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THE EFFECTS OF ANTICOAGULANT TYPE ON THE SURVIVAL AND INFECTIVITY OF *TRYPANOSOMA BRUCEI* IN MICE

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SUMMARY

The effect of anticoagulant types (heparin, disodium ethylene diamine tetra-acetic acid (EDTA) and sodium citrate on survival time of *Trypanosoma brucei* in refrigerated sheep and goat blood and infectivity to mice was investigated. Blood samples from experimentally infected sheep and goat were collected in different anticoagulant types and preserved at 4°C. The survival period and infectivity to mice was monitored. Sodium citrate supported the survival of *T. brucei* at high parasitaemia level in both sheep and goat blood samples. However, at low parasitaemia, EDTA preserved samples supported the survival of *T. brucei* best. *T. brucei* was infective to mice up to 24 hours post storage of samples in all anticoagulant types but only infective 48 hours in sample preserved with sodium citrate anticoagulant. The mean prepatent period was least when sodium citrate was used.

KEY WORDS: Anticoagulants, Infectivity, Mice, Survival, *Trypanosoma brucei*

INTRODUCTION

Trypanosomosis is one of the most important diseases of livestock and man in sub Saharan Africa (Swallow, 2002). It had been recognised as a major constraint to the growing livestock industry in Africa (Shamaki *et al.*, 2002). It is often necessary to transport blood over a long distance to the diagnostic laboratory for parasitological examination and identification during field investigation of natural outbreaks of trypanosomosis (Ekwureke *et al.*, 1985).

This practice necessitates the understanding of factors which might influence the survival times of trypanosoma species in different mammalian blood. Princewill (1980), reported that *Trypanosoma brucei* and *T. congolense* survived longer in artificial laboratory media. Similarly, Otesile (1990) reported that *T. congolense* survived for more than 48 hours in refrigerated pig blood. The survival periods of *T. vivax* and *T. congolense* in cattle blood and *T. brucei* in caprine blood has also been investigated (Otesile *et.al.*, 1990).

The aim of this study was to examine the effect of anticoagulant types on the survival and infectivity of *T. brucei* in mammalian blood kept at 4°C.

MATERIALS AND METHODS

Experimental animals

Small ruminants

Two Kano Brown goats and two Yankasa sheep were obtained from a local livestock market in Ibadan. The animals were housed in the Large Animal Unit of the Veterinary Teaching Hospital,

University of Ibadan, and provided with grasses (*Panmicum maximum*), concentrates and water *ad libitum*. The animals were treated prophylactically with levamisole hydrochloride at 5mg/kg body weight and oxytetracycline long acting 20mg/kg intramuscularly. The animals were allowed to adjust to their new environment for four weeks. They were screened for trypanosoma infection during the first week of arrival and after the adjustment period.

Mice

Albino mice were purchased from the Anatomy Department of the Faculty of Medicine, University of Ibadan. A total of sixty mice were used for the experiment.

Trypanosoma brucei

Trypanosoma brucei isolated from sheep at Lafia, Nigeria in 1995 and stored in liquid nitrogen was obtained from the Nigerian Institute for Trypanosomiasis Research Vom, Nigeria.

Anticoagulants

Heparin sodium salt (Sigma Laboratory) was purchased commercially and prepared into 1 % w/v solution and used at 20 IU per ml of blood.
Disodium ethylene diamine tetracetic acid (EDTA) was purchased and prepared into 10 % w/v solution and used at 500 ug/ml of blood.
Sodium citrate (BDH chemicals) was purchased and prepared as 3.8 % w/v solution and used at one part sodium citrate solution to 9 parts of blood.

Experimental infection with *Trypanosoma brucei*

After the period of adjustment, one goat and a sheep were experimentally infected with $1x10^8$ motile *Trypanosoma brucei* through the jugular vein.

Monitoring of parasitaemia

Beginning from two days post infection, parasitaemia was monitored by rapid matching method (Herbert and Lumsden, 1976) using blood from the ear vein. Blood samples positive for trypanosomes were collected aseptically by jugular venipuncture. For each anticoagulant type, 5ml of blood was collected into bijou bottles and taken to the laboratory on ice. In the laboratory, the blood samples collected in each anticoagulant type was placed into two bijou bottles in aliquots of 2.5ml and kept at 4°C. Beginning from the day (day 0) of blood sample collection, the parasitaemia was monitored by rapid matching method (Herbert and Lumsden, 1976). When the parasitaemia became low, the buffy coat scoring method of Paris *et al.* (1982) was used.

Effect of anticoagulant type on infectivity of *Trypanosoma brucei* to mice

Blood samples positive for *T. brucei* used for monitoring the survival above was used for the infectivity studies. Beginning from day of sample collection, approximately 30 minutes post collection; five albino mice were inoculated intraperitoneally with 0.1ml of blood preserved with each anticoagulant type. Daily inoculation was done after monitoring the survival using new group of mice for four days. Every group of mice was bled daily from the tail to monitor parasitaemia. Positive mice were euthanized. Detection of parasitaemia was discontinued after 30 days.

