Oral route of transmission: *Trypanosoma evansi* in a mice model experiment

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Abstract Twelve Swiss albino mice of either sex and equal body weight were randomly divided in 2 groups (I and II), consisting of 9 and 3 mice respectively and were used to conduct the study. A dose of 2.5×10^4 number of *Trypanosoma evansi* was instantly fed to each mouse of group I. Each mouse of group II was inoculated intraperitoneally with same dose of parasites through infected mice blood and kept separate. The tail blood of each mouse was examined daily up to 30 days post infection by examination of wet blood film and Giemsa-stained blood smears for presence of any trypanosomes. Out of 9 mice of group I those were infected orally, 3 (33.33%) mice became positive for presence of *T. evansi* both by examination of wet blood film and Giemsa-stained blood smears after 4, 6 and 7 days post infection. After 2 days post infection all intraperitoneally infected mice were found positive for *T.*

evansi. Thus incubation period in orally infected mice was longer than the intraperitoneally infected mice. All the positive mice of both the groups died with high parasitaemia after 3–4 days of first appearance of parasitaemia. From the present study, it can be concluded that besides mechanical or parenteral means of transmission, *T. evansi* could also be transmitted through oral route. Thus zoo carnivores might be infected with *T. evansi* and develop disease by eating infected blood or flesh of the infected animals, as a prey and predator relationship.

Keywords Mice · Oral · Transmission · *Trypanosoma evansi*

Introduction

Trypanosoma evansi, an intercellular haemoprotozoan parasite is the causative agent of the disease ‘surra’ in livestock. This disease is common and widely prevalent amongst livestock in India (Laha et al. 2004; Laha and Sasmal 2008a, b; Shahardar et al. 2008). Human trypanosomiasis caused by *T. evansi* has been reported first time from an Indian farmer at Maharashtra State (Joshi et al. 2005) and the parasite has also been established for the first time in pigeon nestlings (Mandal et al. 2008) suggested its adaptation in unnatural hosts. Demonstration of the parasite in various organs and in blood of the suspected animal could be of interest for diagnosis of the *T. evansi* infection in dead animals (Mandal et al. 2014). The disease is responsible for considerable economic losses in tropical and subtropical countries. Cattle and buffaloes mostly suffer from chronic form of the disease, causing of low productivity as well as draught power resulting into huge economic losses to the livestock industry. Equines, camels

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and dogs are highly susceptible to the disease and suffer from acute as well as chronic form and nearly always fatal if treatment is not given. The transmission of *T. evansi* among animals has been occurred mechanically by haematophagus flies mainly *Tabanus* spp. (Gill 1991). But there are occasional reports of transmission of the disease ‘surra’ among carnivores of the zoo. It is not still confirmed in those doubtful cases whether the transmission has been occurred by the haematophagus flies or by other means like through oral routes with the meat supplied to those animals. Hence, in the present study an attempt has been made to observe whether oral route of transmission of the parasite is possible.

Materials and methods

Twelve healthy Swiss albino mice were procured from Institute of Animal health and Veterinary Biologicals (R&T), Belgachia, Kolkata for the present study. These mice were kept in mice cages and were fed with bread, vegetables and water carefully to avoid any injury to GI tract throughout the experiment. They were randomly divided in 2 groups (I and II), consisting of 9 and 3 mice, respectively, and then numbered for identification. From a naturally infected horse, *T. evansi* was isolated and maintained by sub-inoculation in Swiss albino mice. At the height of parasitemia (5×10^6 parasites per ml of blood), tail blood of infected mice was collected and immediately diluted with Alsever’s solution (pH-7.4). The mice of group I was orally given with diluted blood containing approximately 2.5×10^4 parasites drop by drop with plastic dropper. This type of feeding practice was made very carefully and gently to avoid any tearing or injury to the oral cavity or GI tract of mice. The same doses of parasites were inoculated intraperitoneally into mice of group II and maintained as infected control group. These two groups of mice were kept separately under careful observation for 30 days. Tail blood of all the infected mice of these two groups was examined daily by wet blood film (WBF) method and Giemsa-stained blood smears (GSBS) up to a period of 30 days post infection (PI) for detection of infection. At least 2 blood smears (both stained and wet) per mouse were prepared and examined daily under microscope for detection of the parasites.

Ethical guidelines

Approval and necessary guidelines of Institute Animal Ethics Committee (IAEC) was obtained and followed during animal experimentation in this study.

