J Vector Borne Dis 48, June 2011, pp. 93–95

Storage time effect on blood diet for tsetse mass production in sterile insect technique

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ABSTRACT

Background & objectives: Different methods have been developed for tsetse and trypanosomiasis control or eradication but all these have their specific advantages and limitations. However, a combination of methods could be used as part of an Area-wide Integrated Pest Management (AWPM). The sterile insect technique (SIT) as a technique of choice for eradication of tsetse flies requires mass production of flies in the laboratories. Since tsetses are exclusively haemotophogous insects, a quality blood diet is needed for maintenance of flies with optimum production. The aim of this study was to establish the optimum storage time of blood diet for tsetse colonies.

Methods: A total of 450 flies *Glossina austeni* were fed on different batches of blood (collected in 2004, 2005, 2006, 2007 and 2008) three times a week. Pupae were collected daily and mortality checks were done on weekly basis. The quality of the blood diet was measured by observation of tsetse production parameters including survival, pupae production and size.

Results: The survival of flies fed on 2004 and 2006 batches had lower survival compared to the rest of the batches. Also the 2005 batch had a significant higher number of pupae compared to the production in other treatments. The 2004 and 2005 batches had more of small pupae (class A & B) compared to the other batches.

Interpretation & conclusion: There was a significant difference between the blood batches and the production parameters were better in the last three years, i.e. 2008, 2007 and 2006. Therefore, this study recommends three years to be an optimum storage time for blood diet under regional conditions. Also the storage temperature conditions should remain stable at –20ºC.

Key words Blood quality; bovine blood; *Glossina austeni*; mass rearing; SIT; storage time

INTRODUCTION

Tsetse fly is a vector of Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT). Both sexes depend on blood for survival and reproduction. In the process of feeding, they acquire and transmit trypanosomes from infected to uninfected hosts. Both wild and domestic animals have been found to play a significant role in the epidemiology of sleeping sickness[1]. However, rural people living in regions where transmission occurs due to the nature of their activities (agriculture, fishing, animal husbandry or hunting) are most exposed to the bites of the tsetse fly and therefore to the disease.

Tsetse flies are largely responsible for an uneven distribution of cattle in Africa, leading to overgrazing and severe environmental degradation in some areas and preventing the introduction of productive farming and livestock systems in other areas[2]. According to the World Health Organization, over 55 million people living in rural areas of sub-Saharan Africa are at risk of contracting sleeping sickness (human trypanosomiasis)[3]. In Tanzania, on an average of 400 cases are reported annually[4].

There are different methods developed for tsetse and trypanosomiasis control or eradication but all have their specific advantages and limitations. The currently available and environmentally acceptable methods include parasite control by trypanocides, vector intervention using traps and insecticide-treated devices and special formulations of insecticides on livestock. Others are entomological and intend to disrupt the transmission cycle by reducing the number of tsetse flies. These include Sequential Aerosol Technique (SAT) and Sterile Insect Technique (SIT). A combination of several of the above methods could be used as part of an Area-wide Integrated Pest Management[5].

SIT as a technique of choice, requires mass production of tsetse flies in the laboratories. Therefore, quantitative amount of blood is needed for maintenance of fly colonies[6]. In Tanzania, colonization of tsetse species started 1972 at the Tsetse and Trypanosomiasis in Research Institute (TTRI) in Tanga. The first species to be colonized was *Glossina morsitans morsitans* using *in vivo* feeding (live animals such as goats, rabbits and guinea pigs). However, it was difficult to maintain big colonies of tsetse flies by using the *in vivo* system due to high costs. Further, the development of a new technique, the *in vitro* feeding en-

abled the Institute to rear >900,000 *G. austeni* in collaboration with joint IAEA/FAO from 1994–97 for the eradication of *G. austeni* in Zanzibar using SIT[7]. The successful completion of the tsetse eradication project in 1997 declared Zanzibar a tsetse free zone, and this led the African Heads of States to launch a tsetse eradication campaign for all tsetse infested countries called "Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)".

Currently, other eradication programmes are being planned employing large-scale SIT on the mainland of Africa like Ethiopia. Further, TTRI is continuing to rear flies for local use and at the same time providing expertise to people from different countries on tsetse mass rearing techniques.

Mass production of good quality sterile males for SIT requires the blood diet with good quality. This is very important due to the fact that both males and females (also the larvae within the uterus) depend on the blood for their survival and nourishment. However, there are several factors that could influence the quality of the blood diet, like nutritional status of animals, contamination (bacteria or chemicals) and storage time/conditions. This work investigated on the effects of storage time under local conditions on the blood quality.

MATERIAL & METHODS

Five batches of blood collected from three different geographically located abattoirs in 2004, 2005, 2006, 2007 and 2008 as a control were gamma sterilized, tested and frozen at –20°C. Experimental flies *G. austeni* (n = 450) were kept in cages (each 30 flies) and fed for a period of 30 days. Each batch was replicated three times. In order to avoid contamination, sterillium or normal spirit was used to clean the hands before pouring of the blood on feeding plates. Fly feeding was done three days per week on the blood heated at 36.7°C. After feeding, the plates were cleaned and oven sterilized at 120°C for approximately 12 h. Flies were maintained under laboratory conditions of temperature 23 ± 1°C with the humidity ranging between 75 and 80% RH. Mortality check was done on weekly basis and pupae collection was done daily while checking on abortions.

