FINAL TECHNICAL REPORT

Title of Project: **Incrimination of vectors of *Trypanosoma vivax* in the new outbreak zone of Santa Cruz, Bolivia**

R Number: **R7356**

Executive Summary

Bovine trypanosomiasis, a debilitating and sometimes fatal disease caused by the blood parasite *Trypanosoma vivax*, was first recorded in Bolivia in 1996, spreading rapidly to most of the cattle rearing areas of lowland Santa Cruz and Beni Departments. The purpose of this project was to incriminate the vectors and thereby improve our understanding of the epidemiology of the disease, and provide information that could be used to improve disease control (e.g., by strategic application of prophylactic and insecticidal treatments of cattle to periods when flies were more abundant).

The project activities were focused on a smallholder farm, Chocolatal, with a history of bovine trypanosomiasis, located in Guarayos Province, on the borders between Santa Cruz and Beni. During a monthly trapping programme 25,000 tabanid flies (horseflies) of 33 species were caught and identified. Other biting flies were caught in negligible numbers. The most abundant tabanid was *Tabanus occidentalis* (77% of the total). All three species of tabanid that have been demonstrated as vectors elsewhere in South America were collected on the farm. Tabanid seasonality was influenced by climate. They were more abundant in the warmer and wetter months of summer (November-March) and less abundant in the cooler, drier months of winter (May-August). Tabanid populations were much more abundant in Guarayos Province than in the central Province of Sarah. Significantly, Guarayos Province also had the highest record of cases of bovine trypanosomiasis in Santa Cruz.

Cattle on the farm reacted to increasing numbers of tabanid flies by increasing their defensive activities (tail flicks and hoof stamps), a response that would have produced an increased number of interrupted feeds, an important factor in mechanical transmission. Thus, cattle response to high numbers of tabanids would exacerbate disease transmission. Insecticide treatment of cattle (cypermethrin pour-on) had significant benefits: it reduced the proportion of alighting flies that fed and reduced the feeding time, therefore blood intake, of those that did feed by 40%. However, studies are needed to determine whether or not the partially fed flies would continue their feed on another host, thereby increasing the risk of mechanical transmission. Further studies are also needed on insecticidal treatments, in order to recommend an appropriate, long lasting and economical application.

Molecular studies demonstrated the presence of trypanosome DNA in the bloodmeals of seven species of tabanid, including *T. occidentalis*. Trypanosome DNA was present in 7% of completed bloodmeals, but was also found in the abdominal contents of flies with no apparent bloodmeal. The possibility that trypanosomes can survive in tabanids for extended periods requires further study.

Tabanid flies were incriminated as the most likely vectors of bovine trypanosomiasis and, therefore, control regimes based on established fly avoidance techniques could result in a significant reduction in the risk of transmission of *T. vivax*. These include the separation of infected from uninfected animals by 200m and the avoidance of pasture/forest edges for grazing, measures of low cost to smallholders. Additionally, livestock movement controls are a major means to control spread of the disease.

Background

Trypanosoma vivax is a blood parasite that originates in Africa, where it causes significant losses to the livestock industry, through loss of productivity, abortions and deaths of infected animals. The most important vector of trypanosomes affecting ruminants in Africa is the tsetse fly, *Glossina* species, in which the parasite undergoes a cyclical development. *Trypanosoma vivax* was introduced into South America as early as the last century, possibly in a shipment of Zebu cattle from Senegal. It was first described in French Guyana, but was soon found to be present in many other countries in northern South America. Until very recently, *T. vivax* affected livestock only in regions north of Amazonia. However, outbreaks of trypanosomiasis due to *T. vivax* were reported from the Pantanal region of Brazil in 1995 and from Santa Cruz Department, Bolivia, in 1996. The spread of bovine trypanosomiasis in Bolivia appeared to follow the movement of infected cattle along relatively well defined livestock trekking routes. The first cases were recorded to the south of San Matias in the Province of Angel Sandoval in March 1996. Outbreaks were then recorded showing a progressively westerly spread, from San Vicente, to San Ignacio de Velasco to San Javier and finally to around Santa Cruz de la Sierra city itself. By 1997, the distribution of outbreaks involved most of Santa Cruz Department to the north and east of the capital, plus a large area of Beni Department. In these areas are concentrated approximately 60% of the cattle population of Bolivia, a significant portion of the livestock industry, which is worth approximately US$224 million per annum (1998 values). This production was threatened by the introduction of bovine trypanosomiasis as a new disease in the region. A significant problem identified for the future was the potential for spread of trypanosomiasis to the herds of smallholders, where loss of a few animals (kept for milk and draught power) would have high impact, and to the dairy herds, which are composed of less tolerant exotic breeds and cross breeds.

It appears that *T. vivax* in South America has become well established in the absence of the biological vectors found in Africa, species of tsetse fly. The vectors in Bolivia were unknown, but elsewhere in South America tabanid flies, in which *T. vivax* does not undergo a cyclical development, have been implicated directly and indirectly as vectors. Thus, mechanical transmission of the parasite from one bovine to another has been successfully achieved with the tabanids *Cryptotylus unicolor*, *Tabanus importunus* and *Tabanus nebulosus*, all of which are found in Santa Cruz Department. Indirect evidence of transmission comes from epidemiological studies in Colombia that demonstrated a significant temporal relationship between the feeding activity of tabanids and *T. vivax* incidence, and which showed that *T. vivax* infections were associated with low-lying swampy areas, where tabanids breed.

It was against this background that it was considered essential to undertake studies to incriminate the vectors of *T. vivax* in Bolivia. There were two main reasons: (i) to improve strategic timing and targeting of prophylactic treatments, through knowledge of vector seasonality and distribution, (ii) to enable vector control to be planned, as an element in an integrated control programme. Few studies on tabanids had previously been undertaken in Bolivia other than preliminary survey work funded by the Animal Health Programme (Project R5407).

Evidence for a demand for the project was identified in the demands for studies of bovine trypanosomiasis in Bolivia following its introduction, made by a range of groups concerned with the local livestock industry. These included the livestock farmer unions of Bolivia, who represent the full range of livestock owners from smallholders upwards (e.g., FEGASACRUZ and FEDEPLE), by government veterinary organizations (e.g., LIDIVET and UNIVEP) and by the Ministry of Agriculture itself (to FAO).

Project Purpose

The main purpose of this project was to initiate a series of studies of biting flies in Santa Cruz Department with the objective of incriminating the vectors of bovine trypanosomiasis. Incrimination of the vectors of this newly introduced disease would contribute to improved control by enabling more effective targeting of prophylactic applications to livestock and development of vector control strategies that could be integrated into overall strategies for control of bovine trypanosomiasis. Control of this disease would lead to improvement of the performance of livestock, which would be of significant benefit to smallholders.

Research Activities

Fly survey

In order to record the diversity, abundance and seasonality of tabanid flies, monthly trapping studies were carried out on two farms of smallholders in Santa Cruz Department, Chocolatal farm in Guarayos Province and San Diego farm in Sarah Province. Both had a recent history of cases of bovine trypanosomiasis. Trapping studies were carried out by the use of two types of trap proven as effective for tabanid capture: (1) malaise traps (Figure 1), that act primarily as flight intercept traps and, (2) canopy traps (Figure 2), that attract flies by visual and olfactory cues. The malaise traps were of standard design (Marris House Nets, Bournemouth, UK) having black walls and white roofs that provided some visual target for flies. Canopy traps (constructed locally) were fitted with an attractive black sphere below and augmented by the release of octenol from plastic sachets. Flies were collected from the traps twice daily over a four-day period each month. After collection they were killed and identified. Voucher specimens were pinned and are maintained in the entomological collections of the Museo de Historia Natural "Noel Kempff Mercado", Santa Cruz, Bolivia, and of The Natural History Museum, London, UK.

