INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

DURATION OF STORAGE AND TEMPERATURE ON THE VIABILITY AND INFECTIVITY OF *TRYPANOSOMA BRUCEI* IN OUTRED ALBINO MICE

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ABSTRACT
A study was conducted to investigate the adequate temperature and duration of preservation of *Trypanosoma brucei* organisms either from the field or from one laboratory, university or research institute to another for purposes of diagnosis, studies or cryopreservation. The viability and infectivity of *T. brucei* preserved at both room temperature (27ºC) and refrigerator temperature (4ºC) at different time intervals was examined. The viability test was based on the detection and mobility of the trypanosomes *in vitro* while the infectivity was based on the demonstration of patent infections in mice infected with trypanosome-infected blood. Trypanosome-blood preserved at room temperature was both viable and infective for up to six hours following collection from the donor mouse. However, it was viable for up to twelve hours post collection from the donor mouse The blood preserved at 4ºC was viable for up to thirty six hours post collection from the donor mouse and was still infective at twelve hours post collection. Parasitaemia in both storage temperatures decreased with time though this was faster in the trypanosomes stored at room temperature. It was therefore concluded that in the absence facility for cryopreservation facility, trypanosomes could be transported from fields or from one university, research institute or laboratory to another safely for periods of up to twelve hours at 4ºC. Researchers and other laboratory workers are warned of the risk of being infected if they contaminate themselves with infected blood since the trypanosomes can survive even in blood of dead animals for some hours. For the same reason, the need for proper disposal of carcases is reiterated.

KEYWORDS: Trypanosomes, preservation, viability, infectivity.

INTRODUCTION

Emphasis is usually laid on the importance of quick examination or inoculation of samples into laboratory animals or transfer to the laboratory especially field samples following collection. This requirement is so for larvae, trypanosomes and some other microorganisms as delay may lead to lose of viability and infectivity with possible escape of detection or proper identification. The samples are usually transported and/or preserved under some conditions including adequate temperature and storage medium. A lot of insect tissues and parasitic protozoa are usually stored at low temperatures (Minter and Goedbloed, 1971).Trypanosomes and other parasitic protozoans are usually stored in liquid nitrogen (-196ºC) using dimethylsulfoxide (DMSO) or glycerine as cryoprotectants (Mieth, 1966) as cited by Schuster and Mehlhorn (1996); Minter and Goedbloed, 1971).

Deep-frozen organisms are preferred in that changes in their infective, genetic or biochemical characteristics may not occur. Conventional methods of keeping parasites require i*n vitro* cultivation or frequent animal passages, which may cause alteration in virulence and/or genetic changes. However, the maximum period of storage for parasitic protozoa is not yet known. Racther and Siedenath (1972) as cited by Schuster and Mehlhorn (1996) have reported that trypanosomes were still both infective and virulent after 6 years of storage in liquid nitrogen. However, Schupp *et al.* (1980) as cited by Schuster and Mehlhorn (1996) reported that after another 7 years interval the said parasites had lost their infectivity as a result of freezing and thawing and also due to ultrastructural changes. Infective larvae of *Dipetalonema viteae* have been shown to produce infections in Mongolian jirds (*Meriones unguiculatus*) after storage of infected ticks (*Ornithodoros tartakovskyi*) in the presence of dimethyl sulfoxide (DMSO, 5%) for 7 or 595 days in liquid nitrogen (-196 ºC) (McWall *et al.*, 1975). Also, infective larvae of *Heligmosomoides bakeri (polygyrus)* stored in aqueous suspension for a few days at room temperature or up to 8 months at 4ºC (in the refrigerator) were still viable and infective in mice (Kerboeuf, 1978).

Although cryopreservation have obvious advantages as stated above it may not be readily applicable in some environments due to the fact that it requires very low temperatures which can only be maintained with low uninterrupted power supplies. Such continuous electricity supply may not be easily maintained in some developing countries. Apart from the commonly known cryopreservation and animal passage, other studies have not been well documented for storage/handling of parasites especially trypanosomes at least for short term purposes especially in the tropical environments where temperatures are usually high and electricity supply needed to maintain low temperatures is usually erratic. The present study was therefore designed to investigate the feasibility of developing a more flexible short term method of storing and/or transporting trypanosomes in environments with erratic electricity supply. Furthermore, the risk of possible accidental infections of researchers handling these samples such as during necropsy/autopsy

was examined. Also examined was the possibility of improperly disposed infected carcases serving as sources of infection hours after death since *Trypanosoma brucei* have been detected the in the blood of *T. brucei*-infected mice hours following death and was also found to be viable and infective (Ngongeh and Musongong, personal communication).

