FINAL TECHNICAL REPORT

Improving the control of tsetse: the use of DNA profiling to establish the feeding responses of tsetse to cattle.

DFID Animal Health Programme
Project R7364: 1999-2001

S.J. TORR[1.], P.J. WILSON[2.], S. SCHOFIELD[2.], T.N.C. MANGWIRO[3.], S. AKBER[2.] & B.N. WHITE[2.]

[1]Natural Resources Institute, University of Greenwich, Chatham, UK:

[2]Wildlife Forensic DNA Laboratory, Trent University, Peterborough, Canada:

[3]Tsetse & Trypanosomiasis Control Branch, Harare, Zimbabwe

Executive Summary

Project Purpose:

The purpose of this project was to develop and promote environmentally beneficial and cost effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa. In particular, the project aimed to increase the cost-effectiveness of using insecticide-treated cattle to control tsetse in Zimbabwe and tsetse-affected areas of Africa.

Outputs:

In Zimbabwe, field studies were made of the feeding behaviour of tsetse attracted to small herds of cattle. The experimental herds comprised various combinations of calves (<100 kg), cows + steers (~300 kg) and oxen(>400 kg); the composition of the herds reflected the size, condition and age structure of herds found in the tsetse-affected communal areas of northern Zimbabwe.

Tsetse were captured as they fed on herds of cattle. Sub-samples of the flies were analysed using primer sets for seven different ungulate loci and these DNA profiles were matched with those previously recorded from the individual cattle. The results showed that feeding was greater on older and/or larger animals. For instance, in herds comprising a mixture of 2 oxen, 4 cow/steers and 2 calves, ~80% (range, 67% - 91%) of meals were from the two largest animals within the herd and only 0 – 3 % were from the calves.

These findings indicate that the practice of not treating young animals, to allow the development of natural resistance to tick-borne disease, does not compromise the efficacy of using insecticide-treated cattle to control tsetse. Moreover, confining insecticide treatments to the larger adults will improve the cost-effectiveness of the technique.

Contribution of Outputs to Project Goal:

The project goal was to improve the performance of livestock of poor people through the control of disease. The outputs contributed towards developing a sustainable, environmentally beneficial and cost-effective strategy for controlling trypanosomiasis

using insecticide-treated cattle. The work indicated that a selective dipping strategy, based on the treatment of the larger animals within a herd can achieve more cost-effective control. Such a strategy will be particularly cost-effective for poorer livestock-keepers in Zimbabwe, Tanzania and Ethiopia who typically have small heterogeneous herds of cattle. The findings are being used in the design of community-based tsetse control operations being conducted in Ethiopia and Tanzania and government-funded operations in Zimbabwe.

Dissemination of Results.

During the visits to Zimbabwe, presentations on various aspects of the project were made to staff of the Zimbabwe Tsetse and Trypanosomiasis Control Branch and the Regional Tsetse and Trypanosomiasis Control Programme. The findings were also reported at seminars presented to staff from Tanga Dairy Development Project, in Tanzania, and scientists and livestock specialists concerned with controlling tsetse in the Konso District of Ethiopia.

In addition, presentations were made at scientific meetings in Kenya, Canada and the USA and a paper was published in the international journal *Medical and Veterinary Entomology*.

PROJECT LOGICAL FRAMEWORK

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATOR(S)	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Goal 1. Performance of livestock of poor people improved through the control of disease.	1.1 Maintenance of tsetse free areas, provision of new areas for cattle rearing and extended use of both draught power and animal manure in areas currently tsetse infested from 2001 onwards	1.1 Reports of Zimbabwe DVS and Tanzanian LC 1.2 Reports of DVS/RTTCP and FITA/LC land-use economists.	(Goal to Supergoal)
Purpose 1. Sustainable, environmentally-beneficial and cost-effective strategies for control of trypanosomiasis developed.	1.1 Suitable farming systems identified by 2001. 1.2 Insecticide treatment regime applied in suitable farming systems by 2001.	1.1 Project reports. 1.2 DVS, RTTCP, TDDP, HIAP and FITA reports	(Purpose to Goal) Optimal cattle-dipping regime is not constrained by tick-disease management. Tsetse continues to be a significant constraint to livestock productivity. Zimbabwe TTCB and Zimbabwean farmers use bait technology to control tsetse.
Outputs 1. Quantitative relationships between herd structure and cattle-tsetse contact in Zimbabwe established. 2. Recommendations on the suitability of insecticide-treated cattle to control tsetse in Zimbabwe in particular and tsetse-infested areas of Africa in general.	DNA microsatellite profiles for Mashona cattle (20 by Dec 2000) and wild-caught tsetse (300 by Mar 2000, 1000 by Sep 2000) produced Source of blood from 500 fed tsetse identified and feeding preferences of tsetse inferred (Sep 2000). Recommendations on the feasibility of treating specific types of cattle to control tsetse (Dec 2000).	Publication in refereed scientific journals, conference proceedings and reports and presentations made to TTCB and RTTCP.	(Output to Purpose) Veterinary authorities in Zimbabwe maintain adequate facilities and staffing levels. Continued active interest of veterinary authorities The outputs must be combined with those from AHP project R7173 (Cattle management practices in tsetse-affected areas) and the RTTCP project concerned with insecticide performance.
Activities 1. Samples of blood from various Mashona cattle collected for DNA analysis. 2. Tsetse sampled as they approach and depart from single oxen and groups of oxen with known DNA profiles. 3. DNA extracted from field-collected samples and profiled using PCR and DNA microsatellite analysis to determine the source(s) of blood in fed tsetse. 4. DNA data analysed to determine whether tsetse feeding in a group of cattle feed on particular individuals and, if so, what the characteristics of these attractive individuals are with respect to age, size and behaviour. 5. Make recommendations on the feasibility of treating specific types of cattle to control tsetse.	Inputs/Resources: Project Budget Staff costs £14774 Overheads costs £20701 Equipment costs 0 Overseas travel £8285 Consumables £35657 TOTAL £79418 (over 2 years)	Quarterly, annual and final reports.	(Activity to Output) Active collaboration of DVS and involvement of trained field personnel. Provision of research facilities at Rekomitjie Research Station and DVS headquarters, and access to cattle from DVS experimental herd. Availability of analytical facilities and expertise at Trent University, Canada.

Background

Livestock production and trypanosomiasis

Trypanosomiasis, transmitted by tsetse (*Glossina* spp.), is a major constraint to agricultural production over ~10 million km^2 of sub-Saharan Africa. The disease prevents or greatly reduces the productivity of domestic livestock and its effects are particularly severe in some of the most deprived regions of the continent.

In Zimbabwe, tsetse (primarily *G. pallidipes* Austen and *G. morsitans morsitans* Westwood) could occupy about half the country. However, a rinderpest epidemic in the last century and tsetse control operations in this have restricted the infestation to ~10% of the country, i.e. an area of ~30 000 km2 (Lovemore, 1994). Populated areas presently infested or threatened by trypanosomiasis are primarily the poorer communal areas in northern Mashonaland. The predominant farming system in this area is mixed crop-livestock agriculture and within this system, cattle are particularly important. Cattle are the primary source of draught power, as well as providing milk and meat for domestic consumption. They are also important as a source of manure and for their role in various social and economic aspects of rural life (Barrett, 1992).

Control of Trypanosomiasis

Currently, trypanosomiasis control is dependent on either the use of trypanocidal drugs, to treat or prevent the disease, or on controlling tsetse and thereby breaking the disease transmission cycle (Jordan, 1986). To maintain a low level of trypanosomiasis within the communal areas of Zimbabwe, the Zimbabwe Department of Veterinary Services (DVS) conducts continuous control operations costing ~£1million/year. Most of this expenditure is directed at controlling tsetse and, along with most of tsetse-affected Africa, 'bait technology' is currently the main tsetse control method (Vale, 1993). This technology relies on attracting adult tsetse to traps or insecticide-treated targets, sometimes baited with artificial attractants, or to natural baits such as insecticide-treated cattle. Tsetse attracted to the bait are killed, either by contacting it and picking up a lethal dose of insecticide or by being retained in the trap. The low reproductive rate of tsetse means that a low density (~4/km^2) of evenly-spaced baits can eradicate tsetse populations within two years (Vale, 1993; Willemse, 1991; Dransfield *et al.*, 1990).

Bait technology has become the preferred control option throughout Africa largely because it is the most cost-effective method (Barrett, 1998), it is environmentally benign (Vale, 1993) and it is amenable to community-based approaches to control (Okali & Barrett, 1998). This latter point is particularly important due to the funding and infrastructure constraints faced by most countries with tsetse-infested areas.