Fly-cattle interactions

To study the effect on cattle of the biting nuisance caused by tabanid flies, observations were made of two defensive responses of cattle to tabanid flies, active tail flicks and hoof stamps, relating these responses to the numbers of tabanids on the cattle (Figure 3). Observations were made by a single observer standing approximately 2-3 m to one side of the experimental animal. The experimental animals were not tethered in any way but were grazing naturally in the pasture at Chocolatal farm. Because it was not possible to record fly numbers and cattle activity simultaneously, the following procedure was adopted for each observation session:-

- Record fly numbers on cattle six times within one minute, i.e. at intervals of approximately ten seconds (each recording consisted of a visual "sweep" of one side of the cattle, up one leg, across the head and body and then down the next leg), then...
- Record tail and hoof activity for one minute, then...
- Record fly numbers on cattle again, six time in one minute, then...
- Repeat to obtain ten tail/hoof observation periods in each session

In preliminary analyses (Figures 16-18), the numbers of tail or hoof movements in any one minute were plotted against the average number of flies seen in any one observation “sweep” during the minutes before and after the tail/hoof observation period (i.e. each value for number of flies was a mean of 12 observations). In a final analysis (Figures 19-21), the mean cattle activity and mean fly numbers were calculated for each observation session, with an average of ten replicates per session.

Effects of insecticide application on tabanid feeding behaviour

The most widely used tabanid suppression and control technique in Bolivia is the direct application of insecticide formulations to the body of the animal, by spray or pour-on. To evaluate the effect of these on tabanid feeding behaviour, feeding activity was monitored before and after insecticide applications. Feeding activity was measured in terms of the duration of feeding and whether or not the feed was clearly made to completion, i.e., whether or not the fly appeared to be full (Figure 4). All instances in which flies terminated a feed after being clearly disturbed during feeding, either by the host animal itself or by other flies and predatory wasps, were excluded from the analyses.

The insecticide used in this study was one that is widely available in Bolivia and commonly used, a cypermethrin pour-on (5g a.i. per 100 ml, Tehuelche Pour-On). The recommended method of application as a fly repellent is 30 ml, distributed as 10 ml along each side, 5 ml to the shoulder area and 5 ml to the rump. However, due to the observed preference of tabanids for the legs, in our studies 30 ml of cypermethrin was applied as 5 ml along each flank and 5 ml to the outer surface of each leg (Figure 5).

Insecticide bioassay

To evaluate the toxicity of insecticide applications to flies landing on treated cattle, flies were collected by hand-net within 5-10 seconds of alighting on cattle treated at least two hours previously. They were transferred after capture to a cylindrical cage, with 15-20 flies per cage (Figure 6). The cage was kept cool and humid by covering it with tissue soaked in water and keeping it under shade, but otherwise at ambient temperatures. At 0, 3, 6, 9 and 24 hours after collection, the flies were recorded in one of three categories: either, (1) alive, able to fly, (2) knocked down, usually on its back but able to make leg movements, or, (3) dead. Control flies were collected from untreated cattle, but otherwise were treated in the same way. The insecticides used were cypermethrin and fipronil (1 g a.i. per 100 ml), both applied as pour-ons as outlined in the preceding section.

Molecular studies

Biting flies alighting on individual cattle were trapped by hand at monthly intervals for up to 10 months (Figure 7). Abdominal squashes were prepared from these flies in the field, by extracting the abdominal contents via the terminal abdominal segment onto Whatman filter paper using clean forceps (Figure 8). The contents were pressed into the paper using the forceps and, after air drying, they were fixed in acetone. The flies were pinned and subsequently identified. The abdominal squash preparations from these flies were sent to CTVM and subjected to a two-step elution method and assay protocols developed as an output of an earlier DFID-funded project - R7162 (Figure 9). The eluates were then analysed for the presence of *Trypanosoma evansi* and *T. vivax* DNA using a Polymerase Chain Reaction Assay (PCR) (Figure 10) and for host blood components by an Enzyme-Linked Immunosorbent Assay (ELISA), both of which were also developed as outputs from R7162.

Figure 1: Malaise trap

Figure 2: Canopy trap with Guido Zarate changing apical fly collector

Figure 3: Procedure for observing fly-cattle interactions

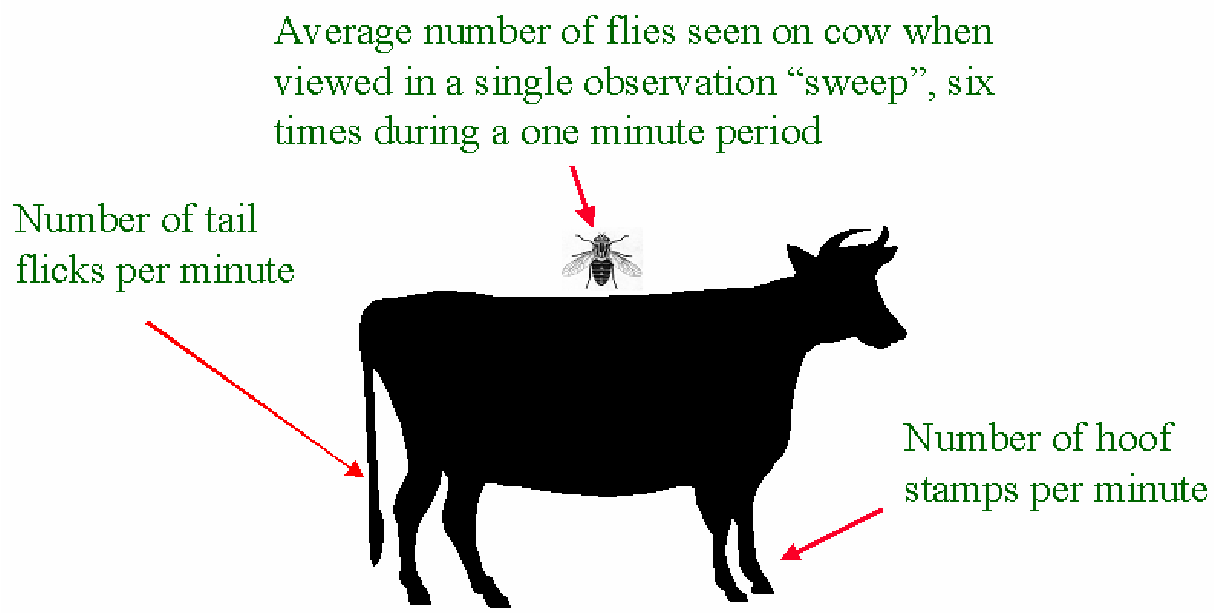


Figure 4: Procedure for measuring duration of fly feeding

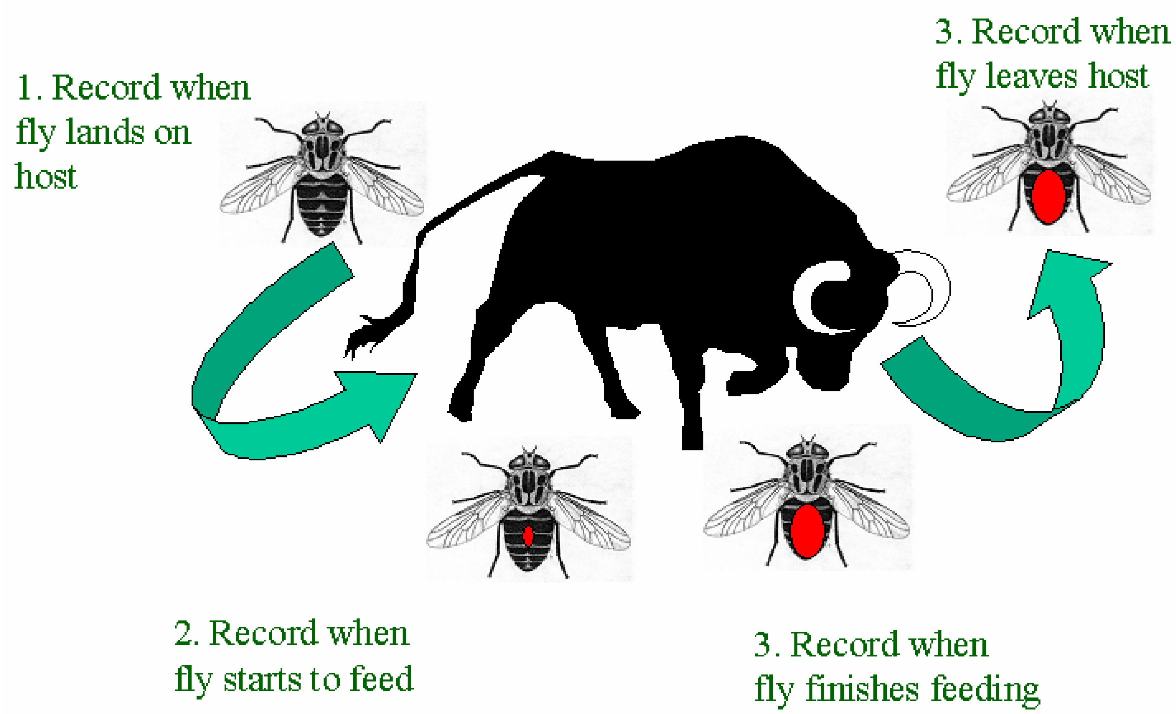


Figure 5: Method of application of cypermethrin pour-on to cattle.