MATERIALS AND METHODS

Infection with *Trypanosoma brucei*

A donor mouse with high parasitaemia of $\log_{10}$ 8.1 was anaesthesized with diethyl ether (May and Baker, Ltd., England). 1ml of blood was taken from the heart of the mouse and put into a bijou bottle containing 500µl of ice-cold normal saline, which contained 2µl of heparin. The parasitaemia of the diluted heart blood was assessed according to the method of Herbert and Lumsden (1976) and adjusted to the working dose of 2 x 10^6 organisms in 0.2ml of blood. The blood dose preparations with normal saline were carried out on ice. Each of the three *T. brucei*-infected mice received this dose by intraperitoneal inoculation on D0. The remaining diluted blood containing the parasites was then divided into two parts. A fraction was preserved at room temperature while the other fraction was preserved at 4ºC in the refrigerator. Some of the blood stored at 4ºC was inoculated into three mice each at 6, 12, 18, 24, 30, 36, 48, 54 and 60 hours period. The same was done for the blood preserved at room temperature (25ºC). Each time before inoculation the blood was examined for the presence and motility of *T. brucei*.

Trypanosoma brucei parasitaemia

Mice were bled from the tail for the estimation of parasitaemia according to the method of Herbert and Lumbsden (1976). At higher levels of infection, microscopic fields (x400 magnification) of wet blood film were matched against standard charts, but where fewer organisms were present, the number of *Trypanosoma* in 5, 10, or 20 such microscopic fields were counted and the equivalent number in one millilitre of blood was read from a standard table (Herbert and Lumbsden, 1976). The number of organisms per field was estimated and expressed as $\log_{10}$ per millilitre of blood. The parasitaemia of infected mice was monitored daily from day (D) 3 following infection with *T. brucei* until the trypanosome infection became patent. Thereafter, parasitaemia was monitored every two days for 10 days.

Packed cell volume

Packed cell volume (PCV) was carried out after every four days beginning from day zero (D0) till the end of each study (D20). Mice were bled from the tail directly into heparinized capillary tubes (Camlab Ltd, Cambridge).

RESULTS

The parasitaemia was estimated to 8.1 at 0, 6 and 12 hours and 7.8 for blood preserved at 4ºC. At 24, 30 and 36 hours the parasitaemia dropped to 7.8, 7.5 and 7.2 respectively for blood preserved at 4ºC. Blood stored for 48-60 hours at 4ºC post bleeding showed no trypanosomes. Trypanosomes were detected at 400 magnification (microscopically) only at 0 and 6 hours for blood preserved at room temperature (25ºC) and the parasitaemia was estimated at 8.1 and 7.5 at 0 and 6 hours respectively. Motility of the parasites decreased with increased duration of storage of the blood until 36 hours when trypanosomes were lastly detected under the microscope for blood stored at 4ºC. The parasitaemia decreased with time up to when it could no longer be detected microscopically for blood preserved both at 4ºC and 25ºC.

All mice inoculated with blood preserved for zero and six hours at 4ºC and 25ºC had patent infections on D4 while the mice inoculated with blood preserved for 12 and 18 hours at 4ºC became patent on D6 and D8 respectively post inoculation. None of the mice inoculated with blood preserved for 24 hours and above had patent infection. Once patent the mice inoculated with blood 6, 12 and 18 hours post collection had the same pattern of parasitaemia and PCV which was similar to those of mice inoculated with the blood just following collection (blood stored for zero hour) as shown on Figures 1 and 2.

Packed cell volume

The packed cell volume of all infected mice depreciated in comparison to those of the uninfected control mice. The pattern of PCV was the same in infected mice though it tended to be lower in as the duration of storage of blood decreased.

FIGURE 1. Parasitaemia of mice inoculated with *T. brucei*-infected blood stored at different time intervals following collection from the donor mice.

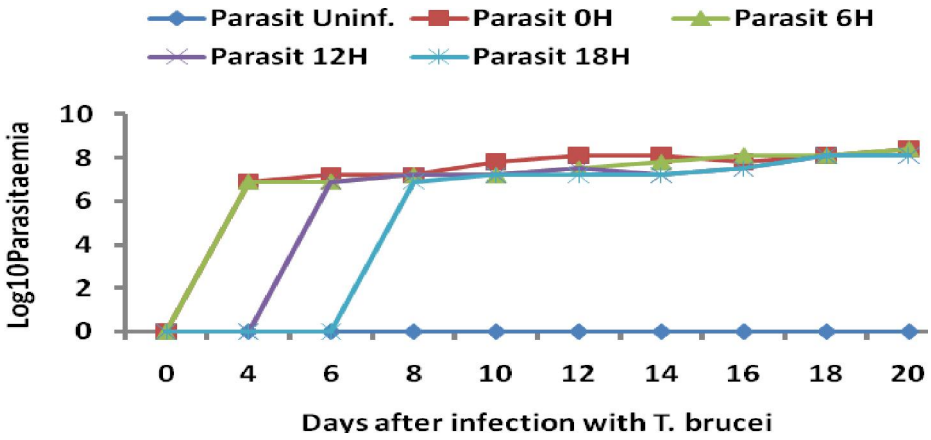
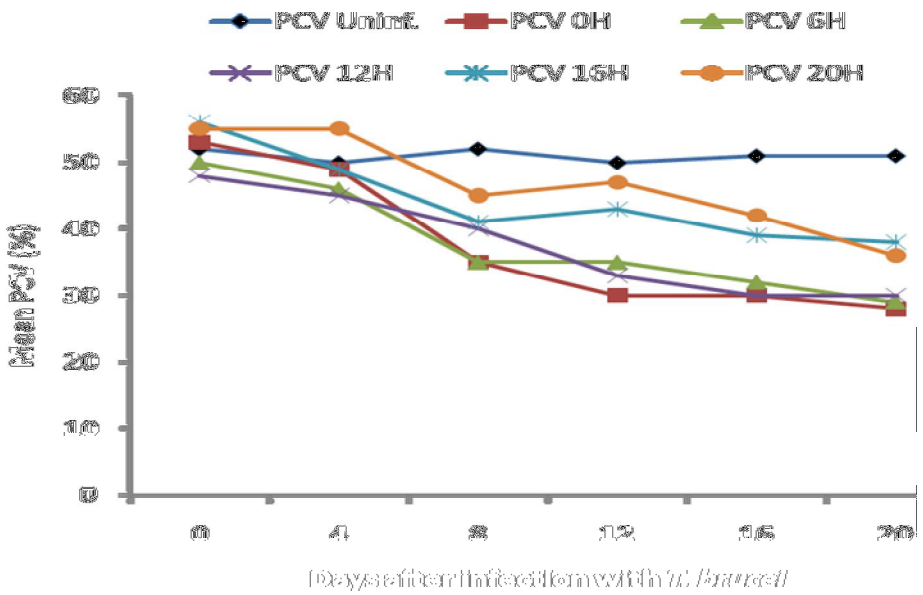


