Experimental studies on survivality and degenerative changes of *Trypanosoma evansi* after death of host

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Abstract Twenty adult Swiss albino mice, 20 rats and 10 rabbits were artificially infected with *Trypanosoma evansi* and killed at the peak of parasitaemia to know the period of survivality of *T. evansi* and degenerative changes of the parasite after death of these hosts. Examination of Giemsa stained blood smears and wet blood smears revealed the presence of parasites and live trypanosomes along with motility in the heart blood of mice and rats up to 14 h and in rabbits up to 13 h post death. Mouse inoculation test (MIT) conducted with heart blood up to 13 h post death of mice and rabbits became positive. MIT with both heart blood and portal blood of rats became positive up to 14 h post death. The liver and lung impression smears could detect the parasites up to 14 h of death of mice and rats and up to 13 h post death of rabbits whereas spleen impression smears revealed the presence of parasites up to 12 h post death of these animals. It is confirmed that *T. evansi* infection in animals may be diagnosed after post mortem examination of hosts by demonstration of parasites.

Keywords *Trypanosoma evansi* · Survivality · Degenerative changes · Death · Host

Introduction

Trypanosoma evansi, the causative agent of a disease in animals, popularly known as ‘surra’. The disease is not

only restricted to animals, because a case of human trypanosomiasis caused by *T. evansi* has been reported from India (Joshi et al. 2005). The disease can be diagnosed in live animals by parasitological, biochemical and molecular methods. Due to non-specific gross lesions of the disease, diagnosis of the parasite after post mortem examination in suspected animals is very difficult. 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. Besides, the disease is reported to transmit mechanically by haematophagus flies (Juyal 2007). Carnivore animals may be infected after ingestion of the infected meat (Raina et al. 1985). Although there are reports of histopathological changes in laboratory animals due to *T. evansi* infections (Da Silva et al. 2012; Bal et al. 2012), but very little information is available as how long parasites remain viable and demonstrable in various organs and in blood following death of the host. Thus, there is a need for detailed study on viability period of *T. evansi* in the host body after death, as well as nature of changes in *T. evansi* in host body after death. With this aim, the present study was conducted to determine the length of parasite survivability and the nature of possible degenerative changes of the parasite itself in experimentally infected mice, rats and rabbits after death of these hosts.

Materials and methods

Experimental animals and method of infection

Twenty adult Swiss albino mice, 20 rats and 10 rabbits were procured for the experiment. They were maintained on balanced ration during the entire period of observation.

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Mouse and rat were experimentally infected through intraperitoneally (IP) and rabbits were infected through subcutaneous route with 0.5 ml of blood diluted with Alsever's solution containing approximately 2×10^6 *T. evansi*. Blood from tail of infected mice and rat and from ear vein of rabbit were examined daily by wet blood film method to determine the extent of parasitaemia.

Time of experimental animal death

Mice were killed on 3rd day of inoculation when the level of parasitaemia was found to be high (approximately 4 to 6×10^6 parasites ml^{-1} of blood). Rats were killed at a fixed time on day six post infection (PI) when parasitaemia was found to be 5×10^8 to 7×10^8 parasites ml^{-1} of blood. Similarly, rabbits were also killed on day 27 PI when parasitaemia reaches to 2×10^5 parasites ml^{-1} of blood. Post mortem (PM) examination was immediately executed for detection of viable parasites in heart blood and hepatic blood and in different visceral organs like liver, lung and spleen by impression smears. PM examination was accomplished by taking each group of mice and rat separately at 2 h intervals up to 12 h after death and subsequently at 1 h interval. In case of rabbits, PM examination was performed by taking only one rabbit at a time, at intervals similar to rats and mice.

Examination of heart blood and impression smears

Wet blood film (WBF) of heart blood of mice, rats, and rabbits was examined at the specified time intervals for monitoring the viability of parasite. Smears of heart blood were prepared for Giemsa stain. Impression smears from different visceral organs (viz. liver, lung, and spleen) were prepared at the specified time intervals mentioned earlier and stained with Giemsa. Stained smears were examined thoroughly under oil immersion ($1,000\times$) lens of microscope.