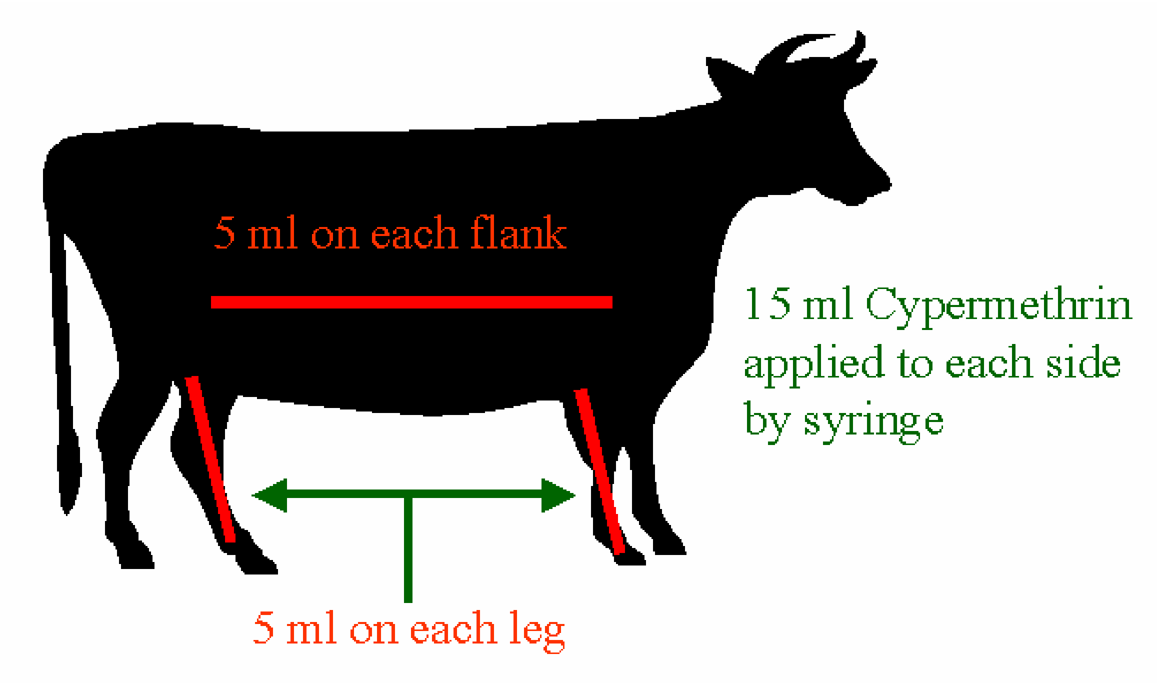


Figure 6: Cage of *Tabanus occidentalis* collected from insecticide treated cattle for bioassay study.

Figure 7: Jose Luis Aramayo Bejarano capturing tabanid flies using hand net

Figure 8: Guido Zarate preparing abdominal squashes of hand collected tabanids.

Figure 9: Processing of fly squash material at CTVM

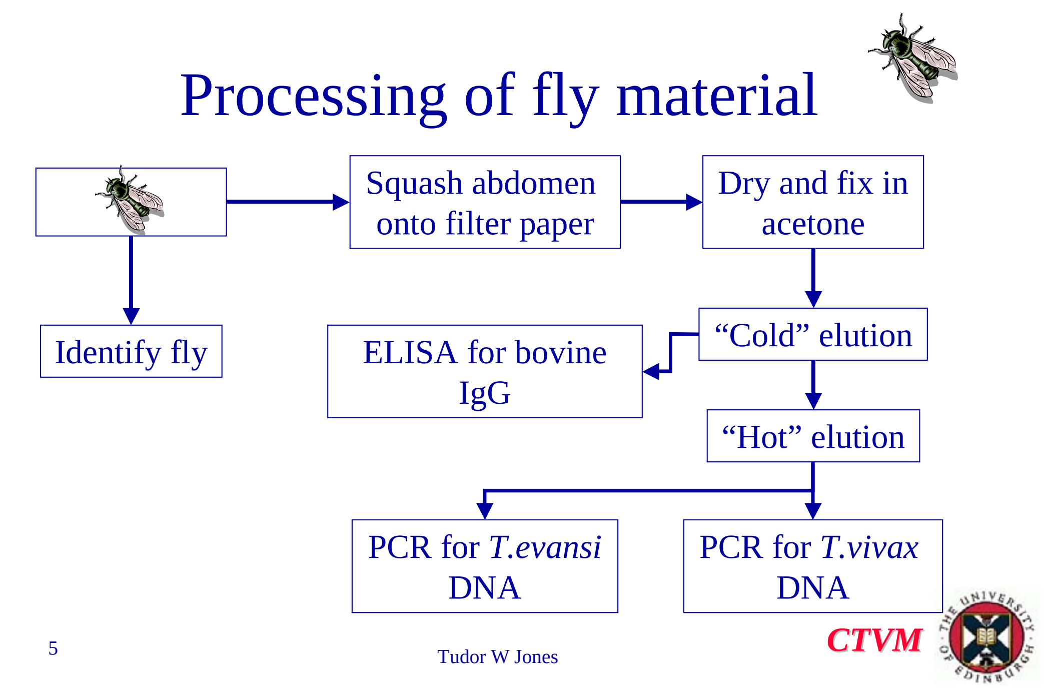
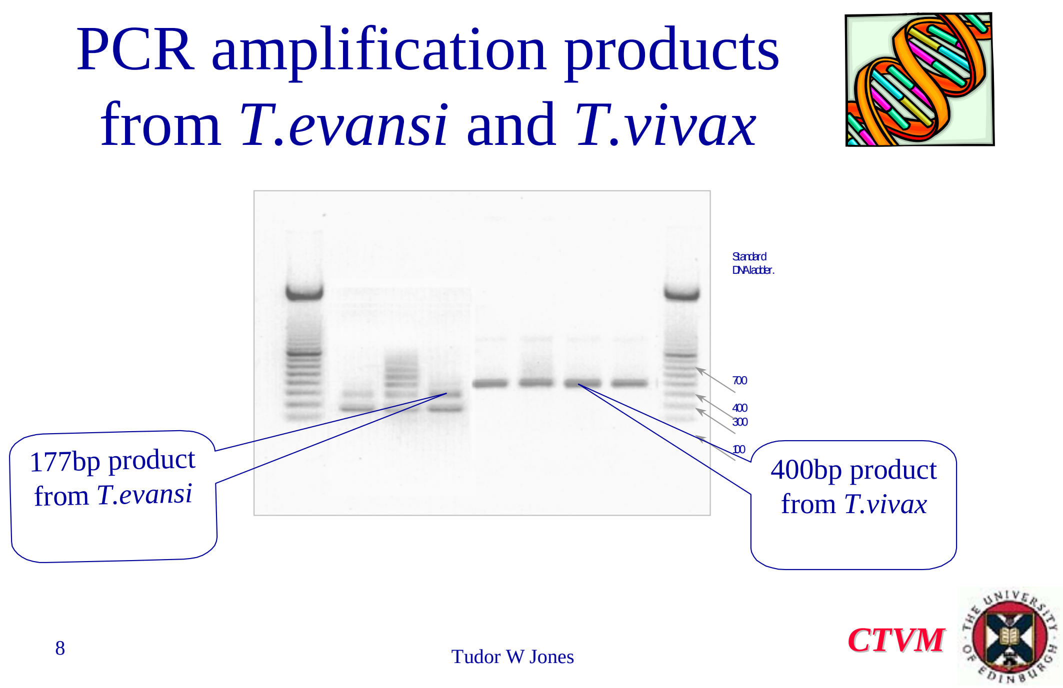