Insecticide-treated cattle

The technology based on the use of traps and targets is underpinned by a large body of knowledge and practical experience (see review by Green, 1994). Consequently, there is a sound theoretical and practical basis for predicting the effect of using artificial baits to control tsetse. The use of insecticide-treated cattle on the other hand has developed very rapidly and largely empirically; from the initial work conducted in 1985 showing that tsetse alighting on deltamethrin-treated cattle were killed (Thompson, 1987), to the widespread use of this technique as part of Zimbabwe's everyday tsetse control strategy (by 1988, > 160000 cattle were being treated) (Thompson *et al*., 1991, Torr *et al*., 1998a). Equally rapid uptake of the technique occurred throughout many tsetse-affected countries including: Burkina Faso (Bauer *et al*., 1992), Ethiopia (Leak *et al*., 1995), Kenya (Stevenson, 1991), Tanzania (Fox *et al*., 1991) and Zambia (Chizyuka & Liguru, 1986). The rapid and widespread proliferation of the technique has occurred with surprisingly little supporting research. Even the impact of such basic matters as: the density and distribution of cattle as well as variables relating to cattle and tsetse ecology and physiology are still largely unknown. Consequently, there is a paucity of information on which to base the application of this control technique. It is particularly important to establish the limits of the technique since government agencies and donors are vigorously promoting the use of this technology for use by resource-poor farmers and communities.

he DFID Animal Health Programme is attempting to address this problem by supporting a collaborative tsetse research programme in Zimbabwe and Tanzania. The programme is aimed at quantifying the effects of both biological (e.g. cattle and tsetse physiology and ecology) and socio-economic (e.g. management practices) variables on the efficacy of the technique. In the first phase of this programme, research was mainly concerned with quantifying the effects of host and tsetse

physiology on the efficacy of cattle as baits. This research produced two findings that have particular significance to this proposal.

Firstly, there were very marked differences in the attractiveness and efficiency of individual cattle as baits (Torr *et al*., 1998b). In particular, younger and smaller animals attracted fewer tsetse than older and larger ones, and of those tsetse attracted to young animals, fewer landed and fed; these effects can result in a 10-fold difference in the efficacy of an ox and a calf as a bait.

Secondly, studies of the bloodmeal size (Torr & Hargrove, 1998) and alighting times (Torr *et al*., 1998b) of tsetse feeding on oxen suggested that a 'feed' may consist of a number of small meals undertaken over a short period. The results suggest that the feeding process is interrupted by the host's behaviour or by direct interactions with other species of fly. Taken together, these results indicate that tsetse feeding from a herd of cattle may actually feed and contact several individuals in the process, and that older and larger hosts are more likely to be contacted.

More recently, studies have focussed on the effects of cattle management practices such as cattle herding. In studies of the responses of tsetse to groups of cattle, it was found that the numbers of tsetse attracted to the group increased with herd size and that when the group comprised calves ~10% percent fed compared to ~50% when the group was comprised entirely of oxen. These results are in line with data from previous studies. Intriguingly however, when the herd comprised a mixture of young and old animals, the percentage of tsetse feeding successfully was ~50% and was not significantly different from the percentage feeding in a group comprised entirely of oxen. This suggests that tsetse attracted to a heterogeneous herd of cattle feed preferentially on the older and/or larger cattle. If this inference is correct, then the cost-effectiveness of using insecticide-treated cattle could be improved by treating only the larger and older animals.

Preliminary data on herd structures in tsetse-affected areas of Zimbabwe indicate that, typically, households owning cattle have a small and heterogeneous herd of animals. Such findings are in line with previously published data. Abel & Blaikie (1988) reported that in Mashonaland, 90% of household's owned <10 cattle and Barrett's (1992) summary of herd structure data for the communal areas of

Zimbabwe shows that communal herds typically comprise 34% bulls/oxen, 31% cows, 17% heifers and 18% calves. A similar pattern is seen in other tsetse-affected countries. For instance in the Changara district of Mozambique, of those that households that owned cattle, 67% kept <10 animals and the percentages of bulls/oxen, cows, heifers and calves were 23%, 37%, 18% and 22% respectively (Doran, 1998). Thus a dipping strategy based on treating only "attractive" animals such as the large oxen would be particularly appropriate for poorer communal farmers in tsetse-affected areas.

The inference regarding tsetse feeding strategies within a herd of hosts is derived from indirect evidence. If we are to make sound recommendations to farmers regarding novel dipping strategies, we need to determine directly which individual animals tsetse feed on within a herd of cattle. Hitherto, this has not been technically possible using the standard methods of studying the host-orientated behaviour tsetse based on direct visual observation (Hargrove, 1976), video techniques (Gibson & Brady, 1985; Packer & Warnes 1991) or using arrangements of electric nets (Vale, 1974). However, combining the use of electric nets with a novel method of identifying the individual source of a bloodmeal should enable us to determine, unequivocally, from which individual host a tsetse has fed.

Genetic-profiling of bloodmeals

Veterinary and medical entomology has long been concerned with determining the specific sources of a bloodmeal, primarily because of the epidemiological implications of the information. For instance, one of the most important parameters controlling the epidemiology of insect-borne diseases such as trypanosomiasis or malaria is the vector's biting rate. Specifically, the vectorial capacity of an insect changes as the square of the biting rate. Previous research has often assumed that an insect's biting rate is equivalent to its feeding interval (i.e. period of time between successive full blood meals). The interruption of feeding leads to repeated probing of different hosts and hence increases the biting rate. Consequently there has been much interest in determining the frequency of multiple bloodmeals and a variety of serological (e.g. Guzman *et al.*, 1994) and histological (e.g. Wekesa *et al.*, 1995) techniques have been used in studies of various vectors.

Coulson *et al.*, (1990) described a methodology for identifying the individual source of a blood meal from a mosquito that had fed on a human. Amplification of the human DNA in the bloodmeal allowed for the characterisation of the individual source of the meal through minisatellite analysis. The study was entirely laboratory-based and was mainly intended to illustrate the potential and problems of the technique. More recently, Koella *et al.* (1998) used DNA micro-satellite analysis of human blood to characterise the sources of meals from mosquitoes resting in houses and thought to have fed on humans in the village. Using this technology, Koella *et al.* (1998) quantified the frequency of bloodmeals that were derived from multiple hosts. In both studies, the small size of bloodmeals taken by mosquitoes, and the delay between the mosquito feeding and its collection, meant that a relatively high frequency of meals could not be identified. In Koella *et al*s (1998) study for instance, 35% of collected meals could not be identified.

In 1998, scientists from Trent University and NRI developed a technique to characterise the source of tsetse bloodmeals based on the DNA micro-satellite analysis of bovine blood (Schofield *et al.*, 1998). In preliminary studies, tsetse were allowed to feed on particular cattle with known microsatellite profiles. All meals were correctly ascribed to the host(s), even when the blood was from a mixed meal derived from two different cattle. The high success rate of the methodology is probably due to the rapid (<10s) collection of tsetse, by electric nets, as they left the host and the relatively large volumes of blood taken by tsetse (40-80 mg; Taylor, 1976, Torr & Hargrove, 1998) compared to mosquitoes (~3 mg; Knaus *et al.*, 1993). Accordingly, the uncertainties raised by unidentified meals in the mosquito studies are likely to be less of a problem with tsetse. This project aimed to use this technology to identify the sources of bloodmeals from tsetse that have fed within a herd of cattle and, in particular, test the hypothesis that most meals are taken from the older and larger animals. This information was then used to make recommendations about the technical and social feasibility of a dipping strategy based on the selective treatment of just a few readily identifiable 'attractive' animals within a livestock owner's herd.

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Project Purpose

The purpose of this project was to develop and promote environmentally beneficial and cost effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa. In particular, the project aimed to increase the cost-effectiveness of using insecticide-treated cattle to control tsetse.

Generally, livestock owners using this technique treat all their cattle but this can be prohibitively expensive and leave livestock susceptible to tick-borne diseases. This project used a form of DNA fingerprinting to identify the individual source(s) of blood taken by tsetse as they fed on herds of cattle. The results show that tsetse feed preferentially on the older and larger animals in a herd. These findings should enable farmers to control tsetse more cost-effectively by treating only the 'attractive' animals within an owner's herd.