RESULTS

The initial parasitaemia used for this study was classified into two levels as follows:
High parasitaemia (HP) = $10^7 ml^{-1}$
Low parasitaemia (LP) = $10^7 ml^{-1}$.

Effect of anticoagulant types on the survival of *Trypanosoma brucei* in sheep blood

Trypanosoma brucei in sheep blood sample with initial high parasitaemia survived up to seventh day in both samples preserved with sodium citrate and heparin ,two days longer than samples preserved with EDTA(Table I). However, samples with initial low parasitaemia preserved with EDTA survived for 24 hours longer than for same samples preserved with either sodium citrate or heparin (Table I).

Effect of anticoagulant type on the survival of *Trypanosoma brucei* in goat blood

Trypanosoma brucei with initial high parasitaemia survived up to the sixth, seventh and eighth days in EDTA, heparin, and sodium citrate preserved samples respectively (Fig.1). However, at low parasitaemia, *T. brucei* survived longer in EDTA preserved sample up to day 3 compared to 1 and 2 days in the same sample preserved in heparin and sodium citrate respectively. At all levels of parasitaemia in each anticoagulant type, goat blood tends to support the survival of *T brucei* longer than sheep blood (Table II).

Effect of anticoagulant type on infectivity of *Trypanosoma brucei* to mice

All samples preserved in different anticoagulants were infective to mice on the day of collection approximately 30 minutes after collection showing the same incubation and prepatent periods. However, after preservation at 4°C for 24 hours, the prepatent periods differed with anticoagulant types although all mice were infected. The mean prepatent period was least for sodium citrate followed by heparin and EDTA. Only samples preserved with sodium citrate was infective to 75% of mice at 48 hours post storage (Table III).

TABLE I: Survival times of *Trypanosoma brucei* in refrigerated sheep blood preserved with anticoagulant type

Anticoagulant type	Sample number	Mean Parasitaemia ($log10ml^{-1}$) on day								
		0	1	2	3	4	5	6	7	8
Heparin	HP	7.1	7.1	6.3	6.0	4.9	4.5	3.6	3.1	0
	LP	6.0	4.7	3.3	0					
EDTA	HP	7.1	6.3	6.0	5.6	4.7	3.4	0		
	LP	5.4	3.8	3.0	2.9	0				
Sodium citrate	HP	7.1	6.6	6.3	5.7	5.2	4.7	3.8	3.2	0
	LP	5.4	3.0	0						

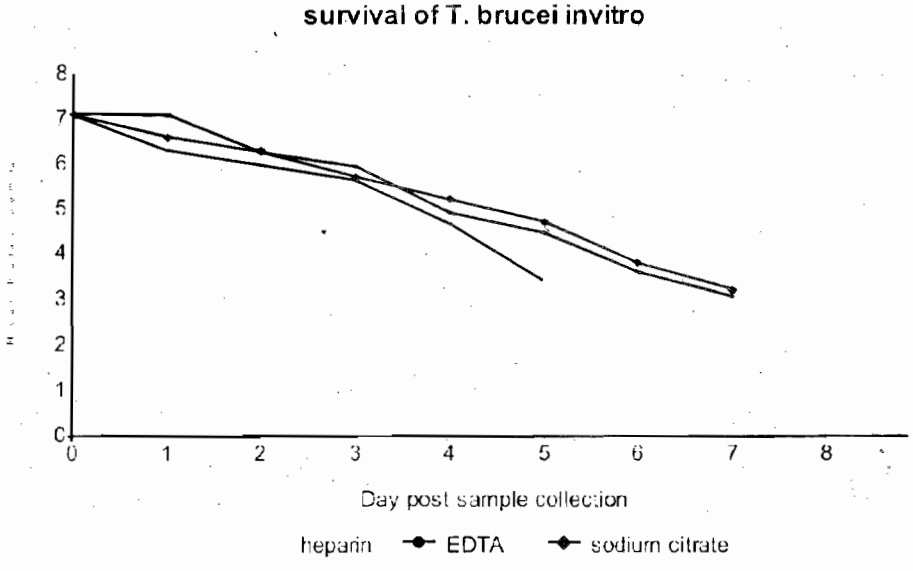

Fig 1.Survival of *T.brucei* in sheep blood with high parasitaemia in vitro at 4°C