Figure 10: PCR products for *Trypanosoma evansi* and *T. vivax* after amplification of eluted fly squash material



Outputs

Fly survey

Due to a delay in the arrival of the project vehicle, monthly trapping of tabanids was not started until November 1999 and it was continued up to February 2001. The abundance of flies at San Diego farm was only 4% of that at Chocolatal farm. Therefore activities were abandoned at San Diego to concentrate at Chocolatal where, in addition to survey work, all of the related studies of fly behaviour were carried out. Chocolatal Farm (*c.* 15.4ºN 63.4ºW) is located in a forested area alongside the Rio San Pablo, which forms a part of the border between Santa Cruz Department and Beni Department to the north. In addition to this river that forms the boundary to the farm, the area is rich in other water courses and still waters (Figure 11). These provide an ideal breeding habitat for tabanids. An undergraduate student of the Universidad Autonoma "Gabriel Rene Moreno", Santa Cruz, Lucia Malisa Coca Bruno, is currently conducting a study of tabanid larval habitats with the support of the project.

Chocolatal farm was relatively small with a herd of 20±5 cattle throughout the majority of the project. The cattle were maintained primarily for breeding and the herd consisted of one adult bull with the remainder being approximately 50:50 breeding cows and their young. The herd grazed on the single major pasture of the farm and also crossed the road to another area of pasture within 2 km of the farm (Figure 12). Most trapping studies were carried out within the main farm, with four canopy and two malaise traps being positioned in the same spots, at the edges of the pasture, during each monthly trapping period. An additional canopy trap was located in the forest area between the two pastures.

During the project 24,846 tabanid flies were captured in traps and identified at Chocolatal. These belonged to 33 different species (Table 1) the most common of which were *Tabanus occidentalis* Linnaeus, 1758, *Tabanus pungens* Wiedemann, 1828, *Tabanus claripennis* (Bigot, 1892) and *Lepissela crassipes* Fabricius, 1805. By far the most numerous species, accounting for 76.8% of the total catch, was *Tabanus occidentalis*, an aggressive feeder on the lower body and legs of cattle. The seasonal trend was for two broadly separable periods, with higher fly abundance in the warm and wet summer months of November to March and lesser fly abundance in the cool and dry winter months of May to August. This was less apparent for *T. occidenatalis* (Figure 13) than for the other species (Figure 14), due to the unusually large capture of *T. occidentalis* in November 2000. Due to the position of Chocolatal at the edge of the Amazon region of Bolivia the climate was favourable to tabanids for much of the year and seasonal trends were much less apparent there than further south. An example of the more marked seasonality at a farm nearer to Santa Cruz, in a similar zone to San Diego, is demonstrated from previous work based on hand netting flies from a horse bait (Figure 15; J.L. Aramayo Bejarano UAGRM Ing. Thesis 1996).

Fly-cattle interactions

Eight different cattle were used for studies of cattle responses to tabanid flies and two main categories of tail flicking response were noted: either no change in response or an increasing response to increasing densities of flies (Figure 16).

Two cattle showed virtually no change in response to changing densities of flies (LB and VN). One of these was very old (LB, 15 years) and had a characteristically low level of response (<10 tail flicks per minute) to a wide range of fly densities (1-6 flies per observation period). The other cattle was much younger (VN, 2 years) and showed a very variable response (0 to>30 tail flicks per minute) to a narrow range of fly densities (only 1-3 flies per observation period). It is possible that a change in its responsiveness would have been recorded if it had been subjected to higher densities of flies. Its high responsiveness was typical of younger animals.

Six cattle showed an increasing response to increasing densities of flies. For five of these cattle (all 3 years old except for LC, 9 years) the increase was at almost the same rate (i.e. parallel lines), although at different overall levels of responsiveness. For one cow (PM, 1.5 years old), the rate of increase with increasing density of flies was greater, possibly due to it being younger and therefore more easily disturbed by the tabanid activity.

When the two cattle that showed no change in response to changing fly densities were removed from the analysis, the pooled data (n = 224) of the other cattle showed a clear and significant relationship between fly numbers and tail flicks (Figure 17; $r^2 = 0.2682$, $P<0.0001$). When the two activities, tail flicks and hoof stamps, of these cattle were analysed, a significant regression was observed (Figure 18; $r^2 = 0.3891$, $P<0.0001$) as might be expected, with hoof stamps increasing in frequency as tail flicks increased in frequency. However, there were far fewer hoof stamps than tail flicks and so this activity provided a less sensitive scale against which to measure tabanid activity. Therefore, the analyses reported here concentrated on tail flicks as a measure of cattle responsiveness.

In a final analysis, the mean tail activity was calculated against the mean number of flies in each observation session (Figure 19) demonstrating a highly significant relationship ($r^2 = 0.4146$, $P<0.001$). This relationship could be improved slightly if the cattle response, the tail flicks, were computed on a logarithmic scale (Figure 20; $r^2 = 0.4667$, $P<0.0005$). Fitting a two-degree polynomial curve to the data indicated that the number of tail flicks was reaching a maximum in the experimental system studied (Figure 21).

Effect of insecticide application on tabanid feeding behaviour

The previous studies on fly-cattle interactions had demonstrated that one older cow (LB, La Baya) showed virtually no change in defence responsiveness to changing densities of flies (Figure 16). Because the studies of feeding behaviour required minimal disruption of fly feeding by external factors, such as cow activity, La Baya was chosen for these studies. In addition, because of the preponderance of *Tabanus occidentalis* at the study site, all data analysed here refer only to that species.

It was clear that cypermethrin applications had a significant effect on fly activity. Whereas 40-75% of alighting flies fed to completion before application, only approximately 20% of flies alighting 1-3 hours after application did so (Figure 22). Of those flies that did feed there was also a significant difference in the durations of feeding before and after application of insecticide. A mean feeding duration of 145-165 seconds before application fell to 85-100 seconds after application of cypermethrin, a 38-41% reduction (Figure 23). There were no significant changes in the time after landing when flies first started to feed, nor in the time after landing when non-feeders left (Figure 23).

To observe the effect of time since application of insecticide on feeding behaviour, fly activity was recorded before application, after application and then at intervals over a 70-hour period until and after a second application of pour-on was made. As expected from the previous studies, after the first application of cypermethrin a significant fall was seen in the percentage of fully fed flies (Figure 24) and in the mean duration of feeding (Figure 25). However, these values slowly recovered over the 70-hour period until they were approaching, but still below, pre-treatment levels. The second application of insecticide reversed this recovery, but not to the extent of the first application.

Insecticide bioassay

Over the 24-hour period of observation, the percentage of flies from cypermethrin treated cattle (n = 49) that were recorded as alive decreased gradually, until after 24 hours only 14% were in that category, compared to 72% of control flies (n = 40) (Figure 26). The trends with fipronil were very different. Thus after 9 hours both control (n = 40) and treated (n = 68) groups had 100% survival. However, at 24 hours none of the treated flies were recorded as alive compared to 83% of control flies (Figure 27).

Molecular studies

Sixteen species of flies were caught on the cattle, with *Tabanus occidentalis* accounting for over 70% of flies caught over the study period (Figure 28), a similar proportion to that in trap catches. *Tabanus occidentalis* was also found as part of each monthly catch, ranging from 30-100% of each monthly population (Figure 29). Trypanosome DNA was detected by PCR in seven of the sixteen fly species caught on the cattle, again with the highest proportion represented by *T. occidentalis* (Figure 30). The majority of those files in which trypanosome DNA was found by PCR also contained host blood (73%) and these represent flies that had recently fed on an infected animal – either the one on which they were caught or another animal in the herd. However, nearly a quarter of PCR-positive flies showed no evidence of host blood, which suggests that trypanosomes might persist in the fly gut for longer than previously thought. Again, *T. occidentalis* was the predominant fly species in this group. No significant differences were found between the two trypanosome species in the proportion of PCR-positive, unfed flies. The importance of this persistence of trypanosome DNA in relation to transmission is unknown, but if this DNA represents viable trypanosomes, then biting flies such as Tabanids might be able to transmit trypanosomes for hours rather than minutes after taking an infective feed, either by regurgitation or, potentially, as biological vectors. Confirmation of any biological transmission role for tabanid flies can only come from controlled feeding studies.

