Nigerian Veterinary Journal
VOL:33 (2) 505-510
ARTICLE

The Sensitivity of Ice Pack Preserved *Trypanosoma Evansi* to Different Parasitological Diagnostic Methods.

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SUMMARY

The aim of this study is to determine the sensitivity of ice pack preservation of *Trypanosomaevansi* to some parasitological diagnostic techniques like wet blood film (WBF), haematocrit centrifugation test (HCT) and mice inoculation test (MIT). Three millilitres each of blood containing approximately 10 x 10^3 *T. evansi*/ml was placed in four different sample bottles and preserved in an insulated flask containing ice at 5 ^{0}C. WBF & HCT were used prior to ice preservation and at 4, 8, 12, 24, and 36 hrs post ice preservation to observe for the presence of motile trypanosomes. A total of 8 mice, two per sample were inoculated intraperitonealy with 0.2mls of the blood samples at each time interval of preservation. The level of parasitaemia was also estimated at each time of preservation by counting the number of live trypanosomes in wet film preparation of the buffy coat materials of each sample under phase contrast microscopy. Decrease in the sensitivities of the tests were noticed as follows: from 100% at 0 – 8 hrs to 75% at 12 hrs and 50% at 36 hrs post preservation for WBF; from 100% at 0 – 12 hrs to 75% and 50% at 24 and 36 hrs post preservation respectively for HCT; and from 100% at 0 – 8 hrs to 63%, 38% and 25% at 12, 24 and 36 hrs post preservation for MIT. Increase in pre-patent periods from 3 days to 21 days in mice inoculated at 0 hr & 36 hrs post preservation respectively were observed. Significant decrease ($P < 0.001$) in mean parasitemia of the preserved infected blood samples was noticed at 24 and 36 hours post preservation. The duration of preservation of the infected blood sample statistically correlated with parasitaemia in the preserved blood samples ($r = - 0.95$; $P < 0.05$) and with pre-patent period ($r = 0.93$; $P < 0.05$) in the inoculated mice.

KEY WORDS: Ice pack preservation, *Trypanosoma evansi*, viability, infectivity.

INTRODUCTION

Trypanosomoses are among the most important, often fatal haemoparasitic diseases of man and other animals in Africa and in some parts of Asia and South America (Reid, 2002). Among the species that are of Veterinary importance are *Trypanosoma brucei brucei*, *T. congolense* and *T. vivax* (which cause nagana), *T. evansi* (causes surra) *T. equiperdum*, (Dourine), *T. simiae* and *T. equinum* (Stevens and Briesse, 2004). The diagnosis of trypanosomosis is difficult because the clinical signs are varied and non-specific and, in enzootic areas, the natural hosts frequently present chronic forms of the disease (Taylor and Authie, 2004). Confirmation of clinical diagnosis is mainly based on demonstrating the flagellates in blood using various parasitological techniques.

Wet blood film, haematocrit centrifugation technique and buffy coat technique are rapid parasitological techniques often used in the diagnosis of trypanosomosis in a herd or flock of affected animals. The sensitivity of these tests however, depend on the level of parasitaemia and viability of the parasites in the collected blood samples as they are based on detecting the motility of the trypanosomes in the preparations (Woo, 1971; Murray *et al*., 1977; Paris *et al*., 1982). Blood samples collected for diagnoses using these tests need to be examined immediately because most of the parasites may die when the time of laboratory examination of the samples is lengthened (Lawal *et al.,* 2004; Ngongeh and Musongong, 2011). There is dearth of information with regard to the duration at which cryopreserved blood samples from the field will be useful for accurate diagnosis of trypanosomosis and isolation of *Trypanosome* species.

The present work is therefore undertaken to determine the effect of duration of ice preservation of *Trypanosoma evansi* infected blood sample on the sensitivities of wet blood film, haematocrit centrifugation test and mice inoculation test.

MATERIALS AND METHOD

Study area: The study was conducted in the Protozoology laboratory of the Department of Parasitology and Entomology Faculty of Veterinary Medicine Ahmadu Bello University, Zaria Zaria is located between latitude 11° 07'N and longitude 7° 44'E within the Northern guinea savanna zone. It possesses a tropical continental climate with a pronounced dry season, lasting up to seven months (October - May). The rainy season lasts from May to September/October. The average rainfall ranges from 1000 – 1250 mm and the average daily temperature ranges from 19 °C – 33 °C.

