SURVIVAL TIME OF *TRYPANOSOMA EVANSI* IN SAMPLES OF BLOOD TAKEN FROM INFECTED BUFFALOES

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ABSTRACT

The viability and infectivity for mice of *Trypanosoma evansi* in blood collected from buffaloes was investigated to establish an acceptable time limit for storing samples in the field. Heparin and EDTA samples could be stored at 4°C for up to 24 hours without loss in infectivity although heparinised blood showed decreased parasitaemia in the HCT compared to EDTA blood.

INTRODUCTION

Trypanosomiasis caused by the protozoan parasite *Trypanosoma evansi* (Surra) is widespread in Indonesia. The disease is of particular importance in domestic livestock and the animals affected include horses, cattle and buffalo.

Diagnostic methods for trypanosome infections rely on the detection of parasites circulating in the bloodstream (Killick-Kendrick, 1968). Among the most sensitive methods available are the microhaematocrit (HCT) (Woo, 1969) and mouse inoculation tests. Since mouse inoculation is the most sensitive method for detecting *T. evansi* (Verma & Gautam, 1978), although the HCT is more convenient for use in the field, both techniques were included in the investigation.

In order to perform the HCT and mouse inoculation techniques, blood is collected from animals suspected of being infected with trypanosomes for examination either in the field or in a nearby laboratory. Among the problems associated with processing blood samples in the same place as the animals are being bled, are the amount of equipment required in the field and the time available to the investigator if large numbers of animals are involved. It is often more convenient to store blood samples at 4°C until they may be processed in the laboratory. This study was undertaken in order to determine how long *T. evansi* can survive in blood samples in the presence of the anticoagulants ethylendiamine-tetraacetic acid (EDTA) or heparin.

MATERIALS AND METHODS

Five buffaloes (*Bubalus bubalis*) infected with *T. evansi* were selected as a source of infected blood. Samples of blood were collected from each animal into two 10 ml vacutainer tubes, one containing EDTA and the other heparin. The tubes were inverted to mix the blood and stored at 4°C. Aliquots of blood were taken from the vacutainers at different intervals and subjected to the HCT to detect live trypanosomes. Two glass capillaries were filled with blood and centrifuged in a microhaematocrit centrifuge at 12,000 rpm for 4 minutes and the leucocyte/plasma interface was examined for parasites using a x 10 objective. The level of parasitaemia in samples taken at different times was assessed by breaking the capillary tube 1 mm below the surface of the buffy coat and expelling one drop of the buffy zone material onto a microscope slide. A cover glass was applied to the drop and the sample examined for parasites using a x 40 objective with dark field illumination (Murray *et al.*, 1977). All the parasites observed in 20 microscope fields were counted and the parasitaemias converted to log equivalents.

In addition groups of 5 mice were inoculated with blood taken 8, 12, 24, 36 and 48 hours after the samples had been taken in order to determine the infectivity of the parasites. Mice were inoculated with 0.25 ml of blood via the intraperiteneal route and thereafter tail blood was examined each day for the presence of parasites.

RESULTS

Results of HCT examinations (Tables 1a, 1b) showed that parasites were detectable using the HCT for longer in samples containing EDTA than those containing heparin. Forty eight hours after blood was collected trypanosomes were detected in 5/5 of the EDTA samples but only 1/5 of the heparin samples. In both groups of samples the mean level of parasitaemia fell as the period of storage increased.

The results of mouse inoculations (Table 2) showed similar incubation periods when blood containing either EDTA or heparin was injected, however the period of incubation increased in relation to storage time in both groups. In the EDTA group the mean incubation period fell from 2.2 days, when blood which had been held for 8 hours was inoculated, to 4.0 days after 48 hours storage. The mean incubation period following inoculation of

Table 1a. Parasitaemia (Log equivalents) in wet preparations prepared from capillary tubes, EDTA blood

Animal number	Time after collection of blood samples (hours)							
	0.5	2.5	5.0	6.5	8.5	11.5	22.0	48.0
370	2.0	1.8	1.3	1.2	0.7	1.5	0.7	1.2
383	1.9	1.9	2.0	1.8	1.8	1.0	1.6	1.5
385	2.3	2.5	2.2	2.5	2.3	2.7	2.0	2.0
377	2.3	1.8	2.0	1.4	1.7	1.5	1.7	1.0
376	3.4	2.7	2.5	2.5	2.8	2.4	2.6	2.2
Mean	2.4	2.1	2.0	1.9	1.9	1.8	1.7	1.6

Table 1b. Parasitaemia (Log equivalents) in wet preparations prepared from capillary tubes, heparin blood

Animal number	Time after collection of blood samples (hours)							
	0.5	2.5	5.0	6.5	8.5	11.5	22.0	48.0
370	1.0	1.2	1.0	0.7	1.0	0.0	0.0	0.0
383	1.7	1.0	1.2	1.7	1.0	1.5	1.2	0.0
385	1.3	2.0	2.0	1.4	2.0	1.7	1.4	0.0
377	2.1	1.5	1.2	1.0	1.8	1.3	0.7	0.0
376	2.4	2.2	2.3	2.0	2.0	2.0	2.1	1.5
Mean	1.7	1.6	1.5	1.4	1.6	1.3	1.1	0.3

Table 2. Results of mouse inoculations

Sample inoculated	Average incubation period (days)	Average time to death (days)
8 hour E	2.2	6.8
8 hour H	2.0	6.2
12 hour E	3.2	8.2
12 hour H	3.4	8.4
24 hour E	2.8	7.0
24 hour H	3.0	7.4
36 hour E	3.8	7.8
36 hour H	3.8	8.2
48 hour E	4.0 (1 not infected)	8.5
48 hour H	5.5 (1 not infected)	9.3

heparinised blood fell from 2.0 days after 8 hours storage to 5.5 days after 48 hours. Blood which had been stored for 48 hours, failed to induce infection in 1 mouse in both of the inoculation groups.

DISCUSSION

The HCT examinations showed that parasites were detectable after a longer storage period if EDTA was employed as an anticoagulant compared to heparin. It was noted that following centrifugation the leucocyte/plasma interface was more clearly defined when EDTA was used, this greatly facilitated detection of trypanosomes in the capillary tubes.

The results of mouse inoculations indicate that the infectivity of parasitaemic blood declines as the period of storage increases. Niether of the anti-coagulants employed appeared to effect this process and similar results were obtained with EDTA and heparin. It was concluded that blood may be stored at 4°C for up to 24 hours before examination without loss in infectivity, and furthermore that EDTA was the preferred anticoagulant when using the HCT method.

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