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A simple and convenient way of transporting *Trypanosoma congolense*

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ABSTRACT

Objective: In Nigeria Trypanosomes are transported from one research laboratory to another by inoculating into live animals or carried in canisters of liquid nitrogen. This study investigated alternative ways of transporting the parasite.

Methodology and results: Blood obtained from a heavily parasitemic rat ($1x10^8$) per/ml blood was diluted at a ratio of 1:0.5 in phosphate buffer saline glucose and phosphate buffer saline in Eppendorf tubes and stored at 4°C and 27°C, respectively. In both buffers, parasites stored at 27°C did not survive after 12 hr. Parasites in phosphate buffer saline glucose stored 4°C survived for up to 7 days.

Conclusions and application of findings: The findings indicate that *T.congolense* could be kept at low temperatures in blood - phosphate buffer saline glucose or nutrient medium supporting growth of the parasites and transported over long distances instead of using live animals which could be susceptible to stress.

Key word: *Trypanosoma*, dilution buffers, storage temperature, survival.

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INTRODUCTION

African tryanosomes are the protozoan parasites responsible for Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) (Igbokwe, 1990; Onah, 1992; Kuzoe, 1993; WHO 2000; Adewunmi *et al.,* 2001; Hoet *et al.,* 2004). Trypanosomiasis is caused by *Trypanosome congolense, T. vivax, T. brucei, T. siminae* and *T. evansi* in animals and *T. gambiense and T. rhodiense* in man. Due to their great economic importance (Kuzoe, 1993; WHO, 1998; Kristjanson et al., 1999) a lot of research on this parasite is ongoing in Africa presently (Igweh & Onabanjo, 1989; Nok *et al*., 1996; Adewunmi *et al.,* 2001; Awotunde, 2002; Atawodi *et al.,* 2003; Ogunsanmi & Taiwo, 2004; Wurochekke & Nok, 2004; Nok, 2005; Maikai *et al*., 2007).

In Nigeria, inoculated animals, mostly goats, rats and mice are the main means by which trypanosome is transported from the research laboratories of the Nigeria Institute of

Trypanosomiasis (NITR) at Vom, Plateau State to other research institutions in the country. At NITR the parasites are cryopreserved in liquid nitrogen. The parasites are alternatively transported while preserved in canisters of liquid nitrogen.

Transporting inoculated animals by road across the various ecological and climatic zones imposes enormous stress upon them. Even when they are transported by vehicle, the movement of the vehicle can cause motion sickness and stress to the animal (Nicol & Saville-weeds, 1993; Warriss 1996; Hartung 2003; Odore *et al.,* 2004), which leads to morbidity and mortality. This paper describes an alternative simple, convenient and cost effective method of transporting trypanosome without using live animals.

MATERIALS AND METHODS

Parasite: *T. congolense* (Nasarawa strain) was obtained from stabilates maintained at the Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The Parasite was inoculated into rats and transported to our laboratory. The method of Herbert and Lumsden (1979) was used to confirm the presence of the parasite in the rat.

Preparation of buffers: Phosphate buffered saline (PBS) was prepared by mixing Sodium hydrogen Phosphate (13.48g), sodium dihydrogen phosphate (0.78g) and Sodium chloride (NaCl) 4.35g. Ingredients were weighed separately and placed into a flat bottom flask in which they were dissolved and made to the 1000ml mark using distilled water. The pH was adjusted to 7.2. Phosphate buffered saline glucose (PBSG) was prepared by weighing 1g of glucose, dissolving it in 60ml distilled water and making to the 100ml mark with 40ml of phosphate buffer saline.

Experimental design: Blood from heavily infected parasitemic rat was obtained from the tail after pre-sterilizing with alcohol, and mixed immediately with PBS and separately with PBSG at a ratio of 1:0.5. One millilitre of the infected blood diluted with PBSG or PBS was placed in sterile Eppendorf tubes labeled as Group A (1ml PBSG + infected blood stored at 4°C); Group B (1ml PBS + infected blood stored at 4°C); Group C (1ml PBSG + infected blood kept at room temperature, i.e. 27°C) and Group D (1ml PBS + infected blood kept at 27°C). Samples were taken in triplicate and each sample was checked for the presence of the parasites by examining a drop under microscope (x400 magnification) before storage.