Mouse inoculation test (MIT) for confirmation of viability, infectivity and virulence

Mouse inoculation test was conducted for monitoring the viability, infectivity and virulence of parasites isolated during PM. For this, heart blood of mice, rats, rabbits as well as portal blood of rats were collected at specified time intervals and diluted with Alsever's solution. Each diluted blood was inoculated separately through IP route to two healthy mice for each carcass and inoculated mice were examined on every alternative day PI up to 60 days.

Results and discussion

Examination of heart blood by wet blood film (WBF) for detection of viable *T. evansi*

Wet blood film examination of heart blood of mice revealed the presence of live trypanosomes (viability 100 %) up to 4 h following host death. Thereafter on subsequent examinations, the viability was going to be reduced by 80, 60, and 40 % at 6, 8 and 10 h, respectively, after death of the mice. Viability was severely reduced to 10, 5 and <1 % after 12, 13 and 14 h, respectively after death of mice (Table 1). Parasites remaining alive in heart blood up to 14 h of death were also still motile. No live/dead parasites were seen in the heart blood at the end of 15 h following host death. The observations in case of rats were same as like as observations recorded in case of mice (Table 1). In case of rabbits examination of heart blood by WBF for detection of live *T. evansi* in dead rabbits, up to 4 h post death, it was same like mice and rats i.e. 100 % viable trypanosomes were found in the wet film preparation of heart blood (Table 3). Then the viability of trypanosomes gradually decreased to 60, 40, 10 and 5 % at 6, 8, 10 and 12 h respectively, after death of the rabbit. After 14 h of death no live or dead parasites were found in the wet heart blood film. But those trypanosomes remained viable in the heart blood of rabbit up to 13 h of death were strongly motile in the adverse environment of the dead body which did not affect their viability and motility. So in the present study the trypanosomes remained viable in the heart blood of rabbit up to 13 h post death.

Confirmation of live trypanosomes and determination of their virulence and infectivity by MIT

MIT conducted with heart blood of dead mice showed positive up to 13 h post death but it was negative at the end of 14 h after death even though <1 % live parasites were present in the heart blood. A minimum 24 h and maximum 192 h incubation period were observed in inoculated mice (Table 1). All inoculated and positive mice were died after 96 h of first appearance of parasitaemia, indicated that after death of the host, the virulence and infectivity of the parasites remained unchanged. It has also been observed that MIT using inoculum prepared from fresh live mice showed death of inoculated mice after 96 h. At the end of 14 and 15 h post death, MIT was negative. In case of rats, MIT was positive up to 14 h of death (Table 2). A minimum of 24 h and maximum 264 h (11 days) incubation periods was found. The virulence and infectivity of the parasites remained unchanged as observed in case of mice. Though least number of viable parasites was present in the heart blood as well as in portal blood after 14 h of death of host, the virulence and

Table 1 Viability of *Trypanosoma evansi* in WBF as confirmed by MIT and staining characters in different organs following death of infected mice

Group of mice	PM exam (h)	WBF	Live parasitaemia (%)	MIT	Incub. period (h)	Staining characters (smears)			
						Heart	Liver	Lung	Spleen
I	0	+	100	+	24	+	+	+	+
II	2	+	100	+	24	+	+	+	+
III	4	+	100	+	36	+	+	+	+
IV	6	+	80	+	48	+	+	+	+ ^a
V	8	+	60	+	96	+	+ ^a	+ ^a	+ ^a
VI	10	+	40	+	108	+ ^a	+ ^a	+ ^a	+ ^a
VII	12	+	10	+	156	+ ^a	+ ^a	+ ^a	+ ^b
VIII	13	+	5	+	192	+ ^a	+ ^a	+ ^a	— ^c
IX	14	+	<1.0	—		+ ^b	+ ^b	+ ^b	— ^c
X	15	—	0.0	—		— ^c	— ^c	— ^c	— ^c

WBF wet blood film, MIT mice inoculation test, h hours, PM post mortem, + positive, — negative

^a Mildly degenerated

^b Highly degenerated

^c Fully degenerated

Table 2 Viability of *Trypanosoma evansi* in WBF as confirmed by MIT and staining characters in different organs following death of infected Rats