The role of tabanid flies in the transmission of bovine trypanosomiasis in Santa Cruz Department

During the course of this study, very few biting flies other than tabanids were captured at Chocolatal and species in the genus *Stomoxys* (Muscidae) were rarely encountered. It was clear that the major group of biting flies that could be implicated in mechanical transmission of bovine trypanosomiasis was that of tabanids, hence the remainder of this discussion concentrates on those flies. The tabanid flies collected at Chocolatal included all three species proven capable of transmission of this disease in South America (see Background section). There is no reason to suppose that most medium to large tabanids cannot also transmit the disease, including the highly abundant species, *Tabanus occidentalis,* studied here.

Tabanid flies were much more numerous and occurred over a longer season of flight activity at Chocolatal farm in Guarayos Province than at San Diego farm in Sarah Province. The risk of mechanical transmission of bovine trypanosomiasis by tabanids must therefore be greater in the northern regions and it is of considerable significance that the greatest numbers of positive cases of *T. vivax* reported by the Veterinary Diagnostic Laboratory, LIDIVET, were from Guarayos Province.

Mechanical transmission of bovine trypanosomiasis is facilitated by the following conditions:-

- when the parasitaemia of the peripheral blood supply of the infected host is high
- when the population of biting flies is high
- when there is a high proportion of interrupted feeds of flies, due to their disturbance during feeding (e.g. disturbance by host defensive behaviours or by competing flies)
- when there is a minimal delay between feeding on an infected host and resumption of feeding on a second, uninfected host – this is most likely when hosts are closely associated, as in herd animals
- when biting flies are highly mobile
- when a large amount of infected host blood remains on the mouthparts of biting flies between interrupted feeds.

There will be interaction between all of the above factors. For example, a host with an acute infection and high parasitaemia might be considered a good source of infectious agents, but if that host is listless due to the disease and unable to respond to fly attack with strong defensive behaviours, then interrupted feeds and subsequent mechanical transmission are unlikely to occur.

With regard to the entomological factors listed above, it is widely accepted that tabanid flies are highly mobile and that they retain greater amounts of blood on their mouthparts that all other biting flies. From the survey work at Chocolatal it was also clear that the population of tabanids was high there. Due to the herd behaviour of cattle, moving around the pasture in a group rather than as individuals, it was again clear that there was the potential for minimal delay between feeding on two hosts. Therefore, research studies concentrated on investigating the remaining entomological factor, that of interrupted feeds.

One of the major causes of interrupted feeds is disturbance, usually by host defensive behaviours, by predatory insects or by other, competing flies. Studies at Chocolatal demonstrated clearly that host defensive behaviours increase as the density of tabanid flies increases (Figure 19). This means that as tabanid numbers increase, the proportion of feeds that are interrupted is likely to rise, leading to a greater number of interrupted feeds than would be predicted if the proportion of interrupted feeds stayed at the same level.

A common response of farmers, including smallholders, to tabanid attack is to apply an insecticide, such as the cypermethrin pour-on studied here. This has the beneficial effect of significantly reducing the number of flies that start to feed (Figure 22). For those flies that do feed, there is a significant reduction (*c.* 40%) in the duration of feeding. This again is of benefit, because tabanids can cause significant blood loss to cattle (Figure 31) and the reduced duration of feeding will reduce blood loss. However, what would not be of benefit would be if these incompletely fed flies left the treated animal to immediately seek the outstanding 40% of the bloodmeal they required on another animal. In such a situation the problem of mechanical transmission might actually be exacerbated by the insecticide application, due to the increased number of what are, in effect, interrupted bloodmeals. On the other hand, it is possible that the reduced feeding on treated livestock is a physiological response to insecticide contamination and that no subsequent feeding would occur, thereby eliminating the risk of an interrupted feed. This important question requires further study.

Although there appeared to be immediate benefits of cypermethrin treatment of livestock, these were short-term and within three days of treatment the effects had almost disappeared (Figures 24 and 25). Such short-term benefits are costly, both in economic terms and in the time needed to maintain insecticidal effectiveness by repeated applications. Future studies should examine alternative formulations of cypermethrin and different insecticides.

Non-feeding flies left treated animals within 20-30 seconds of alighting (Figure 23). When captured after a shorter interval (5-10 seconds), the 24-hour mortality of flies from a cypermethrin treated cow was greater than the mortality of flies caught on an untreated cow (Figure 26). However, the 24-hour mortality of flies from a fipronil treated cow was greater (Figure 27). Fipronil would be a worthwhile chemical for further testing against tabanids, because it is claimed to exhibit much longer activity on cattle than pyrethroids (deltamethrin) against a range of ectoparasites such as the horn fly, *Haematobia irritans*.

Relating the results of this project to what is already known about the feeding behaviour of the fly species suggests that mechanical transmission by tabanid flies plays an important part in the epidemiology of both *T. evansi* and *T. vivax*. Consequently adopting fly avoidance methods which have been shown to work with other mechanically-transmitted pathogens should be evaluated as a potential means of control for both trypanosome species, such as:

- Movement controls on infected animals to prevent spread of infection into new areas
- Separation of infected animals from uninfected animals by 200m to prevent transfer feeding of flies

- Separation of animals from preferred habitats of host seeking flies, e.g. forest margins, by 200m to discourage adult flies from attacking animals (Figure 32)
- Introduction of needle management systems to prevent iatrogenic transmission

The successful implementation of these methods also requires effective diagnostic facilities to identify infected animals and monitor the success of control regimes. Trypanosome diagnostic technologies were established at LIDIVET, Santa Cruz, Bolivia as part of the DFID-funded project R7162.

Two information leaflets providing a background to, firstly, bovine trypanosomiasis and, secondly, tabanids, their identification, biology and control were produced for distribution to veterinarians and livestock owners. Several scientific publications are presently being prepared for publication based on the data presented in this report. Other means of dissemination of the results of this project, scientific meetings, video and WWW site, are listed in the Project Completion Summary Sheet.

Table 1: List of species of tabanid recorded at Chocolatal, Guarayos Province, Santa Cruz Department, November 1999 to February 2001

Tabanus occidentalis Linnaeus, 1758
Tabanus claripennis (Bigot, 1892)
Tabanus pungens Wiedemann, 1828
Tabanus importunus Wiedemann, 1828
Tabanus nubulosus ?
Tabanus guyanensis Macquart, 1846
Tabanus sorbillans Wiedemann, 1828
Tabanus discifer Walker,1850
Tabanus wokei Fairchild, 1983
Tabanus restrepoensis Fairchild,1942
Selasoma tibiale Fabricius, 1805
Poeciloderas quadripuctatus Fabricius, 1805
Phaeotabanus cajennensis Fabricius, 1787
Phaeotabanus fervens Linnaeus, 1758
Stenotabanus cinereus Wiedemann, 1828
Lepiselaga species ?
Lepiselaga crassipes Fabricius, 1805
Lepiselaga albovarius Walker, 1854
Lepiselaga exaeustuans Linnaeus, 1758
Lepiselaga procallosus Lutz, 1912
Philipotabanus species ?
Esenbeckia erebea Wilkerson and Fairchild, 1983
Esenbeckia griseipleura Chainey and Hall 1996
Diachlorus bimaculatus Wiedemann, 1828
Diachlorus curvipes Fabricius, 1805
Diachlorus bicinctus Fabricius, 1805
Chrysops bulbicornis Lutz, 1911
Chrysops inanis Fabricius, 1794
Chrysops variegatus De Geer, 1776
Chrysops patricia Pechuman, 1953
Chrysops unicolor Wiedemann, 1828
Chrysops peruvianus Krober, 1925
Acanthocera species ?