RESULTS AND DISCUSSION

On day 1 after storage all the parasites were active and motile in both the PBS and PBSG medium (Table 1). However, from day 2 to day 7 only parasites in Group A were alive and active, though their population was steadily decreasing over time (Fig.1). All parasites in groups B, C and D were dead on day 2 after storage.

Parasite motility constitutes a relatively reliable indicator of viability among most zooflagellate parasites (Peter *et al*., 1976). Atawodi *et al.,* (2003) and Maikai *et al*. (2007) reported that trypanosomes could survive in phosphate buffer saline for up to 4 hours or more. Our findings showed that *Trypanosome congolense* was able to survive in phosphate buffer saline glucose and phosphate buffered saline at room temperature (27°C) for 12 and 5 hours, respectively. When stored at 4°C in a refrigerator, the trypanosomes survived and remained active in phosphate buffer saline glucose for up to 7 days as compared to 1 day in phosphate buffer saline. At room temperature (27°C) the parasites could not survive for long in phosphate buffer saline due to active movement and high metabolic activities which rapidly exhausted glucose supplies in the blood. The parasites in phosphate buffer saline glucose survived for much longer periods due to the additional glucose in the PBSG medium.

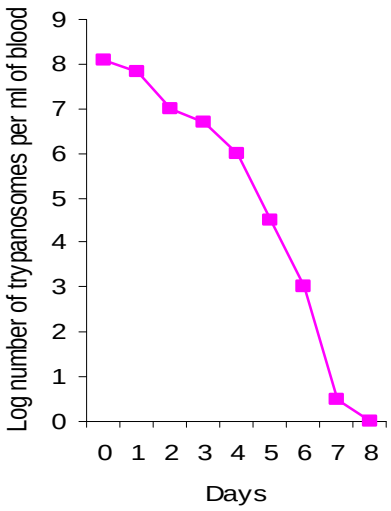

Figure 1: Graph showing the relationship between population of parasites in PBSG and time.

Trypanosomes depend on the host for supply of carbohydrates, protein, lipids and some micronutrients (Igbokwe, 1995). Trypanosomes in the blood stream generate energy primarily by breaking down exogenous glucose during metabolic activities in the glycosomes (Gutteridge & Combs, 1977; Fairlamb & Opperdoes, 1986). In our study, the parasites could not survive for more than one day in PBS at 4°C possibly because they had exhausted the glucose available in the blood. In PBSG medium, the parasites survived for upto 7 days because the low temperature (4°C) substantially reduced their metabolic activities, thus also slowing down enzymatic breakdown of glucose. Thus at the low temperature the extra glucose lasted for a longer time period. Besides of the rapid depletion of nutrients, production of toxic metabolic waste by the parasite could also contribute to their decline in the preservation media.

The findings of this study are important in that animals do not have to be used as transport conduits for trypanosome over long distances between laboratories. The parasite can be inoculated into blood diluted with PBSG in small Eppendorf tubes which are light and much more safer to carry over long distances in an ice cool box or an ice packed thermos flask. This will not eliminate stress to animals but also the potential risks of spreading other animal diseases between different regions in the country.

Table 1. Survival of trypanosome parasite in blood-PBSG and blood-PBS.

Treatment group	Sampling days						
	1	2	3	4	5	6	7
A	++++	++++	+++	+++	++	++	++
B	++++	-	-	-	-	-	-
C	++++	-	-	-	-	-	-
D	++++	-	-	-	-	-	-

++ : parasites very motile and active; -parasites absent.

REFERENCES

Adewummi CO, Agbedahunsi JM, Adebajo AC, Aladesanmi AJ, Murphy N, Wando J, 2001. Ethno-veterinary medicine: Screening of Nigerian medicinal plants for trypanocidal properties. Journal of Ethnopharmacology 77: 18-24.

Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M, 2003. In vitro trypanocidal effect of methanolic extracts of some Nigerian savannah plants. African Journal of Biotechnology 2 (9): 317-321.

Awotunde J0, 2002. Trypanocidal effect of *Ricinus communis* on *Trypanosoma brucei* invivo. The zoologist 1 (1): 95-99.