Parasite: The *T. evansi* used for the study was isolated from the blood of naturally infected camels during slaughter at Kano abattoir, Kano State, Nigeria and maintained in albino rats in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

Donor animals: Four donor rats bred in the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria were used to multiply the parasite. The rats were housed in a rat cages at room temperature, fed with commercial chick mash and given unrestricted access to clean water.The rats were acclimatized for 72 hours before the commencement of the experiment.

Experimental animals: A total of 48 albino mice bred in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were used. The mice were housed in cages at room temperature, fed with commercial chick mash and clean water was provided *ad libitum*. The mice were acclimatized for 48 hours before the commencement of the experiment and thereafter grouped into six groups of eight mice each.

Blood sample collection from the donor rats and cold preservation on ice: Following inoculation of the donor rats with the parasite, parasitemia was monitored using wet mount technique and by 72 hours post inoculation the parasitemia was high. The rats were then humanely sacrificed and their blood was pooled into a sterilized Bijou bottle containing salt of Ethylene Diamine Tetra-acetic Acid (EDTA). Three milliliters each of the pooled blood containing approximately 10 x 10^3 motile trypanosomes / ml (Murray *et al.,* 1983)

was placed into four different sample bottles and preserved in an insulated flask containing ice blocks at an average temperature of 5 ^{o}C to simulate field condition. Melted ice was replaced with new ones after every 6 hours of preservation to maintain the cold storage condition.

Determination of effect of duration of ice pack preservation on the sensitivity of wet mount and HCT: The infected blood samples collected from the donor rats were immediately examined for motility of viable trypanosomes before preservation (0 hour) and at the 4th ,8th ,12th ,24th and 36th hours post preservation using wet mount and Haematocrit Centrifugation Technique (HCT) (Soulsby, 1982).

Parasitaemia estimation: Level of parasitaemia was estimated at each of the time intervals of preservation by counting the number of motile trypanosomes per wet film preparation of the buffy coat materials of each sample under phase contrast microscopy as described by Murray *et al.,* 1983.

Determination of infectivity of the *Trypanosoma evansi* prior and post preservation on ice, using mice inoculation test: At each time interval of preservation, a total of 8 mice (2 per blood sample) were inoculated with 0.2ml of the infected blood containing approximately 2 x 10^{3} trypanosomes (Murray *et al.,* 1983). The infected mice were then monitored for parasitemia daily using wet mount technique.

Data analysis: Data were summarized as mean ± SEM and analyzed by ANOVA, Tukey's multiple comparison tests and Pearson's correlation test using Graph pad software version 5.0 (Graphpad Prism, 2010). Values of $p<0.05$ were considered as statistically significant.

RESULTS

Table I shows effect of duration of ice pack preservation of *Trypanosoma evansi* infected blood on sensitivity of wet mount and haematocrit centrifugation technique. The sensitivities of the tests were not affected for up to 8 hours post preservation. However, decrease in sensitivity were noticed from 100% to 75% at 12 hour and to 50% at 36 hour post preservation for wet blood film; and to 75% and 50% at 24 and 36 hours post preservation respectively for the HCT (Table I).

Decrease in sensitivity of mice inoculation test was also observed from 100% at 0, 4 and 8 hours of preservation to 63%, 38% and 25% at 12, 24 and 36 hours post preservation respectively. More so, as the duration of ice pack preservation goes beyond 4 hours, progressive increase in pre-patent period in the inoculated mice was observed from 3 days in mice inoculated at 0 and 4 hours post preservation to 21 days in those inoculated at 24 and 36 hours of preservation (Table II).

Table III shows the progressive decrease in mean parasitaemia scores of the preserved blood samples as the length of time of ice preservation increases. However, the decrease was only statistically significant ($p<0.05$) at 24 and 36 hours post preservation. There were statistically significant ($p<0.05$) correlations between the duration of preservation, mean parasitaemia and infectivity of the *Trypanosomaevansi* to mice (Table IV)