Research Activities

Research activities aimed to produce unequivocal evidence that tsetse attracted to a herd of cattle contact just a few 'attractive' individuals. The project investigated this matter directly by sampling flies as they fed on herds of cattle. The individual specific source(s) of the bloodmeal for each fed fly were determined, using a form of DNA fingerprinting, and these results were used to determine whether tsetse consistently feed on certain types of animal within a herd. The results of these studies were combined with those from the AHP-funded project "Cattle management practices in tsetse-affected areas" (R7173) to predict the technical and social feasibility of using a selective dipping strategy for controlling tsetse. The project activities were as follows.

Activity 1. Samples of blood from various Mashona cattle collected for DNA analysis. Blood samples were collected from 34 Mashona cattle, a breed of Sanga indigenous to Zimbabwe, from the research herd at Rekomitjie Research Station in Zimbabwe. Samples were taken from cattle by collecting blood from the ear vein onto a filter paper. The sample was air dried and placed in an aluminium foil envelope that was heat-sealed and then stored in a -10^{o}C freezer, pending analysis at Trent University in Canada.

Activity 2. Tsetse sampled as they approach and depart from single oxen and groups of oxen with known DNA profiles.

All field studies were carried out between January 1998 and April 2001 within 3 km of Rekomitjie Research Station in the Zambezi Valley of Zimbabwe where *G. pallidipes* and *G. morsitans morsitans* occur. The research station is in the Mana Pools National Park where wild ungulate hosts such as buffalo (*Syncerus caffer* Sparrman), bushbuck (*Tragelaphus scriptus* Pallas), kudu (*Tragelaphus strepsiceros* Pallas) and warthog (*Phacochoerus aethiopicus* Pallas) are abundant. Apart from a herd of ~30 research cattle at Rekomitjie, no domestic livestock are found within 50 km of the station.

Phase 1: Developing and testing the technology

In the first phase of this project, experiments were undertaken to establish confidence in the use of microsatellite profiles to identify the source(s) of bloodmeals.

Specimens

Tsetse. Unfed tsetse were caught from Epsilon traps (Muzari & Hargrove, 1996) baited with a blend of acetone (500 mg/h), 1-octen-3-ol (0.5 mg/h), 4-methylphenol (1 mg/h) and 3-*n*-proylphenol (0.1 mg/h) dispensed by the methods of Torr *et al.* (1997). The traps were operated between 1400 and 1800 h, >3 km from Rekomitjie Research Station. The trapped flies were killed within 30 min. of collection by placing them in a freezer for 10 min. Fed tsetse were obtained by three different methods.

First, flies were caught in the late afternoon after they had fed off a bait ox. They were retained singly in a glass tube (2.5 cm x 7.5 cm) with a cork stopper at one end and netting at the other. Caught tsetse were kept in a polystyrene cool box and later transferred to a laboratory where they were killed at various intervals after feeding.

Second, unfed flies were caught from traps in the late afternoon. The flies were transferred singly to glass tubes (2.5 cm x 7.5 cm) with a cork stopper at one end and netting at the other and held alive in an insectary (Temp 23-25^{o}C, RH 60-70%) overnight. The following morning, the tubed flies were allowed to feed on an ox by placing the tube against the flank of the animal. After each fly had partially fed, it was moved to a second animal where it completed feeding. The flies were killed within 30 min of engorgement.

Third, tsetse that had fed naturally in the field on cattle were sampled following the method of Vale (1977). One or two cattle were placed at the centre of an incomplete ring (8 m diameter) of six electric nets (Vale, 1974). Flies that struck the net were killed or stunned and fell onto metal or plastic trays. Tsetse caught on the outside or the inside of the ring were presumed to be approaching or leaving the ox, respectively. Feeding efficiency was estimated as the number of fed tsetse on the

inside of the ring of nets expressed as a percentage of the total catch from the inside of the ring. To enhance the numbers of tsetse attracted to the cattle, a blend of carbon dioxide (120 l/h), acetone (500 mg/h), 1-octen-3-ol (0.5 mg/h), 4-methylphenol (1 mg/h) and 3-*n*-proylphenol (0.1 mg/h) was dispensed ~2 m downwind of the animal.

Flies were collected from the trays at 30-minute intervals and placed individually into single tubes that were then stored in a fridge at 4^{o}C. Tsetse were classified according to the side of the net where they were caught and as fed or unfed according to the presence or absence of fresh red blood visible through the abdominal wall. On days when a calf + ox were in the ring of nets, observers viewed the cattle from a tower (Torr, 1994) and counted the number of tsetse seen on each animal.

Phase 2: Assessing the feeding responses of tsetse to cattle herds

In the second phase of the project, experiments were undertaken to assess the responses of tsetse to larger heterogeneous groups of cattle. For these studies, the animals were placed in a large kraal (6 x 6 m) placed within a ring (16 m dia) of 12 electric nets (1.5 x 1.5 m).

In the first experiment, studies were made of the feeding responses to:-

1. single ox,
2. four oxen,
3. four calves,
4. three calves and ox

This experiment repeated one undertaken in 1998 which showed that the feeding rate for tsetse attracted to four oxen was ~50% compared to <10% for four calves but that feeding rates for groups comprising a mixture of calves and oxen was ~50% (Torr *et al.,* 2000). In this experiment we aimed to test the hypothesis that the high feeding rate with heterogeneous groups resulted from tsetse feeding selectively on the oxen.

Following on from this experiment, studies were made of the responses of tsetse to a group of 8 cattle comprising seven calves and one adult.

Finally, studies were made the responses of tsetse to groups of 8 cattle comprising two oxen, four cows/steers and 2 calves. These groups reflect the mean size and composition of herds found in the tsetse-affected communal lands of northern Zimbabwe. Studies of these 'natural' herds were undertaken during the wet and dry seasons since previous studies (Torr & Mangwiro, 2000) indicated that the large numbers (e.g. >1000 flies/hour) of other biting flies during the wet season affects the feeding behaviour of tsetse.

In all experiments, different treatments were compared using a repeated random block design. Groups of adjacent days were regarded as different blocks and treatments were be randomly allocated to days within these blocks.

All experiments will be analysed using GLIM4 (Francis *et al.,* 1993) which fits models using a maximum likelihood method. To compare the number of tsetse coming to each treatment, the catches (n) were transformed to $\text{Log}_{10}(n+1)$ and then subjected to analysis of variance. To assess the proportion that feed, the numbers of tsetse caught on the inside of the ring and the proportion of these that are fed were recorded. These proportional data were analysed using a binomial model with a logit link and the significance of changes in deviance were assessed by χ^2 (Crawley, 1993).

Activity 3. DNA extracted from field-collected samples and profiled using PCR and DNA microsatellite analysis to determine the source(s) of blood in fed tsetse.

DNA from samples of tsetse blood meals and cattle blood was extracted following a modified Qiagen (Qiagen Inc, Mississauga, Canada) extraction protocol using lysis buffer (Guglich *et al.,* 1994).

DNA from samples of tsetse fly blood meals was extracted and amplified with four bovine-specific microsatellite primer sets (Research Genetics, Huntsville, Alabama, USA) and one ovine microsatellite set (Paterson *et al.,* 1998) (Table 1) as follows.

Table 1. Ungulate primer sequences for 5 microsatellite loci.

Primer	Primer Sequences	Reference
BM4513-F	5'- GCG CAA GTT TCC TCA TGC -3'	Bishop *et al.* 1994
BM4513-R	5'- TCA GCA ATT CAG TAC ATC ACC C -3'	
BM1225-F	5'- TTT CTC AAC AGA GGT GTC CAC – 3'	Bishop *et al.* 1994
BM1225-R	5'- ACC CCT ATC ACC ATG CTC TG - 3'	
MAP2C-F	5' – TTT ACC AGA CAG TTT AGT TTT GAG C - 3'	Moore *et al.* 1994
MAP2C-R	5' – AAG GAT TCT GTC TGA TAC CAC TTA G - 3'	
IGF-1-F	5' – GCT TGG ATG GAC CAT GTT G – 3'	Kirkpatrick *et al.* 1992
IGF-1-R	5' – CAC TTG AGG GGC AAA TGA TT – 3'	
OLDARB- int	5' – CGT ACC CAG AKT GAG TGA AGT ATC – 3'	Paterson *et al.* 1998
ODARB- schw	5' – TGK GCA GCG GCG AGG TGA G – 3'	

Samples were amplified using 4.6 pmol λ^{33}P T4 polynucleotide kinase end labeled primer ATP in a total reaction volume of 10μl per tube using 25ng of genomic DNA, 200 μM dNTPs, 1x amplification buffer, 2.0 mM $MgCl_2$, unlabelled primer (0.2 mM), labeled primer, and 0.5 units of *Taq* polymerase (Life Technologies, Burlington, Canada). Products were amplified under the following conditions: 94°C for 5 min., 55-65°C for 30 sec., 72°C for 15 sec. 1 cycle; 94°C for 15 sec., 55°C for 30 sec., 72°C for 15 sec. 30 cycles; 94°C for 15 sec., 55°C for 30 sec., 72°C for 2 min. 1 cycle. Products were then mixed with 0.4 volume of formamide loading buffer and were heated at 95°C for 5 minutes before loading onto a 6% sequencing gel containing 50% (w/v) urea. A control sequencing reaction of phage M13 DNA was run adjacent to the samples to produce size markers for the microsatellite alleles.