Fairlamb AH. and Opperdoes FR, 1986. Carbohydrates metabolism in African trypanosomes with special reference to the glycosome. In: Carbohydrate metabolism in cultured cells (Morgan MJ. (ed)). New York, USA, Plenum.

Gutteridge WE. and Coombs GH, 1977. Biochemistry of parasitic protozoa. Macmillan Press, London.

Hartung J, 2003. Effects of transport on health of farm animals. Veterinary Research communications 27: 525-527.

Herbert WJ. and Lumsden WHR, 1976.*Trypanosoma brucei*. A rapid matching method for estimating the host's parasitemia. Experimental parasitology 40 :427-431.

Hoet S, Opperdoes F, Brun R, Adjakidje V, Quetin L' eclercq J, 2004. In vitro antitrypanosomal activity of ethnopharmacologically selected Beninese plants. Journal of Ethnopharmacology 91: 37-42.

Igbokwe IO, 1990. Biochemical approaches to the pathogenesis of trypanosomisis. OBABS proceedings, University of Maiduguri, Nigeria 3:94-103.

Igbokwe JO, 1995. Nutrition in the pathogenesis of African. Trypanosomiasis.*Protozoloogy Abstracts*. Vol. 19 (12): 797-808.

Igweh AC. and Onabanjo AO, 1989. Chemotherapeutic effect of *Annona Senegalensis* in *Trypanosoma brucei brucei*. Annals of Tropical Medical Parasitology 85 (5): 527-534.

Kristjanson PM, Swallow BM, Rowland G, 1999. Measuring the cost of African Animal Trypanosomosis, the potential benefits of control and returns and research. Agr. Sys 59: 79-98.

Kuzoe F, 1993. Current Situation of African Trypanosomiasis. Acta Tropica 54: 153-162.

Maikai VA, Nok AJ, Adaudi AO, Alawa C, 2007. *In vitro* antitrypanosomal activity of aqueous and methanolic crude extracts of stem bark of *Ximenia americana* on *Trypanosoma congolense*. African Journal of Biotechnology 2 (3): 55 - 58.

Nicol C. and Sville–Weeks C, 1993. Poultry handling and transport. In Livestock Handling and Transport. Grandlin T. (Ed). Wallingford, CAB International.

Nok AJ, Williams S, Onyenekwe PC, 1996. *Allium sativum* induced death of African trypanosomes. Parasitol. Res. 82: 634-637.

Nok AJ, 2005. Effective measures for controlling trypanosomiasis. Expert Opinion. Pharmacotherapy 6 (10): 1-9.

Odore RD, Anselo A, Badino P, Bellino C, Pagliasso S, Re G, 2004. Road transportation effects blood hormone levels and lymphocytes glucorticoids and andrenergic receptor concentration in calves. Veterinary Records 168: 297-303.

Ogunsanmi AO. and TaiwoVO, 2004. Comparative studies on erythrocyte calcium, potassium, haemoglobin concentration, osmotic resistance and sedimentation rates in grey ducker, sheep and goats experimentally infected with trypanosoma. *Veterinaski ARHIV* 74(3): 201-216.

Onah DN, 1991. Porcive trypanosomiasis in Nigeria: Infections in local and exotic pigs in the Nsukka area of Anambra State. Tropical Animal Health and Production 23: 141-146.

Peter D, Honigberg BM, Fern AM, 1976. An improved method of cryopreservation of *Trypanosoma (Nannomonas) Congolense* brooden in liquid nitrogen.The Journal of Parasitology 62 (1): 136-137.

Warriss PD, 1996. The welfare of animals during transport. The Veterinary Animal 36: 73-85.

WHO, 1998. Control and surveillance of African Trypanosomosis. World Health Organisation. Technical Report Series No. 881

WHO, 2000. African Trypanosomiasis. In: Report on Global surveillance of Epidemic – prone infectious diseases. WHO/CDS/CSR/ISR/2000.

Wurrochekke AU. and Nok AJ, 2004. In vitro antitrypanosomal activity of some medicinal plants used in the treatment of trypanosomosis in Northern Nigeria. African Journal of Biotech. Vol. 3 (9). 481-483.