DISCUSSION

The result of the present study shows that the sensitivities of wet blood film,

haematocrit centrifugation and mice inoculation tests on *Trypanosoma evansi* infected blood can be maintained when infected blood samples are preserved on ice for up to 8 hours. This was evident by the observed motility of the parasite in all the preserved samples using wet mount and HCT and the maintained infectivity of the parasite during the first 8 hours of ice preservation. However, decreases in sensitivities of the tests were observed with increased duration of preservation which was first noticed at 12 and 24 hours post preservation for wet mount and HCT respectively. This observation agrees with previous reports that HCT is a more sensitive technique in detecting live trypanosomes than the wet blood film (Woo, 1971; Murray *et al.*, 1977; Paris *et al.*, 1982). The observed decrease in sensitivities of the tests could be strongly attributed to the significant decrease in mean parasitemia scores of the preserved *T. evansi* infected blood sample that was observed beyond 8 hours post preservation. The reduction in parasitemia could be attributed to inability of the trypanosomes to reproduce; progressive weakness and death of some of the trypanosomes with increased preservation time (Lawal *et al.,* 2004). Decreased infectivity of the *Trypanosoma evansi* observed during the course of preservation which was evident by the decreased number of mice with patent parasitemia and prolonged pre-patent periods in mice inoculated after 8 hours of cold preservation could also be attributed to the decrease in number of viable trypanosomes that will multiply and establish in the blood of the infected mice.

The viability and infectivity of the parasite was however not completely lost throughout the period of ice preservation. Live trypanosomes were detected in some of the preserved blood samples even at 36 hours post ice preservation. This agrees with the report of Lawal *et al.* (2004) who reported presence of live trypanosomes in refrigerated blood samples after 6 days. The maintained viability of the trypanosomes during this period of cold storage may be attributed to the decrease in metabolic rate of the trypanosomes which reduces the utilization of nutrients in the blood (Igbokwe, 1993). Moreso, some of the inoculated mice showed parasitaemia even when inoculated at 36 hours post preservation. This agrees with the finding of Reid *et al.* (2001) who demonstrated that infectivity for mice of blood containing *T. evansi* for up to 21 hours of cold storage was maintained. Lawal *et al.* (2004) also reported maintained infectivity of refrigerated *T. brucei* infected blood samples for up to 48 hours. However, it disagrees with the findings of Ngongeh and Musongong, (2011) who reported complete loss of infectivity of *T. brucei* after 24 hour of cold storage at 4°C.

In conclusion, the result of the present study showed that the survival and infectivity of *T. evansi* under the dry tropical condition can be maintained when infected blood samples are preserved on ice for up to 36 hours.However beyond eight hours of preservation progressive decreased parasitemia and infectivity of the parasite occurs with consequent decreased sensitivities of wet mount, HCT and mouse inoculation test.

We therefore, recommend that for accurate diagnosis of Trypanosomosis in tropical environments like that of Zaria in Kaduna State, Nigeria, blood samples collected from the field should not be preserved on ice for more than 8 hours. However such preserved samples could be useful for isolation of live trypanosomes from field specimens for the purpose of research even at 36 hours of ice preservation.

TABLE I: Effect of duration of ice pack preservation of *Trypanosoma evansi* infected blood on the sensitivity of wet mount and HCT.

Duration of sample storage (hour)	No. of samples examined	Number positive (%)	
		Wet mount	HCT
0	4	4 (100)	4(100)
4	4	4 (100)	4(100)
8	4	4(100)	4(100)
12	4	3(75)	4(100)
24	4	3(75)	3(75)
36	4	2(50)	2(50)

TABLE II: Effect of duration of ice pack preservation of *Trypanosoma evansi* infected blood sample on the sensitivity of mice inoculation test and pre-patent period.

Time of inoculation (hours)	Number of mice inoculated	Number of mice with patent parasitemia	Pre-patent period (days)
0	8	8 (100%)	3
4	8	8(100%)	3
8	8	8(100%)	4±1
12	8	5(63%)	13±1
24	8	3(38%)	21
36	8	2(25%)	21

TABLE III: Effect of duration of ice preservation of *T. evansi* infected blood on parasitaemia.

Duration of sample preservation (hours)	Estimated parasitaemia / mls of blood (Mean ±SE) x 10^3
0	10.0 ± 0.0^a
4	8.8 ± 1.3^a
8	8.8 ± 1.3^a
12	5.3 ± 1.8^a
24	1.8 ± 1.1^b
36	1.6 ± 1.2^b

Mean values with different superscript in same column are statistically significant (P< 0.001)

TABLE IV: Relationship between duration of preservation of *T. evansi* infected blood sample with mean parasitaemia in the samples and pre-patent period in inoculated mice

Correlated Parameters	Pearson's correlation
Duration of preservation and parasitaemia	-0.95*
Duration of preservation and pre-patent period	0.93*

*P< 0.05

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