The production parameters including fly survival, pupae production and size were used to measure the quality of different batches of blood.

RESULTS & DISCUSSION

There was a significant difference in fly survival for the batches of 2004 and 2006, as the flies had lower survival compared to the rest of the batches. Also, 2005 batch was significantly different from the rest and had higher percentage of surviving flies (Table 1). For pupae production, the 2007 batch had significantly high number of pupae compared to the production in other treatments. The average number of pupae decreased from the 2004 batch to 2006 batch and then increased for 2007 batch and then decreased for 2008 (Fig. 1). Pupae size distribution in terms of weight (Fig. 2) the curves in all treatments showed peaks in class C though the 2007 batch had more pupae than other batches.

Bioassays conducted by feeding flies on different batches of blood from 2004 to 2007 compared to the 2008 batch showed a significant difference (p <0.05) in fly survival and pupae production. Survival percentages were low for the batches of 2004 and 2006. The flies that fed on batch 2005 had higher survival percentage but the number of pupae produced was lower compared to batches of 2007 and 2008 respectively (Table 1 & Fig. 1).

In these experiments, it was generally observed that the performances of flies were not good and this was due to the holding conditions in the insectaries. Yet, still ob-

Table 1. Fly survival and production in tsetse fly colonies fed on different batches of blood (Mean ± S.D.)

Year of collection (Batch)	Fly survival (%)	Pupae production
2004	82.0 ± 7.2^{b}	19.3 ± 3.1^{b}
2005	93.0 ± 3.0^{a}	18.7 ± 1.5^{b}
2006	82.7 ± 5.8^{b}	16.7 ± 3.5^{b}
2007	87.7 ± 4.0^{ab}	25.7 ± 1.5^{a}
2008	88.7 ± 2.3^{ab}	20.3 ± 2.1^{b}

Means within column with different superscript letters are significantly different at p <0.05 following means separation by Duncan Multiple Range Test (DMRT); S.D. = Standard deviation.

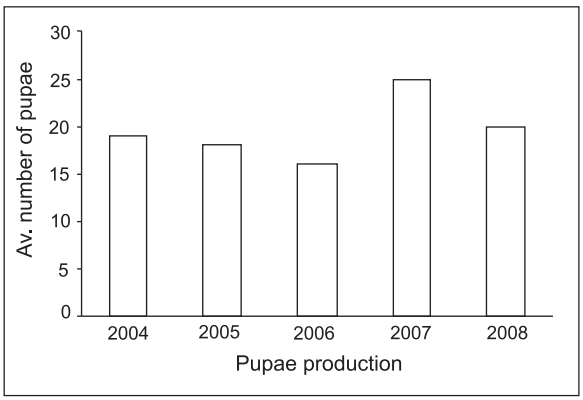

Fig. 1: Average number of pupae produced in colonies fed in different batches of blood.

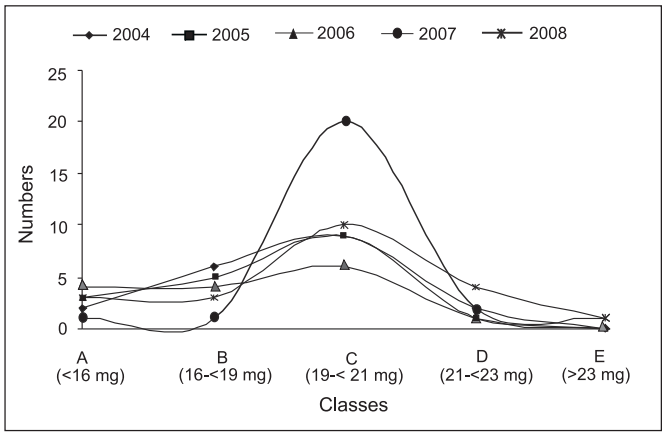

Fig. 2: Average pupae classes (size/weight) fed on different batches of blood.

served differences between the batches gave an indication that the storage time of blood diet has an effect on the fly performance.

For the normal growing colony, it is expected that pupae classes or weights should express a normal distribution curve where most of the pupae are expected to be in class C and <10% in class A. The distribution in Fig. 2 was normal but the numbers of pupae were very few in all the treatments with exception of batch 2007. However, the flies fed on batches 2004 and 2005 had more pupae in classes A and B (which means low pupae weights) compared to batches 2006, 2007 and 2008.

To some extent these results show a trend on the decrease of the measured parameters in relation to the storage time especially with regard to pupae number and sizes. This study concludes that under stable storage conditions the blood diet could be stored for at least three years without affecting the quality. This could be adopted as an optimum storage time under regional conditions. It is important that optimum storage conditions (temperature –20ºC) should be maintained throughout the storage period. In addition to this, blood should be kept intact for that period; no thawing and freezing unless it is to be used at once. Therefore, the size of storage containers should be considered when intending to keep the blood for that long in relation to the fly colony size.

An added advantage of the establishment of the storage time is for easier management of tsetse colonies and plans could be made to collect and have enough amount of blood in stock for a specific time and thus reduce inconveniences and minimize the rearing costs.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to the Director of TTRI, Dr A. Msangi for the guidance provided through this work. Also, the assistance of the IAEA for providing funds under the CRP is highly appreciated.

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Received: 11 October 2009 *Accepted in revised form:* 14 March 2010