Cytochrome b gene sequencing and sequence analysis

For a few bloodmeal samples where the blood was found to be not from cattle, the host species was identified using the following primers to amplify a 305 b.p. region of the mitochondrial DNA gene cytochrome b gene (Kocher 1989).

Primer 1 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'
Primer 2 5'-CCC TCA GAA TGA TAT TTG TCC TCA-3'

The cytochrome b gene was amplified in a total reaction volume of 20μl per tube using 25ng of genomic DNA, 200 μM dNTPs, 1x amplification buffer, 2.0 mM $MgCl_2$, primers 1 and 2 (0.2 mM) and 1.0 units of *Taq* polymerase. Products were amplified under the following conditions: 94°C for 5 min., 55°C for 30 sec., 72°C for 30 sec. 1 cycle; 94°C for 30 sec., 55°C for 30 sec., 72°C for 30 sec. 35 cycles; 94°C for 30 sec., 55°C for 30 sec., 72°C for 2 min. 1 cycle. Products were re-amplified and cleaned through QIAquick (Qiagen, Canada) for DNA sequencing using dye-terminator cycle sequencing using a ABI Prism 373 DNA Sequencer (MOBIX, McMaster University, Hamilton, Ontario, Canada)

Genetic analysis of microsatellite and mitochondrial DNA

Individual genotypes were scored from the samples. Multiple bloodmeals were detected based on the presence of more than two alleles per locus. The confidence in assigning a specific genotype to an individual was assessed using a probablity of identity (POI) (Patkaeu & Strobeck, 1994; Waser & Strobeck, 1994). This measure is used in forensic identification when calculating the probability of a match or the probability that two random and unrelated animals will share the same DNA profile by chance. The POI values were calculated for the herd of cattle (n=29).

DNA sequences obtained for the mtDNA cytochome b region were aligned using the programs Seqpup and ClustalW. Individual sequences were compared to GENBANK/EMBL submitted sequences using the BLAST program (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) to identify sequences with the highest similarity.

Activity 4. DNA data analysed to determine whether tsetse feeding in a group of cattle feed on particular individuals and, if so, what the characteristics of these attractive individuals are with respect to age, size and behaviour.

The auto-radiograph generated from the samples indicated the number of alleles present in each sample and the genetic profiles were compared with the known

cattle genotypes to assess which animal(s) were present within a particular bloodmeal. GLIM4 will be used to analyse these data. For data classed into different categories (*e.g.* numbers feeding on different hosts given a choice of *n* hosts and where $n>2$), a model with a Poisson error and log link was specified (Crawley, 1993). Potential hosts were specified as *n* factors and the significance of changes in deviance was assessed on the basis of a G-test.

Activity 5. Make recommendations on the feasibility of treating specific types of cattle to control tsetse.

The above research established that tsetse feed preferentially on the older and larger animals in a herd, implying that farmers could control tsetse more cost-effectively by treating only the 'attractive' animals within an owner's herd.

The findings and recommendations were disseminated at workshops and various informal meetings with farmers, livestock scientists and tsetse control specialists in Zimbabwe, Tanzania and Ethiopia. In addition, the practical implications of the research were promoted via reports produced for NGOs promoting tsetse control in Tanzania and Ethiopia. The scientific findings were disseminated at scientific conferences in Kenya, Canada and USA and the publication of a paper in the international journal, *Medical and Veterinary Entomology*.

Outputs

Analyses of cattle blood

The results of the microsatellite profiles for 29 cattle show that there were 3-13 alleles for each locus with expected heterozygosities ranging between 0.418-0.905. The OLADRB locus demonstrated the most genetic variability within the herd and IGF-1 was the least variable; the latter primer was consequently the least useful for distinguishing cattle (Table 2). Each of the cattle at Rekomitjie had unique microsatellite profiles despite the herd being isolated and consequently inbred with many closely related individuals as illustrated by the profiles of a cow and two of her calves (Fig. 1). The Probability of Identity (POI) values ranged between 6.35×10^{-8} and 9.55×10^{-4}; the latter probability indicates that in a sample of ~1000 cattle it would be expected that two unrelated animals would share this DNA profile by chance.

Phase 1: Developing and testing the technology

Analysis of tsetse with known feeding histories

Unfed flies. The gut contents of six unfed flies were analysed. No cattle DNA was detected in five of the samples but one sample amplified a weak product at locus BM4513. This could represent the remnants of a blood meal from cattle or a wild ungulate.

Fed flies. Male *G. pallidipes* were caught after feeding on one of three cattle and samples of these flies were killed within 30 min. of feeding or at 24 h, 48 h or 72 h thereafter. Cattle DNA was detected in the gut contents of all flies killed 30 min. (n=15) or 24 h (n=5) after feeding, and the microsatellite profiles from the bloodmeals matched that of the known host. For three of 15 samples taken at 30 min. after feeding, amplified products from other, unknown animals were also apparent. These products could be from other cattle or wild ungulates. Complete microsatellite profiles were achieved for five (n=6) flies killed 48 h after feeding and two (n=3) of those killed 72 h after feeding. Although the samples sizes were small, the data suggest that the technique is less reliable >48h after feeding.

Table 2. Genetic variation, allele frequencies and expected heterozygosity (H_E) of five microsatellite loci in Mashona cattle (n=29).

BM4513	
Allele	**Frequency**
138	0.0172
140	0.0690
142	0.0172
146	0.3621
148	0.2931
150	0.0172
152	0.1552
158	0.0172
162	0.0172
170	0.0345
H_E	0.765

OLADRB	
Allele	**Frequency**
150	0.0172
152	0.0172
158	0.0517
160	0.0690
164	0.1207
166	0.0172
168	0.0690
170	0.1034
172	0.0862
174	0.0517
180	0.1724
192	0.0517
194	0.1724
H_E	0.905

Map2C	
Allele	Frequency
97	0.0172
99	0.2414
101	0.5690
107	0.0690
111	0.0862
113	0.0172
H_E	0.616

BM1225	
Allele	Frequency
227	0.0172
229	0.0172
237	0.0172
241	0.7586
243	0.0862
247	0.0517
251	0.0517
H_E	0.418