Results and discussion

Observations on transmission of *T. evansi* through oral route have been presented in Table 1. It has been observed from the table that a total of 3 (33.33%) mice of group I, those were infected orally became positive both in WBF and GSBS. After 2 days PI, the mice of group II found positive both by examination of WBF and GSBS. The incubation periods were totally different for each orally infected positive mouse of group I like 4, 6 and 7 days post feeding of infected blood. On the other hand, the incubation period was 2 days in case of all 3 mice of group II. In case of all positive mice, the GSBS showed gradual increase of parasitaemia (‘++’ to ‘++++’) i.e. 5–9 parasites/field to more than 15 parasites/field in 1000× of infected mice till death. So level of parasitaemia produced by each of 3 positive mice of group I was similar like group II mice. The clinical signs i.e. jerky and encircling movements showed by these 3 mice of group I just before death were also similar like mice of group II. Two orally infected mice died with high parasitaemia after 3 days of first appearance of parasitaemia and rest one mouse also died with similar parasitaemia after 4 days of first appearance of parasitaemia, like intraperitoneally infected mice. Thus, except the variation in incubation period, no difference was observed in intraperitoneally and orally infected mice. So, it has been observed from this experiment that 33.33% mice were positive for *T. evansi* infection through oral route. It has been confirmed that *T. evansi* could be transmitted through oral route other than mechanical transmission through the vectors.

Generally *Trypanosoma evansi* is transmitted by mechanical means through the bites of haematophagus flies like *Tabanus* spp., *Stomoxys* spp. etc. (Gill 1991). In the field condition, some suspicions remain whether only this mechanical vector transmission is the only means of transmission. In the present study it has been found that 33.33% mice became positive for *T. evansi* infection through oral route by feeding of infected blood and 66.66% mice remained negative both in WBF and GSBS. Thus, it could be mentioned that sometimes *T. evansi* could be transmitted through oral route but not always as observed earlier (Raina et al. 1985). The incubation period of orally infected *T. evansi* positive mice was longer compared with intraperitoneally-infected mice as reported earlier in case of other species of trypanosomes (Maraghi et al. 1995). It might be due to that most of trypanosomes go to stomach after oral feeding and after exposure in acidic environment of stomach; many of the parasites might have died. A few live trypanosomes are able to penetrate the digestive tract or buccal mucous membrane and then enter into circulation. So, longer time is required for flare up of *T. evansi* in

Table 1 Transmission of *Trypanosoma evansi* through oral route and their infectivity

Group	Mice no.	Route	Dose	Results after examination by		Incubation period	Death ^a of mice
				WBF	GSBS		
I	1	Oral	2.5×10^4	Negative	Negative	–	–
	2	Oral	2.5×10^4	Positive	Positive	4 days	3 days
	3	Oral	2.5×10^4	Negative	Negative	–	–
	4	Oral	2.5×10^4	Positive	Positive	6 days	3 days
	5	Oral	2.5×10^4	Negative	Negative	–	–
	6	Oral	2.5×10^4	Negative	Negative	–	–
	7	Oral	2.5×10^4	Negative	Negative	–	–
	8	Oral	2.5×10^4	Positive	Positive	7 days	4 days
	9	Oral	2.5×10^4	Negative	Negative	–	–
II	10	i.p.	2.5×10^4	Positive	Positive	2 days	3 days
	11	i.p.	2.5×10^4	Positive	Positive	2 days	4 days
	12	i.p.	2.5×10^4	Positive	Positive	2 days	3 days

i/p intraperitoneally, WBF wet blood film, GSBS Giemsa stained blood smear

^a After first appearance of parasitaemia

blood circulation. Hence, incubation periods were longer after oral feeding of blood. Further, among all the orally infected animals, different incubation periods were observed due to personal variation and relative condition factors in relationships between parasites and their hosts (Gupta et al. 2016). Followed by penetration of the digestive tract and after reaching the blood circulation, binary fission of *T. evansi* took place as like intraperitoneally inoculated parasites. Hence, the level of parasitaemia, clinical signs and patent period showed by these 3 orally infected mice were similar like intraperitoneally infected mice.

So, on the basis of present investigation it is concluded that besides mechanical transmission, *T. evansi* may also be transmitted through oral route some times but not always, thereby the disease on eating infected blood or flesh of the animals might occur in carnivores.

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