Figure 11: Location of Chocolatal in Guarayos Province

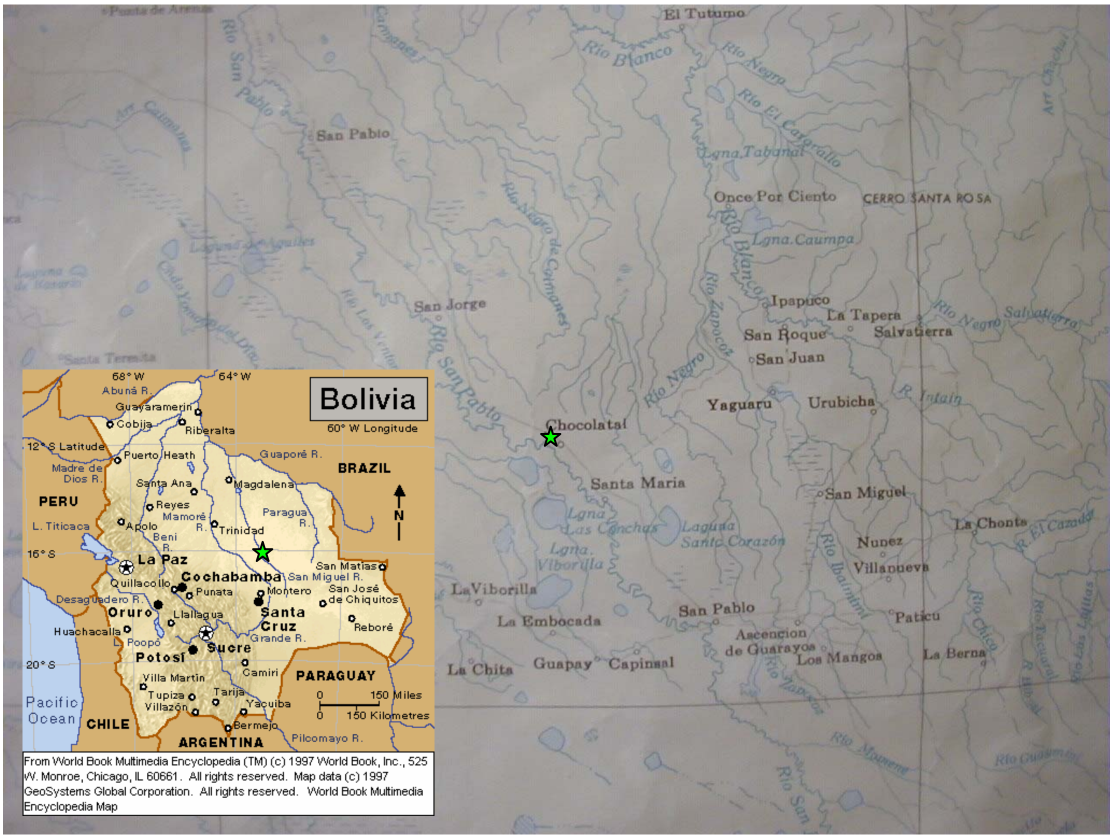


Figure 12: Aerial view of Chocolatal farm

Figure 13: Trap capture of *Tabanus occidentalis* at Chocolatal.

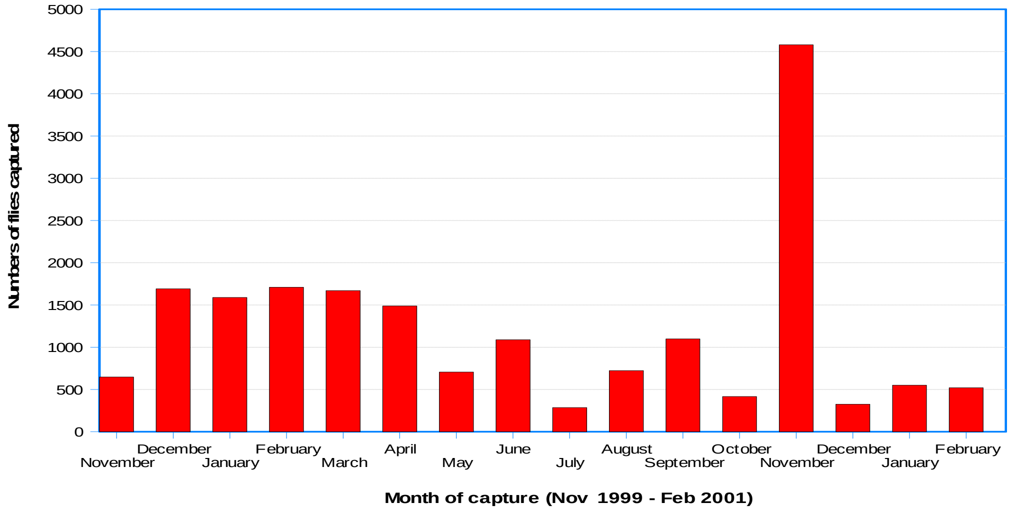


Figure 14: Trap capture of all tabanid species except *T. occidentalis* at Chocolatal.

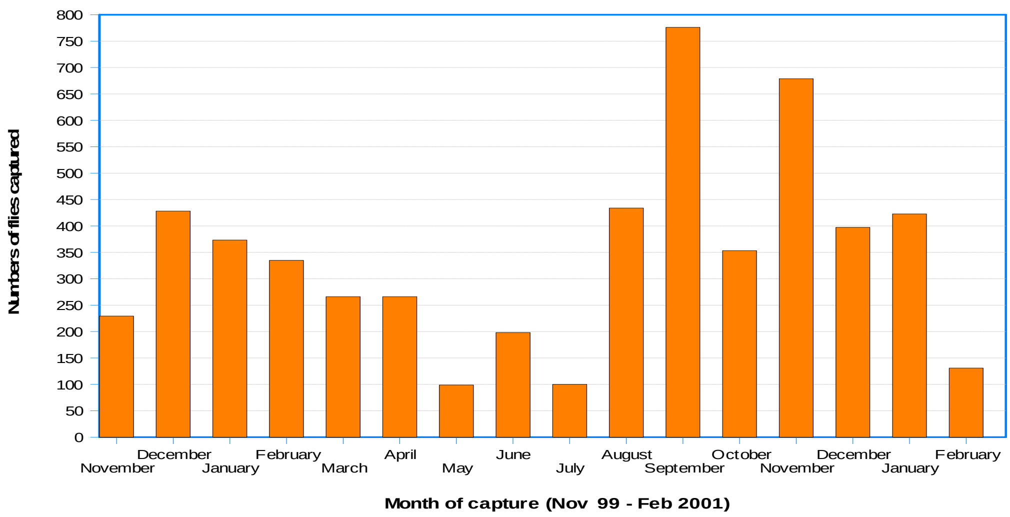


Figure 15: Hand net collection from horse of all tabanid species at Saavedra.

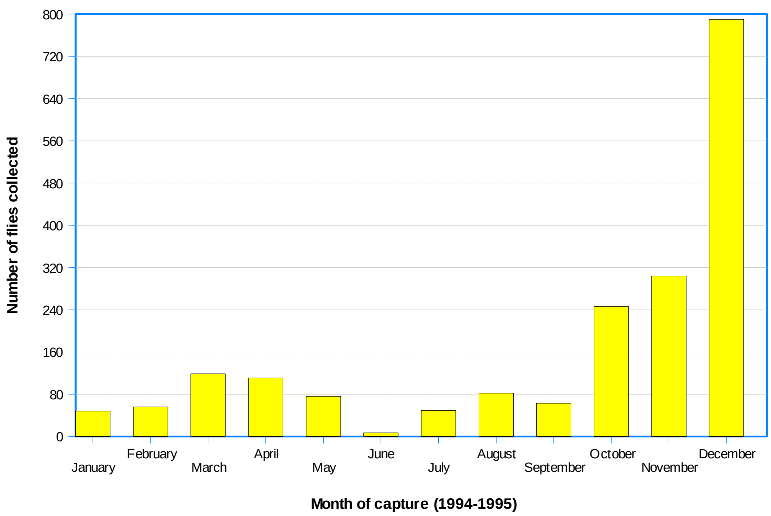


Figure 16: Linear regression plots of tail flicks against fly density recorded for eight cattle. Note that the response levels of cattle LB and VN do not change significantly in response to changes in the density of flies.