IGF-1	
Allele	Frequency
227	0.5345
229	0.0172
231	0.4483
H_E	0.522

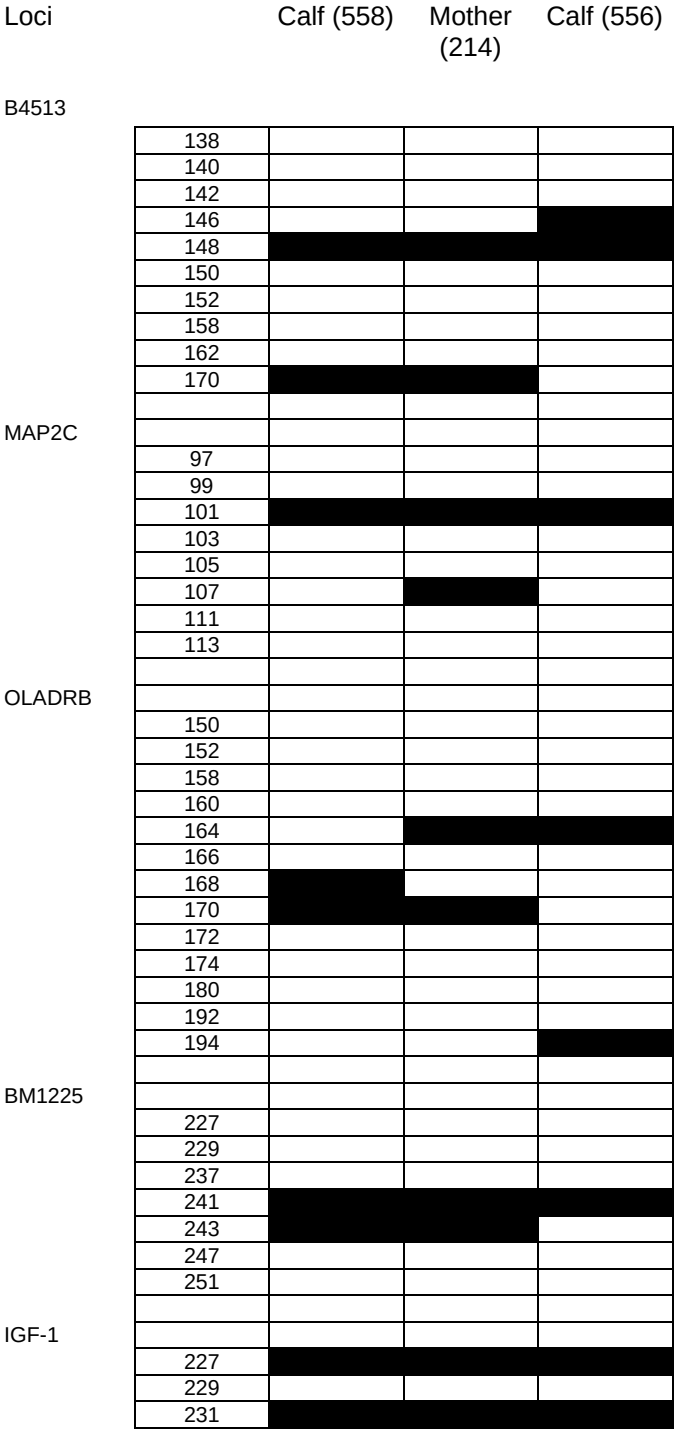

Fig.1 Microsattelite profiles of a cow (no. 214) and her calves born in 1995 (556) and 1997 (558). The father(s) of the calves is unknown. While each calf shares at least one allele at each locus with its mother, and some loci are identical for different animals (IGF-1), the overall profile from the five loci is unique for each animal.

Multiple meals. Analysis of the gut contents of six tsetse known to have fed from two different cattle gave allelic patterns that were consistent with the microsatellite profiles of blood from the two known hosts.

These preliminary investigations suggested that for tsetse feeding on cattle at Rekomitjie, the individual source(s) could be uniquely identified using a suite of five microsatellite primers. Accordingly, studies were undertaken to analyse the feeding responses of wild tsetse feeding naturally on cattle.

Analysis of wild tsetse

Studies were made of the feeding responses of tsetse to either single or paired animals. The pairs comprised either two oxen or a calf and an ox. In 1998, a total of 964 tsetse were caught, of which 97% were *G. pallidipes* compared to 72% (n=516) in 1999.

All studies concerned with the responses of tsetse to either single animals or a calf + ox were carried out in 1998. Pooling the catch data for all tsetse, the mean feeding rate on a single calf was 16% (n=109) compared to 35% (229) on an ox and 39% (170) for an ox + calf. For four days when an ox + calf were in the ring, 96 tsetse were observed on the ox compared to 19 on the calves. The differences in the mean feeding rates for calves and oxen, and the numbers seen landing on oxen and calves, are consistent with more extensive data (Torr & Mangwiro, 2000) showing that tsetse feed more successfully on oxen than calves. In studies of the responses of tsetse to pairs of oxen, the mean feeding rates varied between 27% and 54%.

Microsatellite profiles

Results from the analyses of 413 tsetse attracted to cattle (Table 3) were in general accord with expectations. First, 85% (n=237) of the fed flies were identified as containing blood from the test animal(s) within the ring of nets on that day, compared to 2.8% (176) of the unfed flies. Of the fed flies, 5.5% contained blood from another host and in 9.8% of samples no host DNA was detected. For the unfed flies, 18.8% contained blood from non-test host(s) and 78.4% of samples had no detectable host DNA.

Table 3. Percentages of bloodmeals from tsetse containing DNA from either the test animal(s), other unidentified ungulate or with no detectable bovine DNA. Flies were classed as being fed or unfed and were caught from the inside or the outside of the ring of nets.

Feed status	Catch position	*n*	Test animal (%)	Other ungulate (%)	No amplification (%)
Fed	Inside	219	86.3	5.0	8.7
	Outside	18	72.2	11.1	16.7
Unfed	Inside	70	4.3	28.6	67.1
	Outside	106	1.9	12.3	85.8

A few flies (5/413=1.2%) contained bovine DNA from non-test animals, which were identified, using the profile data for the entire herd, as being from known cattle which were not in the ring of nets on that particular day. Similarly, a few flies (8/413=1.9%) with a clear host identity, were also found to contain DNA from a second unknown ungulate host. The DNA profiles classed as belonging to another ungulate comprised some with alleles found within the cattle as well as some alleles not found in the cattle. Three specimens containing the latter were amplified at the cytochrome b region and identified as being from African buffalo (*Syncerus caffer*).

Tsetse found to have unidentified bovine DNA presumably represent flies that contained remnants of DNA from bloodmeals derived from other wild hosts or possibly small traces of blood from a cattle meal that were not sufficient to obtain a clear identity.

Paired cattle

For fed tsetse caught on days where there was an ox + calf in the ring of nets, 100% of identified meals were from the ox (Table 4). When two oxen were in the ring, the percentages varied between one pairing where all meals were from one animal to three pairings where roughly equal numbers of meals were from each animal. The frequency of mixed meals was very low; overall for days where two hosts were in the ring of nets, only 1.5% (3/202) contained blood from both hosts.

Table 4. Mean feeding rates and bloodmeal identities for tsetse attracted to pairs of cattle. Bloodmeal identities indicate the percentages of bloodmeals identified as being a single feed from either animals #1 or #2, or a mixed feed comprising blood from #1 and #2. Sample sizes for feeding rates and bloodmeal identities are n_f and n_b respectively and *P* indicates the probability that the numbers of meals from the pair of animals are significantly different at the P<0.05 (*) or P<0.001 (***) level of probability. Only data for those bloodmeals that contained blood identified as being from at least one of the animals within the ring of nets are shown. Results for ox *vs*.calf pairings are the pooled results from four separate ox + calf pairs.

Treatment	Animal #1	Animal #2	Feeding rate		Bloodmeal identities				
			%	n_f	#1 (%)	#2 (%)	#1 + #2 (%)	n_b	*P*
Ox *vs*. calf*	Ox	Calf	39	170	100	0	0	38	***
Ox *vs*. Ox	167	225	54	13	100	0	0	6	*
	167	537	52	81	55	40	5	38	ns
	556	219	27	73	58	42	0	12	ns
	167	556	36	83	68	38	4	28	ns
	537	219	52	96	79	21	0	42	***

Practical implications

Present results show that the individual source(s) of a tsetse bloodmeal from cattle can be identified using a suite of five microsatellite primers. For flies that took a full meal, the technique provided clear and unequivocal identification of the source for at least 24 h post feeding and could also be used to identify the hosts in mixed meals that were derived from two hosts. Closely related animals such as a cow and her calf could be readily distinguished. This is particularly important for studies of veterinary pests where there is frequently much in-breeding within a herd of livestock.

Tsetse are thought to digest ~60% of a bloodmeal within 24 h of feeding and ~95% within 48 h (Loder *et al.*, 1998). The fact that we could identify all meals at 24 h post-feeding and >80% at 48 h, provides some evidence that we could have detected DNA from small incomplete meals. Although the sample sizes were small, the present results do suggest that the technique is less reliable for identifying meals >48 h after feeding. This accords with extensive data showing that tsetse completely digest their meals within ~72 h of feeding; during this period host DNA will be degraded so reducing the efficacy of the technique.

When the technique was used to study the responses of flies feeding naturally, the success rate of the technique was slightly less than that observed with the initial laboratory studies (85% vs. 100%). The difference is partly because a proportion (5.5%) of those flies classed as fed had not taken a meal from the host(s) within the ring but from another unidentified animal. The technique described here should be of general use in studies of other vector-borne diseases of cattle. Indeed, the same technique has been successfully used in studies of the feeding behaviour of *Stomoxys* spp (A. Prior, unpublished data).

