Letter to the Editor

Improved protocol for aseptic collection and handling procedures of bovine blood diet in areas with special contamination challenges for use in tsetse rearing

Importation of blood to support tsetse feeding in Africa is costly and marred by delays, quarantine restrictions and other logistic problems,[1] while chemical contamination is likely to be encountered in tsetse diet from drug residues arising from treatment of animal diseases in Africa. Further, blood diet collection procedures are not standardized risking the health of the donor animals. We established a blood collection procedure of bovine blood for *in vitro* tsetse feeding in areas with special contamination challenges that is safe for both donor animals and tsetse flies. Six Orma Boran steers (registered at the Kenya Stud Book, Boran Cattle Breeders Society 2009, Kenya) aged between 12 and 16 months supplied from Kenya Agricultural Research Institute–Trypanosomiasis Research Centre (KARI-TRC), delivered on foot, thoroughly washed with water using a soft brush (PVC, LG Harris and Co (EA) Ltd, Nairobi, Kenya) to remove possible pesticide contamination, de-wormed with 10% albendazole and acclimatized for one week before collection of blood could start. The criteria for the selection of steers were healthy looking animals above six months of age and animals recently exposed to treatment, especially antibiotics, were excluded.[2] The animals were confined in a well-ventilated, fly-proof barn house partitioned into eight metallic cubicles each measuring $3 \times 4.3 \times 2.5$ (height) m with concrete floor and wood chippings provided as bedding material. The steers were kept in two adjacent cubicles in groups of three animals each separated by round metal cross bars. The animals were kept in a locked room under natural light; room temperature and humidity were not regulated. The steers were maintained on 9–10 kg/animal/day dry fodder (Hay, KARI-TRC, Kikuyu, Kenya), 1 kg/animal/day concentrates (Ranch cubes®, Unga Feeds Ltd, Nairobi, Kenya), 2 kg block/animal/month mineral salts (Maclick®, Cooper Kenya Ltd, Nairobi, Kenya), 5 kg/animal/day green fodder (Nappier grass, KARI-TRC). Mains tap untreated water was provided *ad libitum*. Blood collection from the six steers was carried out once a month for a period of four months. Animal handling protocols and procedures used were reviewed and approved by the KARI-TRC Institutional Animal Care and Use Committee. Body weight, pulse rate, rectal temperature, respiratory rate, rumen movement and packed cell volume were monitored before and after the experiment and found to have no clinical abnormalities. Prior to each bleeding session, the steers were transferred to the restraining crush (Tubar® Cattle Crush, manufactured by HJ Urry & Son Ltd, Alveley, Bridgnorth, Shropshire, UK) situated within the barn house. After the study, the steers were recruited back to the resident herd at KARI-TRC. Plastic, glass and metallic ware used were sterilized and stored as described earlier,[2] while personnel wore waterproof overalls, sterile gloves, face masks and head caps. Blood collection conformed to guidelines described earlier[3] to avoid animal exsanguination. We collected blood from the same steer once a month provided 10% of the total blood volume was not exceeded. Bleeding on a weekly basis should not exceed 7.5% of the total blood volume. Total blood volume was calculated by multiplying circulating blood volume (60 mL/kg) by the animal's body weight.[3] Fresh blood was aseptically collected by jugular venepuncture[4] over a period of four months. The distal end of the jugular vein was cleaned with methylated spirit, blocked with the thumb to ensure engorgement then punctured using a sterile 14-gauge needle and blood allowed to freely flow via a foot long delivery tube into a 2 L conical flask containing 800 g of 5–8 mm diameter glass beads with continuous swirling for at least 10 min to ensure complete de-fibrination. The processing and bacteriological screening of the blood diet was carried out as described elsewhere,[2,4] but with modification. The standard nutrient agar plated Petri dishes were incubated at $36 \pm 1^{\circ}C$ for 72 h instead of the normal 48 h prescribed earlier[3] and any contaminated blood discarded. To evaluate its suitability, 200 teneral female *Glossina morsitans morsitans* (Westwood) flies (parental generation colony) were allowed to feed for 10 min daily on the blood diet for five days a week through a silicone membrane.[4] Three-day-old females were mated with 11-day-old males in the ratio 1:1 for 24 h and the females retained for larviposition while the males were discarded. The F_1-generation flies were maintained as described above and the tsetse-specific colony parameters of the two groups determined. The pupae were weighed individually using an electronic balance and the female fecundity, pupal emergence, mortality and abortion rates determined as described earlier.[2,5] Pupal weight class was taken as Class A (<18 mg), B (18–22 mg), C (23–26 mg), D (27–30 mg) and E (>30 mg). The 95% confidence intervals of % blood diet harvest and colony performance parameters were calculated using Microsoft Excel computer program. The mean (standard error) percentage recovery of blood diet (volume of de-fibrinated blood/volume of whole blood) was 79.2 (1.7)% with a 95% confidence interval of 75.9–82.6% after 24 bleeding sessions and the tsetse fly colony parameter values of the parental and F_1-generation flies are shown in Table 1 and which are acceptable for the flies as prescribed earlier.[2,4] The blood diet collection and handling method described here is therefore suitable for small-scale tsetse rearing operations in areas with special contamination challenges. It is optimal for blood collection involving resident/laboratory steers and emphasizes on the welfare issues of these animals. However, since livestock industry in Africa is more challenged by disease(s), it may be necessary to incorporate screening techniques for chemical contamination as a complementary component during tsetse diet collection. We thank Mr John Mungai, Director KARI and the

