magnification of × 400) and that their movements were slow. Under these conditions, 25 infected ticks were given daily as food to the three healthy rats on three consecutive days. When the healthy rats were given the engorged ticks, they ingested them quite easily in less than 2 minutes. Conditions for transmission were then considered to be gathered. However, all three rats remained negative for the 30 days of observation, leading to an infection success rate of 0% ($CI_{95\%}$ 0–71). These results are recorded in Table 1. The four different infection success rates were compared using the two-sided Fisher's exact test, and proved not to be statistically different from each other as reported in Table 2.

Table 2. Results of the two-sided Fisher's exact test for comparing infection success rates.

Population 1	Population 2	*p*-value
Rats fed with blood	Mice fed with blood	1.00
Rats fed with blood	Rats fed with ticks	0.14
Mice fed with blood	Rats fed with ticks	0.20
Rats and mice fed with blood	Rats fed with ticks	0.07

DISCUSSION

The results of the first study showed that oral transmission of *Trypanosoma evansi* was very easy in the rats with 80% ($CI_{95\%}$ 29–99) of successful attempts, and occurred very rapidly even in the absence of any oral lesions. Indeed, the incubation period was very short (4.5 days) and was no different from that generally observed in intra-peritoneal inoculation of parasites. In the mice, the success rate was 60% ($CI_{95\%}$ 15–95) contradicting the results of da Silva *et al.* (2007), who were not successful in infecting mice by the oral route, although it was possible for rats. Conversely, Raina *et al.* (1985) observed 100% infection by the ingestion of meat in 6 dogs and 90% in 10 mice. Consequently, our find-

ings are another argument in support of the hypothesis of Raina *et al.* (1985), who stated that *T. evansi* could reach blood capillaries by passage through the buccal mucosa. We can therefore conclude that oral infection of rats and mice is possible with this Thai isolate, although it was not possible to highlight any differences in the infection success rate as group sizes were too small. Other *Trypanosoma* species have been shown to be transmitted by the oral route, not only that of the Trypanozoon subgenus such as *Trypanosoma brucei* (Moloo *et al.* 1973), but also some of the Herpetosoma subgenus such as *Trypanosoma lewisi*, *Trypanosoma microti*, *Trypanosoma evotomys* and *Trypanosoma grosi* (Maraghi *et al*. 1995), and some of the Schizotrypanum subgenus such as *Trypanosoma cruzi* (Coura 2006). The latter may even survive for a while in fruit juice, regularly inducing human oral infection in Brazil and Venezuela (Cardoso *et al.* 2006).

Attempts to infect naive rats by feeding them with ticks engorged on infected rats was not successful. Another 2 experiments (not published) were carried out, but the parasitaemia in feeder rats was rarely high at the time of the final engorgement of the ticks. For these reasons, although we can conclude that such passage is of low probability, it cannot be definitively ruled out that ticks may act as passive carriers and be a possible source of oral infection in rodents or other animals.

In Thailand, surveys among wild rodents suggest that they are naturally infected by *T. evansi* (Jittapalapong *et al*. 2008), giving rise to the hypothesis that rodents may act as a reservoir for these parasites found in livestock. However, the nocturnal activity of rodents prevents them from being bitten by tabanids and stable flies. To our knowledge, the route of infection in rodents still remains unknown. The first results of this study, like those obtained by Raina *et al.* (1985) and da Silva *et al.* (2007), suggest that wild rodents can be infected by ingesting trypanosomes from contaminated materials, such as infected blood, fresh dead animals, scraps or placenta. However, our second experiment could not confirm the hypothesis of oral transmission by eating ticks engorged on infected hosts, although it cannot exclude it either. Consequently, the possible epidemiological link between small mammals and livestock is still unconfirmed and uncertain. However, experiments with the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* might be more conclusive as regards the link from cattle to rodents, as final engorgement in this tick species is faster than for *Rhipicephalus sanguineus* in rats, which could more effectively preserve the infectivity of trypanosomes in the tick's gut.

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