While the technique is not perfect, the success rate is superior to that obtained in comparable studies of mosquitoes. Gokool *et al*. (1993) found for instance, that only ~35% of microsatellite profiles from fed *Anopheles gambiae* caught in the wild were readable while Koella *et al* (1998) achieved a success rate of ~65%. Assuming that there are no inherent differences in the ability of the appropriate primers to analyse human or bovid blood samples, then the difference must be related to the flies used in the different studies. In particular, the size of bloodmeals in tsetse (30 – 90 mg; Taylor, 1975; Torr & Hargrove, 1998) are greater than those of mosquitoes (~3 mg; Takken *et al*., 1998; Knaus *et al*., 1993). Moreover, in the present study, tsetse were caught and killed within seconds of feeding whereas there was a delay of some hours in the study of mosquitoes. Thus the mosquitoes contained less host-DNA in the first place and a longer period was allowed for the DNA to be degraded.

Biological implications

Although the present study was primarily intended to establish a technique for identifying the sources of bloodmeals, the first application of the method has provided a number of new insights.

First, with regard to the use of Vale's (1977) method of using an incomplete ring of electric nets to study the feeding behaviour of tsetse, the present study shows that flies classed as being fed or unfed on the presence or absence of blood visible through the abdominal wall is providing a fair indication of feeding success. Most of the flies with visible blood did indeed have blood from the host, and flies classed as unfed did not have any bovine DNA products. Thus tsetse seem not to take small meals which are missed by field workers.

Second, the method showed that in situations where tsetse were presented with the choice of feeding on an adult or a calf, all the meals were from the adult. Previous studies, using rings of nets, indicate that tsetse are more successful in feeding on older animals and it is thought that this difference is related to host size and higher rates of defensive behaviour by young animals (Torr, 1994; Torr & Mangwiro, 2000). The present study confirmed these findings by demonstrating that feeding success on the calves and oxen placed individually in the ring were different (16% vs. 35%). Moreover, when a calf and ox were placed together, more tsetse were seen to land on the adult, all the meals were from the adult, and the feeding rate for tsetse attracted to an ox+calf (39%) was closer to that of an ox alone (35%) rather than that of a calf (16%). This suggests either that tsetse were biased towards landing on the adult and/or that those flies which landed on the calf were interrupted before they began to feed and subsequently moved and fed on the neighbouring adult. The paucity of mixed meals suggests that tsetse that moved from the calf to the adult did so before they began to feed on the calf. This has interesting epidemiological implication since it suggests that suckling calves, which will be close to their mothers, will be bitten even less than suggested by Torr & Mangwiro's (2000) results. This phenomenon may partly explain why very young calves can survive long periods in tsetse-affected areas without contracting trypanosomiasis (Torr & Mangwiro, 2000).

Third, when two oxen were placed in the ring we had variable results. In some cases, roughly equal numbers of tsetse fed from each ox while in others significantly more meals were taken from one of the oxen. Torr & Mangwiro (2000) provide data on the feeding responses of tsetse to four of the oxen (numbers 225, 219, 167 and 537) used in this study. Their data show that ox 225 is an unusually poor host with only 39% of tsetse successfully feeding on it compared to 63% for 167. When these two animals were paired in the present study all the bloodmeals were from 167. Similarly the only other treatment where there was a significant difference in feeding success was the pairing of ox 219 with 537 and the mean feeding rates for these two were respectively 51% and 61% (Torr & Mangwiro, 2000). Conversely when oxen 167 and 537 with very similar feeding rates (63% vs. 61%) were paired there were no significant differences in the numbers of meals from each ox. These data provide circumstantial evidence that relatively slight differences in feeding success between hosts translate into larger differences in biting rates and possibly in disease risks.

Lastly, the present data show that mixed meals were relatively rare when tsetse were attracted to pairs of oxen. This paucity of mixed meals suggests that tsetse which were interrupted as they fed on one host did not attempt to complete the meal on a neighbouring one, but returned to the original host or left the vicinity entirely. However, the present indications regarding host choice and the frequency of mixed meals were gained from studying the simplest 'herd' of two animals.

Phase 2: Assessing the feeding responses of tsetse to cattle herds

Ox-calf groups

The mean feeding rates for tsetse attracted to the different groups were broadly in line with previous results (Fig. 2), with a very low feeding rate for groups comprised entirely of calves and higher rates for groups containing at least one ox. For instance, the feeding rates of *G. pallidipes* attracted to a group of calves, or oxen or calves+ox were 6% (n=87), 72% (141) and 40% (105) respectively.

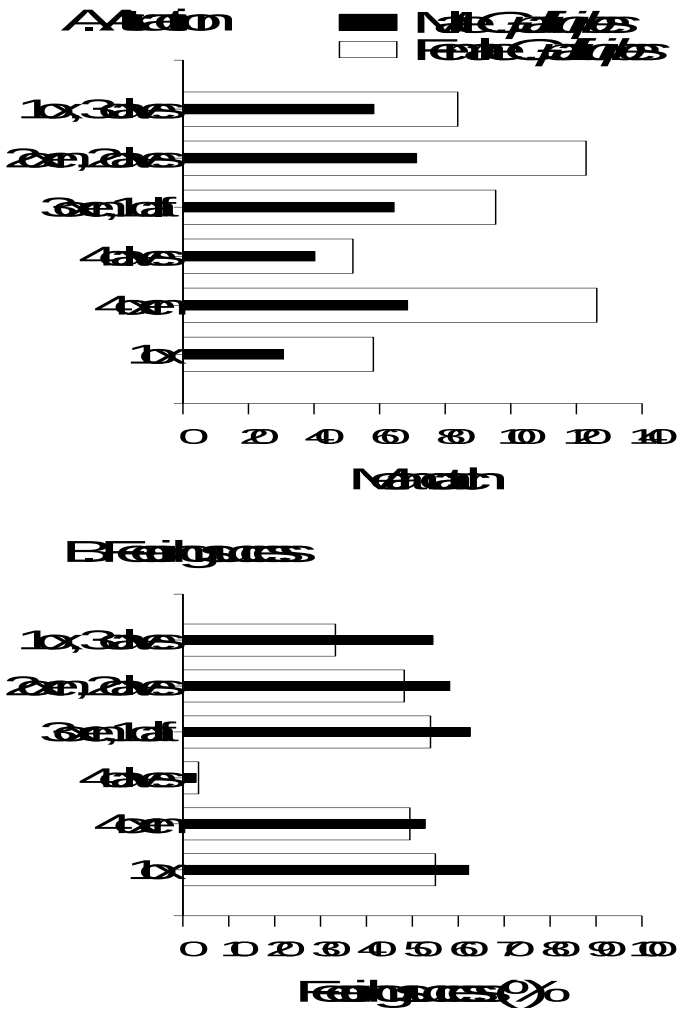

Fig. 2. Detransformed mean catch and percentage feeding success of *G. pallidipes* attracted to a single ox or groups of four cattle comprising various combinations of calves and adults. (Data produced by AHP project R7173)

Analyses of 22 fed tsetse attracted to the single ox showed that most (95%) had fed on the animal in the ring; one (5%) 'fed' fly, and three of the 15 'unfed' flies, were found to have blood from another ox that had been in the ring on a previous day. A total of 21 unfed and 4 fed tsetse attracted to the group of calves were analysed. Surprisingly, the fed flies were found to contain blood from an ox that had been in the ring on a previous day rather than the calves. In three of the cases, it was noted at the time of collection, that the meals were small and/or appeared to be old.

Analysis of the DNA profiles from fed tsetse attracted to the 4- or 8-host groups of three calves and one ox showed that 98% (n=39) and 92% (63) of identified meals were from the single ox respectively. For the 4-host group (1 ox + 3 calves), no meals were from the calves and one meal (2%) was from an ox that had been in the ring previously. For the 8-host group (1 ox + 7 calves), 3% (2/63) of meals were from the calves and 5% (3/63) were from other oxen. These results support the hypothesis that tsetse feed selectively on the older and/or larger animals in a group, even when the smaller hosts are more numerous and/or constitute a larger biomass.

Tsetse did not feed equally on the four hosts comprising the four-ox herds. The results (Fig. 3) show that some hosts were particularly favoured. Oxen 537 and 219 were present in each of the three herds tested and the respective feeding rates for 537 were 55%, 41% and 62% compared to 13%, 24% and 12% for 219. Previous studies have been made of the feeding success of tsetse on these two particular hosts, and the results have indicated that tsetse are generally more successful on 537 (61%) than 219 (51%) (Torr & Mangwiro, 2000). Similarly, the results from studies of the feeding responses of tsetse presented with the option of feeding on either 219 or 537 (above) also showed a significantly greater number of feeds from 537.