Table 1 Performance parameter values of tsetse flies maintained on the bovine blood diet expressed as 95% confidence intervals

Colony parameter	Parental colony	F_1-generation colony
Pupae emergence rate (%)	89.2–99.6	86.7–91.5
Female mortality (%)	1.2–3.9	2.4–6.1
Male mortality (%)	2.8–9.8	–
Fecundity	0.7–0.9	0.6–0.8
Weight of pupae	27.3–27.7	26.2–26.4
Weekly abortion rate (%)	0.3–1.0	0.8–1.8

The Class A pupae portions were 1.1% and 2.6%, respectively, for parental and F_1-generation colonies

Government of Kenya for providing tsetse rearing expertise, permission to publish these findings and funding this work, respectively.

J K Kibugu, J M Kiragu, A M Mumba, D M Mwangangi and M W Muchiri
Kenya Agricultural Research Institute–Trypanosomiasis Research Centre (KARI-TRC), PO Box 362, Kikuyu, Kenya
Email: jkkibugu@yahoo.com
DOI: 10.1258/la.2010.010020

REFERENCES

1 IAEA. Development of cost-effective diets for use in mass production of tsetse flies. Report and recommendations of a Consultants' Group Meeting organized by the Joint FAO/IAEA Division of Nuclear Applications Food and Agriculture, Vienna, Austria, 17–21 July 2000
2 Baker FJ, Silverton RE, Pallister CJ. *Introduction to Medical Laboratory Technology*. 7th edn. London: Oxford University Press, 2001
3 Murdoch University. Blood collection recommendations for Murdoch University Research and Teaching Projects. See www.research.murdoch.edu.au/ethics/arec/BloodSop.rtf (last accessed 18 March 2010)
4 Feldmann U. Guidelines for the rearing of tsetse flies using the membrane feeding technique. In: Ochieng-Odero JRP, ed. *Techniques of Insect Rearing for Development of Intergrated Pest and Vector Management Strategies 2*. Nairobi: ICIPE Science Press, 1994:449–71
5 Feldmann U. Some quality control parameters used in rearing of tsetse flies. In: Ochieng-Odero JRP, ed. *Techniques of Insect Rearing for Development of Intergrated Pest and Vector Management Strategies 1*. Nairobi: ICIPE Science Press, 1994:15–29