Group of mice	PM exam (h)	WBF	Live parasitaemia (%)	MIT	Incub. period (h)	Staining characters (smears)			
						Heart	Liver	Lung	Spleen
I	0	+	100	+	24	+	+	+	+
II	2	+	100	+	24	+	+	+	+
III	4	+	100	+	36	+	+	+	+
IV	6	+	80	+	48	+	+	+	+ ^a
V	8	+	60	+	96	+	+ ^a	+ ^a	+ ^a
VI	10	+	40	+	120	+ ^a	+ ^a	+ ^a	+ ^a
VII	12	+	10	+	156	+ ^a	+ ^a	+ ^a	+ ^b
VIII	13	+	5	+	216	+ ^a	+ ^a	+ ^a	— ^c
IX	14	+	<1.0	+	264	+ ^b	+ ^b	+ ^b	— ^c
X	15	—	0.0	—		— ^c	— ^c	— ^c	— ^c

WBF wet blood film, MIT mice inoculation test, h hours, PM post mortem, + positive, — negative

^a Mildly degenerated

^b Highly degenerated

^c Fully degenerated

infectivity of trypanosomes were restored and could easily infect the mice. MIT with heart blood of dead rabbit was positive up to 13 h of death and MIT was negative after 14 h of death (Table 3). The virulence and infectivity of the parasites remained unchanged after death of the hosts.

Examination of heart blood smears, liver, lung and spleen impression smears by Giemsa stain

In case of mice (Table 1), the trypanosomes were clearly recognized in the blood smears prepared from heart blood

up to 8 h of death without degeneration but at the end of 10 h of death, the parasites could be recognized with variable degree of degeneration of parasites (Fig. 1). After 13 h post death, most of the parasites were degenerated except some remained intact and were easily recognized. The parasites could easily be recognized in the heart blood smears prepared after 14 h of death with massive degeneration (both nucleus and cytoplasm of trypanosomes). But at the end of 15 h of death, there was complete disappearance of trypanosomes in the heart blood. In case of liver and lung impression smears, the parasites were clearly

Table 3 Viability of *Trypanosoma evansi* in WBF as confirmed by MIT and staining characters in different organs following death of infected rabbit

Rabbit ID No.	PM exam. (h)	WBF	Live parasitaemia (%)	MIT	Incub. period (days)	Staining characters (smears)			
						Heart	Liver	Lung	Spleen
R1	0	+	100	+	5	+	+	+	+
R2	2	+	100	+	5	+	+	+	+
R3	4	+	100	+	6	+	+	+	+
R4	6	+	60	+	8	+	+	+	+ ^a
R5	8	+	40	+	10	+	+ ^a	+ ^a	+ ^a
R6	10	+	10	+	11	+ ^a	+ ^a	+ ^a	+ ^a
R7	12	+	5	+	14	+ ^a	+ ^a	+ ^a	+ ^b
R8	13	+	<1.0	+	15	+ ^b	+ ^b	+ ^b	— ^c
R9	14	—	0.0	—		— ^c	— ^c	— ^c	— ^c
R10	15	—	0.0	—		— ^c	— ^c	— ^c	— ^c

WBF wet blood film, MIT mice inoculation test, h hours, PM post mortem, + positive, — negative, ID identification

^a Mildly degenerated

^b Highly degenerated

^c Fully degenerated

Fig. 1 Showing initiation of degeneration of *Trypanosoma evansi* in blood smear collected from heart at 10 h post death of mice (Giemsa stain $\times 1,000$)

Fig. 2 Showing degeneration of *T. evansi* in impression smear of spleen at 10 h post death of mice (Giemsa stain $\times 1,000$)

recognized up to 6 h of death but at the end of 8 h, the parasites were recognized with certain degree of degeneration. At 14 h of death, few parasites could be recognized in liver and lung impression smears with massive degeneration result no trace of parasites after 15 h of death. In case of spleen impression smears (Fig. 2), degeneration of parasites was started 6 h after death and up to 12 h parasites were found with massive degeneration. Then, at the end of 13 h, there was complete disappearance of parasites resulted no existence of parasites in spleen impression smears after 13 h post death. Similar findings were observed in case of rats as mentioned in Table 2. In case of rabbits, the trypanosomes were easily recognized without degeneration in the heart blood smears up to 8 h of death (Table 3). From 10 to 12 h after death, degenerated trypanosomes were found in the heart blood smears but they could easily be recognized morphologically (Fig. 3). At the end of 13 h parasites were recognized with difficulty due to massive degeneration occurred. But no trace of parasite was found after 14 h of death of rabbits. In liver and lung impression smears (Fig. 4), parasites could be detected to 13 h post death. The parasites were easily identified in spleen impression smears up to 10 h of death but at the end of 12 h massive degeneration occurred and those were identified with difficulty. After 13 h of death, no trace of parasite was found in spleen impression smears. Thus, it has been found from the above observations that in cases of mice, rats and rabbits, in spleen impression smears parasites showed degeneration earlier than those of heart blood smears, liver and lung impression smears.