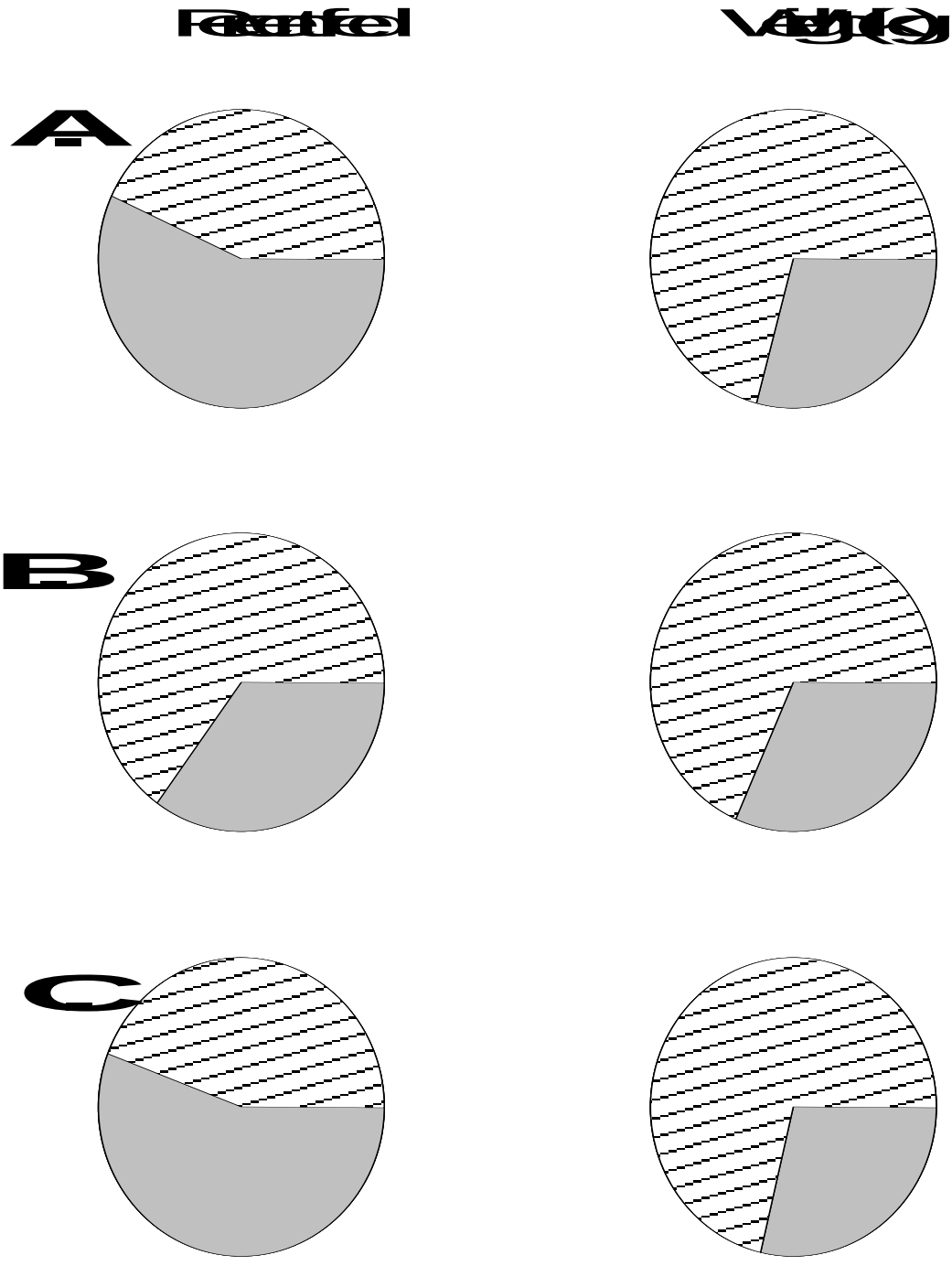

Fig. 3. Source of bloodmeals for tsetse attracted to different groups of four cattle. Results indicate the percentage of meals (left-hand column) taken from individual cattle and their respective liveweights (right-hand column). Black and crosshatched segments of the piecharts indicate animals #219 and #537 respectively. Tsetse sample sizes for herds A, B and C were 24, 18 and 48 respectively and the total herd liveweights were 1605 kg, 1312 kg and 1350 kg respectively.

Natural herds: effect of herd size on mean feeding rate

Larger herds of cattle showed significantly greater rates of feeding success. For instance, an experiment comparing the feeding responses of tsetse attracted to a single ox or a herd of 12 oxen showed that the larger herd attracted a significantly greater number of tsetse and the tsetse showed significantly higher feeding rates.

Table 5. Detransformed mean number and percentage feeding success of tsetse attracted to a single ox or a group of 12. Percentage feeding rates are based on sample sizes of *n* flies. (Catch data previously presented in Torr *et al.,* 2000).

Species	Sex		1 ox	12 oxen	se
G. m. morsitans	Male	Mean	2.9	4.2	
		Transformed mean	0.591	0.713	0.038
		Feeding rate	34.6	59.7	
		n	52	62	
	Female	Mean	4.8	8.9	
		Transformed mean	0.766	0.997	0.063
		Feeding rate	62.8	65.2	
		n	86	161	
G. pallidipes	Male	Mean	12.8	51.1	
		Transformed mean	1.140	1.717	0.043
		Feeding rate	58.5	69.8	
		n	147	650	
	Female	Mean	27	115.5	
		Transformed mean	1.447	2.066	0.039
		Feeding rate	53.5	72.9	
		n	301	1424	

For the single ox, feeding success was negatively correlated with the density of *Stomoxys* ; increasing numbers of *Stomoxys* increase host-defensive behaviour and this in turn reduces the feeding success of tsetse ((Torr & Mangwiro, 2000). The non-linear correlation between herd size and numbers of biting flies attracted to a herd (Hargrove *et al*., 1995) means that the mean number of flies per host is reduced with larger herds of cattle and thus the mean feeding rate is higher with large herds, especially at high densities of biting fly (Fig. 4).

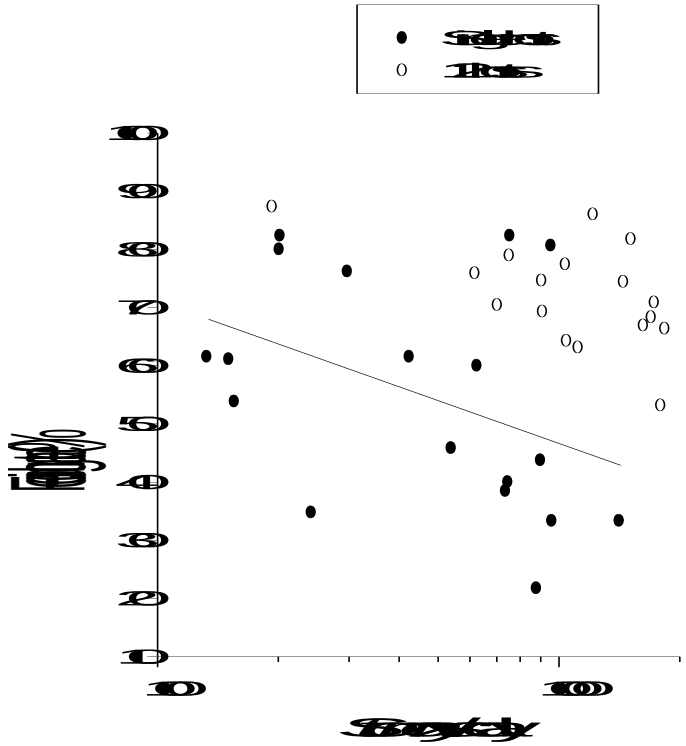

Fig. 4. Scatterplot of percentage feeding rate of tsetse and density of *Stomoxys* for a single ox or a group of 12 oxen. Mean catches of tsetse for single host and 12-host herds are shown in Table 5. Line shows regression for percentage feeding rate on a single ox against the density of *Stomoxys*.

Natural herds: host selection

Analyses of the DNA profiles of flies attracted to heterogeneous herds of 8 cattle showed that <5% of meals were from the calves and >70% were from the two larger oxen in the herd (Table 6).

Table 6. Percentage of meals taken from adult male, adult female or young cattle hosts. The test herds consisted of 8 animals, each comprising two oxen/bulls, four heifer/cows and two calves. Percentages were either based on all identifications, including mixed meals, or only those meals where a single host was identified. The percentages for all meals sum to >100% because a mixed meal comprises blood from at least two hosts.