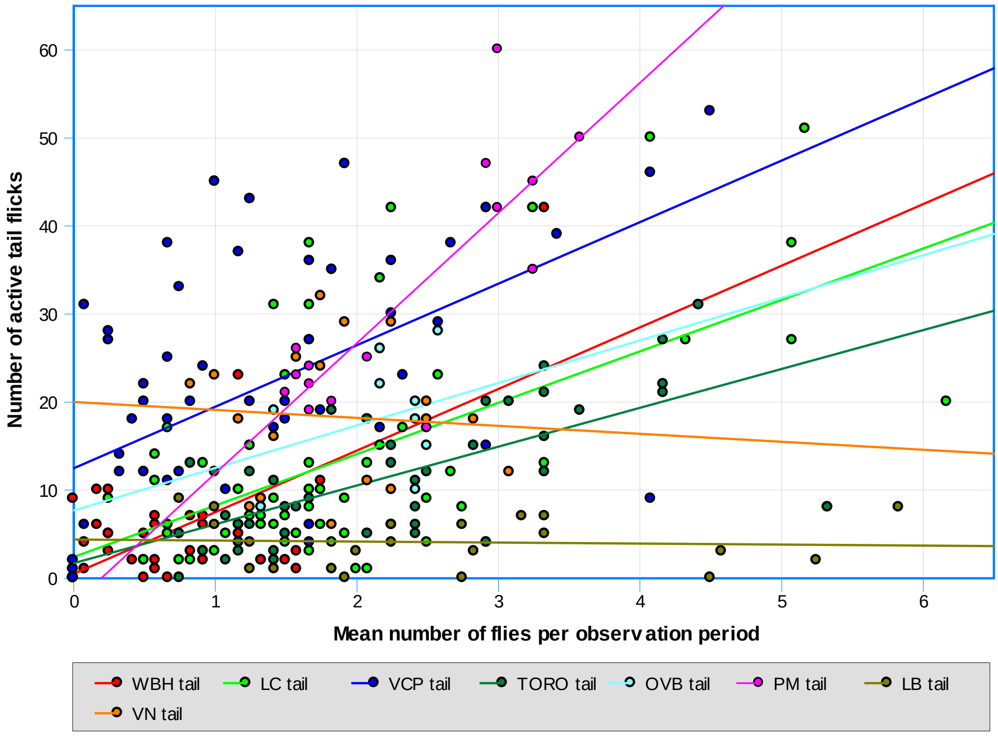


Figure 17: Linear regression analysis of pooled data from all cattle that demonstrated a changing response to changing fly density.

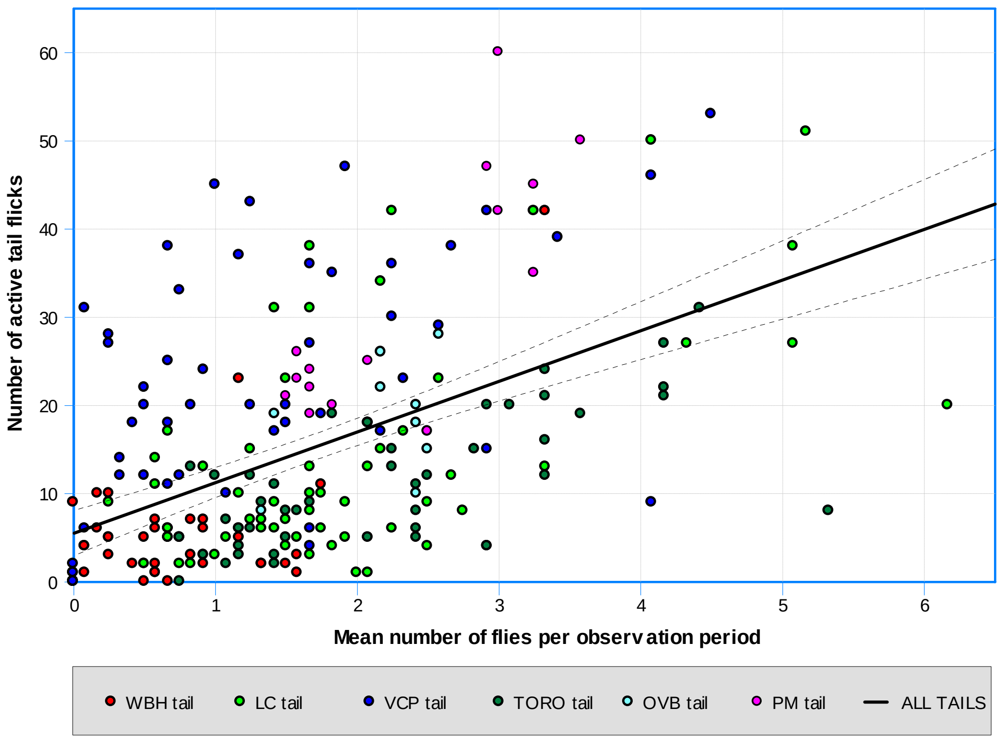


Figure 18: Relationship between the two defensive activities of cattle recorded, tail flicks and hoof stamps.

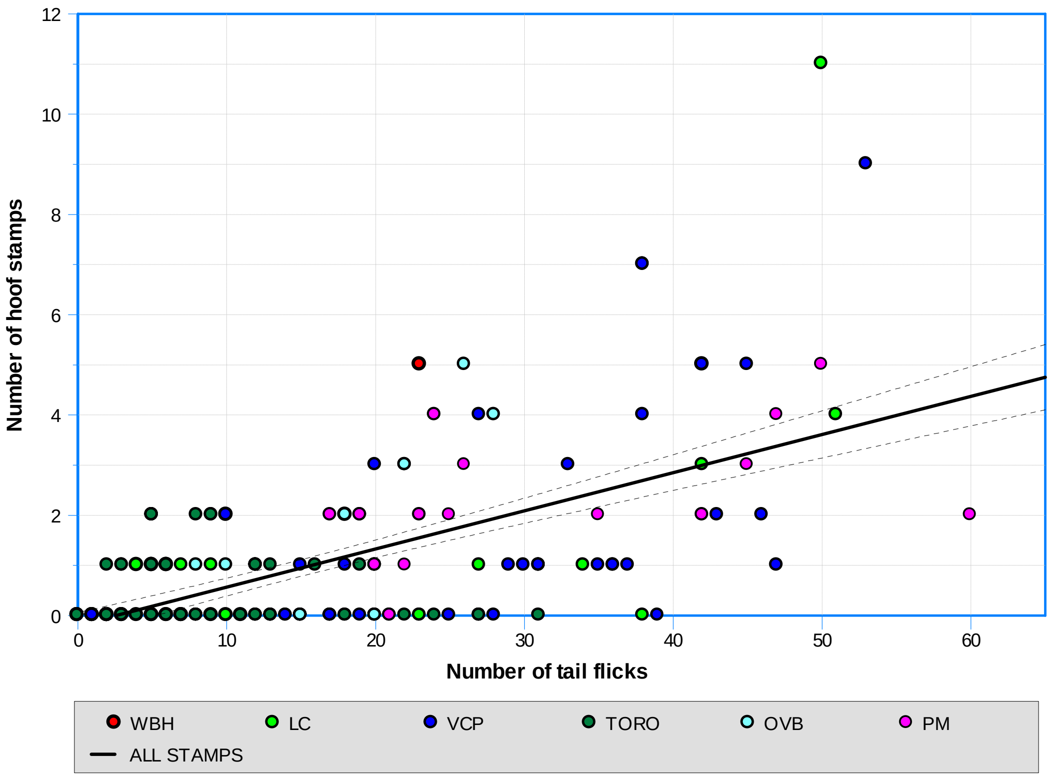


Figure 19: Relationship between fly density and tail flicks, based on means of observation sessions (approximately ten records per session).