FIGURE 2. Mean PCV of mice inoculated with *T. brucei*-infected blood stored at different time intervals following collection from the donor mice.



DISCUSSION

It was observed that the viability and infectivity of *T. brucei* based on motility *in vitro* and subsequent establishment in outbred albino mice respectively were both time and preservation temperature dependent. Motility of the trypanosomes in whole blood preserved at 4ºC decreased with increased preservation period up to 30 hours post collection of blood. After this time trypanosomes were no longer detected microscopically. In blood preserved at room temperature, motility decreased gradually and by 12 hours no parasites were microscopically detectable.

Mice inoculated with trypanosome infected blood preserved at 4ºC and at room temperature (25ºC) for up to 6 hours became patent of the infection reflecting that the parasites were still infective. However, only trypanosome-infected blood stored at 4ºC for up to 12 hours was still infective shown by the fact that mice inoculated with such blood lead to patent infection while mice inoculated with trypanosome infected blood preserved at 25ºC for 12 hours did not develop patent infection. Trypanosomes were also detected in blood preserved at 4ºC both at 24 and 30 hours post bleeding of the of the donor mice but were not more infective as patent infection was not established in the inoculated mice. A patent infection has been established in mice inoculated with blood from *T. brucei*-infected blood collected from a mouse that died 5-6 hours earlier. This is a situation similar to infecting mice with blood stored at room temperature for six hours before inoculation. In a field study that was designed to screen west African dwarf goats for trypanosome infections, trypanosomes were detected in the blood samples collected from the animals and preserved under continuous cold conditions (4ºC) after 30 hours. However, the motility of trypanosomes in whole blood was shown to decrease with increase duration of storage, only highly parasitaemic blood could still yield detectable levels of trypanosomes after such a long period of preservation. Long preservation might thus increase the chances of recording false negatives especially in low parasitaemic blood when the animals were actually positive of the infections.

It is therefore suggested that to ensure fairly reliable results by microscopical detection, blood samples from suspected cases should be examined for the presence of trypanosomes preferably not more than twelve hours following collection of the samples. It also implies therefore that for any trypanosome to establish in experimental animals, blood samples preserved in 4ºC should be inoculated not later than 12 hours after bleeding suspected cases or donor animals. The earlier the inoculations, the better since motility which can be considered partly as an indicator of viability tends to decrease with increasing time of storage. The decreasing motility and hence viability over time might be due to depletion of energy reserves of the parasites as their metabolic activities would be relatively high unlike those stored in liquid nitrogen (-196ºC) where the metabolic activities may be totally arrested. Such energy depletion seemed to be faster at room temperature compared to refrigerator conditions due to the higher activity and hence higher metabolic rate of the former compared to the latter. At both 4ºC and 25ºC the decrease of parasitaemia with time up to when it could be no longer detected microscopically could also be due to energy depletion and subsequent mortality. The number of third stage larvae recovered from faeces of nematode infected sheep exposed to 4ºC was shown to decrease as the initial period of exposure of the faeces to the low temperature increased (McKenna, 1998).

The results of this study also serve as a caution against mechanical transmission of trypanosomes to laboratory workers who usually handle blood or blood contaminated equipments as they can become infected with the parasites and other blood pathogens. It also shows that dead infected animals could serve as sources of infections to other susceptible hosts. Therefore ailing and dead infected animals should be adequately disposed and not just thrown into surrounding bushes as is practiced by some

researchers of laboratory animal handlers. Finally, temperature and duration of preservation of trypanosomes *in vitro* are thus crucial to the viability and infectivity of the parasites. With these results therefore, the movement/transfer/transport of trypanosomes for example from one research institute, university or laboratory to another for study or dianostic purposes should therefore maintained at 4ºC and moved and used within 12 hours for good results to be achieved.

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