The death of trypanosomes is suspected to be due to lack of glucose and oxygen in the blood of dead host, which is

Fig. 3 Showing degeneration of *T. evansi* in blood smear collected from heart at 12 h post death of rabbit (Giemsa stain $\times 1,000$)

Fig. 4 Showing degeneration of *T. evansi* in impression smear of liver at 12 h post death of rabbit (Giemsa stain $\times 1,000$)

required for their metabolism (Gill 1991) and exposure to adverse environment in the dead hosts' body. In a similar study Sarmah (1998) observed $<1\%$ motile parasites at 10 h after death and only dead parasites 12 h post death in wet blood film of rats. This variation in survival time of *T. evansi* in dead host's body might be due to strain variation and/or different environmental stress in hosts. In the present study, no significant variation on survival period of *T. evansi* in the heart blood was found among dead mice, rats and rabbits.

In the present study it has been found that MIT conducted with heart blood materials up to 13 h post death, became positive but it was negative at the end of 14 h of death of mice and rabbits though $<1\%$ viable parasites were present in the heart blood of these mice. The least numbers of live parasites present in the inoculums prepared from the heart blood at 14 h post death of mice could not able to penetrate gastrointestinal mucosa and established themselves in inoculated mice, might be the reason of negative results in MIT. In case of rats, MIT conducted with both heart blood and portal blood became positive up

to 14 h post death indicated trypanosomes remain alive in the hepatic blood as in heart blood and portal blood, could also be used for diagnosis of trypanosomosis at post mortem through MIT. In the present study, it is further observed that incubation periods varied inversely proportional with the number of live trypanosomes present in the inoculums prepared, as mentioned earlier (OIE 2004). After MIT, all positive mice died with high parasitaemia after 96 h of first appearance of parasitaemia indicated that the virulence and infectivity of trypanosomes even exposure in the adverse environment in the heart blood as well as in portal blood of dead host remained unchanged. In contrast to present findings Sarmah (1998) reported that MIT conducted with heart blood up to 10 h became positive although he also observed that all inoculated mice died. So on the basis of present investigation trypanosomosis can be diagnosed through MIT using heart blood up to 13 h of death of host. But no available literature was there on MIT using portal blood for diagnosis of the disease after death. So on the basis of present study, it is concluded that diagnosis of trypanosomosis at post mortem might be possible through MIT using portal blood even up to 14 h of death of host.

The disintegration and disappearance of the parasites after death of host might be due to absence of sufficient blood glucose, oxygen and other metabolic requirements. Sengupta (1974) demonstrated partially degenerated *T. evansi* in the blood smears of two zoo animals during post mortem examination 7 h after death whereas Murali Manohar et al. (2003) reported that the blood smears collected 6 h after death did not reveal the parasites. Sarmah (1998) demonstrated the degenerated parasites in heart blood smears up to 12 h of death of rats in contrast to the degenerated parasites up to 14 h post death of mice and rats. The parasites were degenerated in the heart blood of rabbits earlier than those of mice and rats.

Sarmah (1998) observed the degenerated parasites in liver and spleen impression smears up to 12 h of death of rats. This mild variation might be due to strain variation and environmental condition. Parasites showed degenerative changes in spleen impression smears earlier than liver and lung impression smears. It might be due to adverse environment for *T. evansi* created by lysis of RBCs, defensive mechanisms and comparatively lack of sufficient glucose in spleen than liver and lung. No literature was found about lung impression smears. So on the basis of present investigation, it is opined that like blood smears, liver and spleen impression smears, examination of lung impression smear is also equally important for diagnosis of trypanosomosis after death of host.

So, from this present investigation it is concluded that trypanosomosis can be diagnosed through the demonstration of parasites in blood smears, liver and lung impression

smears up to 14 h of death of host, in spleen impression smears up to 12 h post death of host and through MIT using heart blood up to 13 h. MIT conducted with portal blood may be helpful for diagnosis of trypanosomiasis up to 14 h of death of host.

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