	April 2000		October 2000	
	All meals	Single meals	All meals	Single meals
Oxen/bull	81	76	71	72
Cow/heifer	28	23	29	28
Calves	4	1	1	1
n	224	149	273	257

There is a strong correlation between the liveweight of an animal and the percentage of feeds from that animal and thus an animal's liveweight provides a good rule of thumb to judge which animals to treat. To illustrate the efficacy of selectively treating herds using this rule, animals were ranked within the herd according to their weight and the rank was then plotted against the percentage of meals from the animal. The pooled results (Fig. 5) for four herds studied in April-October 2000 show the proportion of tsetse that would contact insecticide-treated cattle as increasing numbers of cattle were treated. Generally, treating the two largest animals in the herd would kill ~75% of the flies visiting the herd. Slightly higher efficiences of ~90% are obtained with the herds studied in April 2000. This arises because in April, 16% (n=261) of meals were mixed compared to 1% (261) in October. Consequently in April, 16% of tsetse contacted at least two animals in the herd whereas only 1% did so in October. The higher rates of mixed meals are thought to be due to increased rates of host-defensive behaviour elicited by the larger numbers of *Stomoxys* that are present in April at the end of the wet season.

For the herds considered here, treating more than 2/8 animals would only produce slight (1-5%/animal) improvements in the percentage of tsetse 'killed' by the herd. For the herds used in these studies, the two largest animals constituted ~44% of the liveweight of the entire herd. Given that the dosage of pour-ons is directly related to the liveweight of the animal, treating the two largest animals in the herd, rather than the entire herd, would half insecticide costs and, by not treating the young cattle, enzootic stability to tick-borne diseases would be maintained.

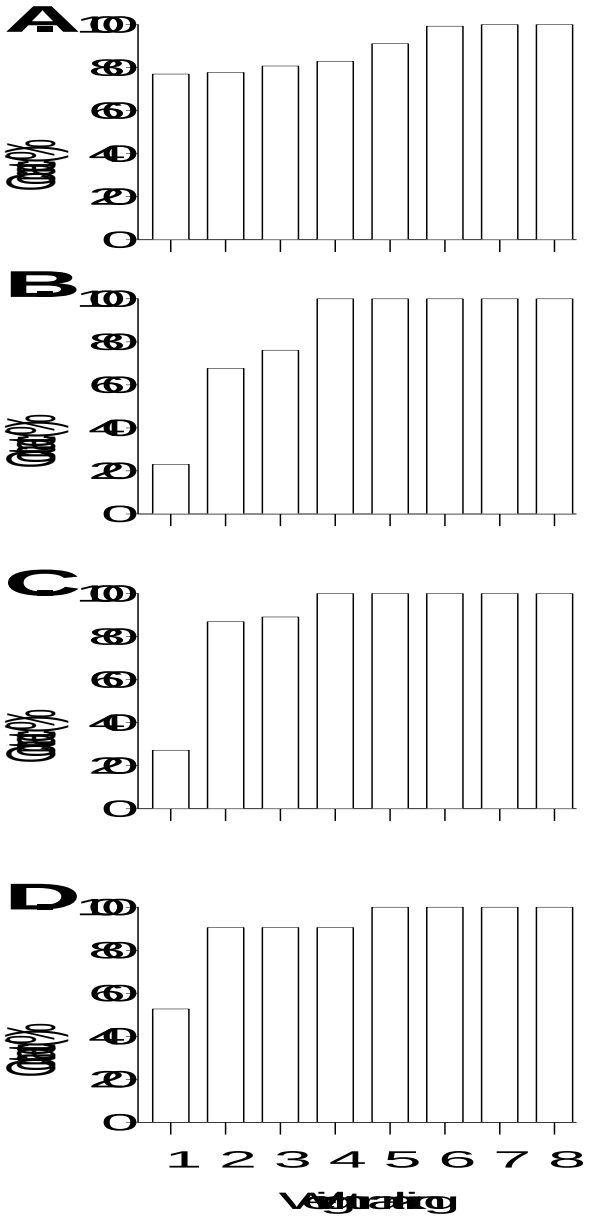

Fig. 5. Percentage of tsetse contacting insecticide-treated cattle assuming that 1-8 cattle were treated with insecticide and treatment was based on the relative weight of an individual within the herd. Thus the heaviest and lightest animals are ranked 1 and 8 respectively. Each herd comprised 8 animals (2 oxen, 4 cow/heifers, 2 calves), studies were undertaken in the dry (A,B) or wet (C, D) seasons and percentages are based on sample sizes of 135 (A), 130 (B), 112 (C) and 81 (D) fed tsetse.

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Contribution of Outputs

The project goal was:-

'Performance of livestock of poor people improved through the control of disease.'

The outputs will contribute towards developing a sustainable, environmentally beneficial and cost-effective strategy for controlling trypanosomiasis using insecticide-treated cattle. The work indicates that a selective dipping strategy, based on the treatment of the larger animals within a herd can achieve more cost-effective control. Such a strategy will be particularly cost-effective for poorer livestock-keepers in Zimbabwe, Tanzania and Ethiopia who typically have small heterogeneous herds of cattle. The findings are being used in the design of community-based tsetse control operations being conducted in Ethiopia and Tanzania and government-funded operations in Zimbabwe.

The findings are particularly relevant to tsetse control operations being promoted by NGOs in East Africa. In Ethiopia for instance NGOs such as FARM-Africa, SOS Sahel and Action for Development are promoting the use of insecticide-treated cattle to control tsetse. The costs of insecticide are to be largely borne by the cattle owners themselves and these cattle owners have not been prepared to treat all their cattle. In operations promoted by FARM-Africa in the Konso district of southern Ethiopia for instance, participating owners initially treated half their cattle (Torr *et al.*, 2001) and this rate of treatment declined to ~25% after a year or so. Similar situations prevail in community-based control operations being undertaken and/or planned in the region. The present findings provide a clear guide as to which cattle should be treated, and what the costs and benefits of selective treatment might be. Farmers appear to be naturally more inclined to treat their larger animals and the extension message fits with this inclination.

The results also have implications for other methods of controlling trypanosomiasis. First, the results suggest that older/larger animals are more likely to be bitten than smaller/younger ones. Consequently, the large animals are more likely to contract trypanosomiasis and be reservoirs for disease. This suggests that more effective use of trypanocides might be achieved by focussing treatments on the larger animals in the herd.

During the course of the project, findings were disseminated to veterinary staff of the Zimbabwe Tsetse and Trypanosomiasis Control Branch and the Regional Tsetse and Trypanosomiasis Control Programme in Zimbabwe. The findings were also reported at seminars presented to staff from Tanga Dairy Development Project, in Tanzania, and scientists and livestock specialists concerned with controlling tsetse in the Konso District of Ethiopia.

In addition, the following presentations were made at scientific meetings:-

Mangwiro T.N.C. (1999). The use of DNA microsatellites to identify the individual sources of bloodmeals in tsetse. Paper presented (by Mangwiro) at the ISCTRC meeting in Mombasa, Kenya (September 1999).

Torr, S.J., Wilson, P.A. & Schofield, S. (1999). Use of DNA microsatellites to identify multiple bloodmeals from tsetse flies feeding on cattle. Paper presented (by Schofield) at the annual meeting of the Entomological Society of America at Atlanta, USA.

Torr, S.J., Schofield, S. & Wilson, P.J. (2001). The effects of herding behaviour on the feeding behaviour of tsetse: who are the winners and the losers? Paper presented (by Schofield) at the annual meeting of the Entomological Society of America at Montreal, USA (December 2000).

In addition, the findings were disseminated to the wider academic community via a paper published in *Medical and Veterinary Entomology* (see Appendix) and further presentations are planned in 2001, at the ISCTRC meeting in Burkina Faso and the ESA meeting in USA.

The outputs from this project have been disseminated via two DFID/ASSC-funded projects in Tanzania and Ethiopia and the results influenced the design of control schemes planned for the Tanga region of Ethiopia and the Konso district of Ethiopia.

These findings, along with outputs from other tsetse research projects, will be promoted by a new AHP-funded project specifically concerned with disseminating tsetse control technologies to NGOs. The geographical foci of this new project will be tsetse-affected regions of Tanzania and Ethiopia and will build on the personal, professional and institutional links established in these countries by various DFID-(AHP, LPP, ASSC) supported projects.

Annex / Appendix

See attached paper (mve_2001.pdf) or follow this link **PDF file**.*

* http://www.gre.ac.uk/~fd03/pdf/mve_2001.pdf