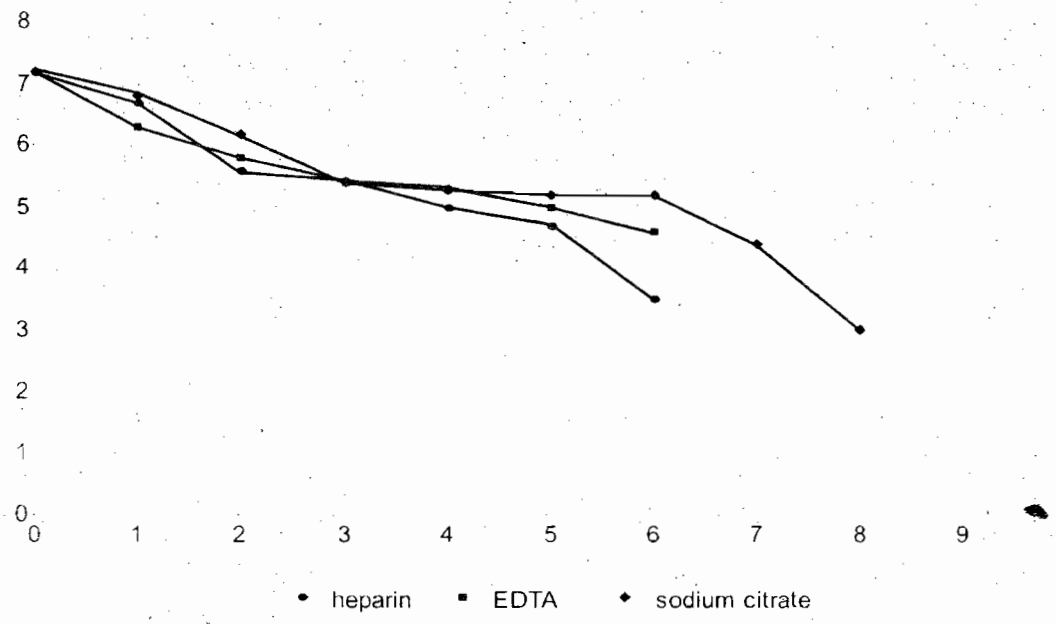

Fig 2.Survival of *T.brucei* in goat blood at high parasitaemia at 4°C invitro.

TABLE II: Survival times of *Trypanosoma brucei* in refrigerated goat blood preserved with anticoagulant type

Anticoagulant type	Sample number	Mean Parasitaemia (log $10ml^{-1}$) on day							
		0	1	2	3	4	5	6	7
Heparin	HP	7.2	6.7	6.1	5.6	5.4	5.0	4.7	3.5
	LP	5.4	3.3	0					
EDTA	HP	7.2	6.3	5.8	5.4	5.3	5.0	4.6	0
	LP	5.4	3.8	4.7	3.7	0			
Sodium citrate.	HP	7.2	6.8	6.2	5.4	5.3	5.2	4.4	3.0
	LP	6.0	5.6	4.9	3.1	0			

TABLE III: Effect of anticoagulant type on the infectivity of *Trypanosoma brucei* to mice

Parameter studied	Anticoagulant type	Period Of Blood Storage			
		30 minutes	24 hours	48 hours	72 hours
Dose of T.brucei injectectd per mouse	Heparin	$10^{5.3}$	$10^{5.0}$	$10^{4.4}$	$10^{4.0}$
	EDTA	$10^{5.3}$	$10^{4.7}$	$10^{4.4}$	$10^{3.6}$
	Sodium citrate	$10^{5.3}$	$10^{5.3}$	$10^{5.0}$	$10^{4.7}$
Proportion of mice that develop parasitaemia.	Heparin	5/5	5/5	0/5	0/5
	EDTA	5/5	5/5	0/5	0/5
	Sodium citrate	5/5	5/5	3/5	0/5

DISCUSSION

The result of this experiment shows that sodium citrate supported the survival of *T. brucei* in goat and sheep blood samples best with initial high parasitaemia (Table I). In samples with initial high parasitaemia, motile *T. brucei* organisms can be seen up to the 7th and 8th days in refrigerated sheep and goat blood samples respectively preserved with sodium citrate. Ethylene diamine tetra acetic acid (EDTA), the anticoagulant used frequently during routine sampling supported the survival of *T. brucei* least at high parasitaemia in goat and sheep samples. However, at initial low parasitaemia of samples, EDTA best supported the survival of *T. brucei* in both sheep and goat samples (Table I).

Refrigerated goat blood samples with any of the anticoagulant types tends to support the survival of *T. brucei* longer than sheep blood as earlier reported for *T. vivax* (Ewkureke *et al.*, 1985). Generally, the higher the initial mean parasitaemia the longer the survival period for each anticoagulant type (Table I and II). This trend was earlier reported (Princewill, 1980; Otesile, 1990; Otesile *et al.*, 1990). The suggestion by Princewill (1980), that dilution of blood samples removed some inhibitory factors for trypanosomes and prolonged their survival seems reasonable in this case of sodium citrate which introduced 10 % dilution in the blood sample.

It is possible that some other factors, especially the anticoagulatory mechanism of these chemicals may influence the survival times of trypanosomes in refrigerated sheep and goat blood.

For the infectivity test, sodium citrate preserved sample maintained infectivity better than the other anticoaguiant types (Table III). At all stages of preservation, the prepatent periods were shorter for sodium citrate preserved samples, indicating its superior quality as anticoagulant over the others.

CONCLUSION

The result of this work shows that sodium citrate supported the survival of *T. brucei* better than EDTA and heparin at high parasitaemia. However, at low parasitaemia which is the norm under field condition EDTA supported the survival of *T. brucei* best in both ovine and caprine samples. EDTA therefore, is preferred for preserving samples during investigation of natural outbreaks of trypanosomosis under field condition.

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