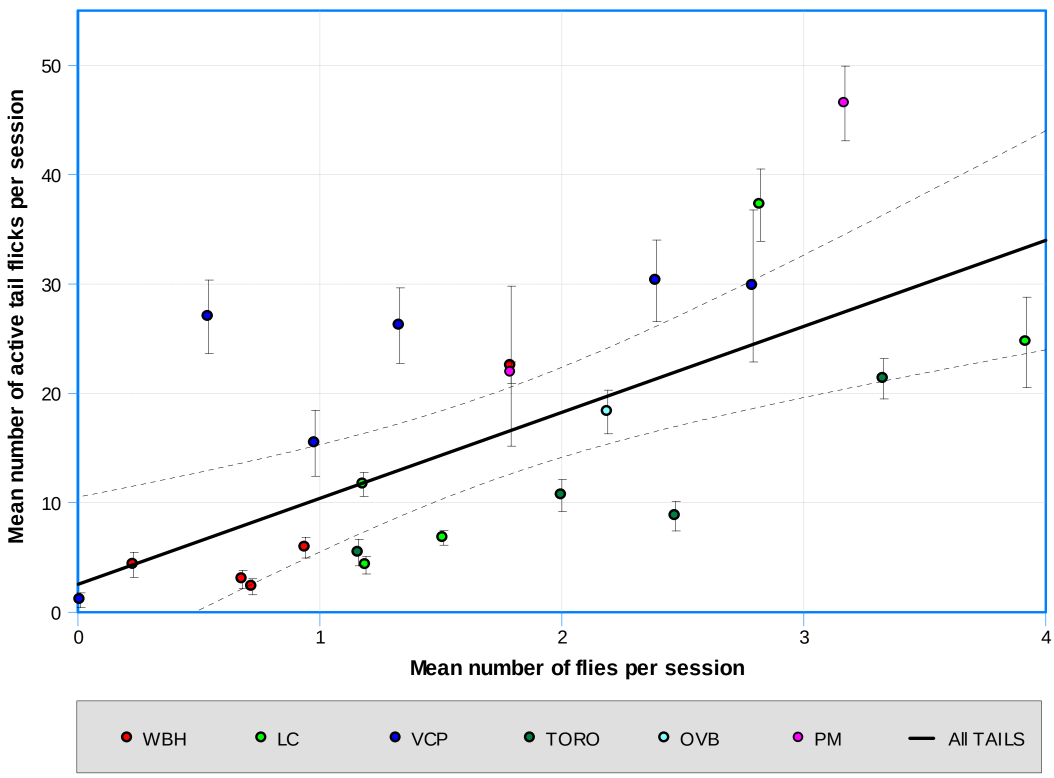


Figure 20: Relationship between fly density and tail flicks, based on means of observation sessions (approximately ten records per session) and with cattle response on a logarithmic scale.

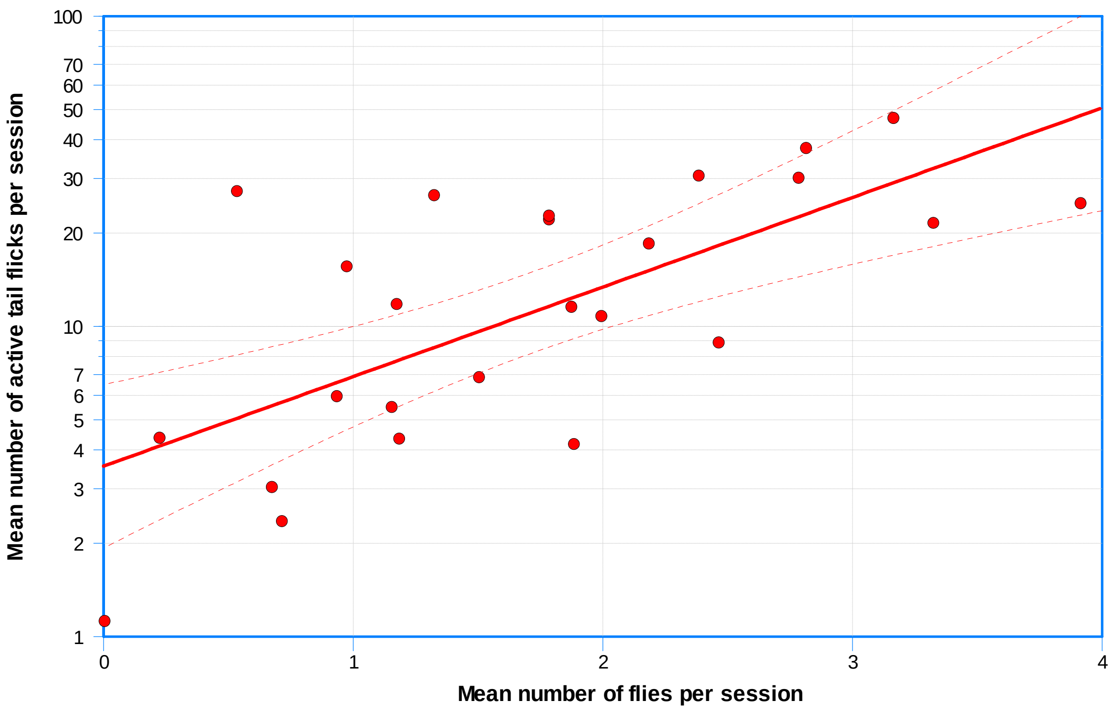


Figure 21: Relationship between fly density and tail flicks, based on means of observation sessions (approximately ten records per session) and with cattle response on a logarithmic scale as in Figure 20, but fitted with a curvilinear plot.

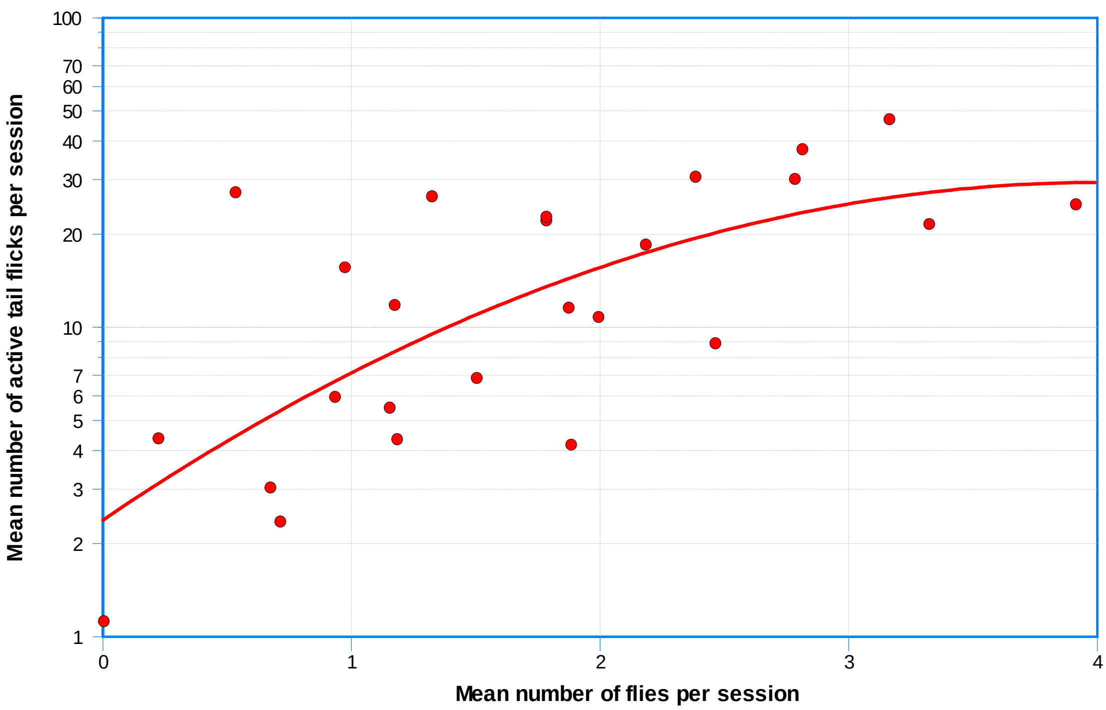


Figure 22: Change in feeding activity (not fed, partly fed or fully fed) of tabanid flies on cattle before and after treatment with cypermethrin pour-on.

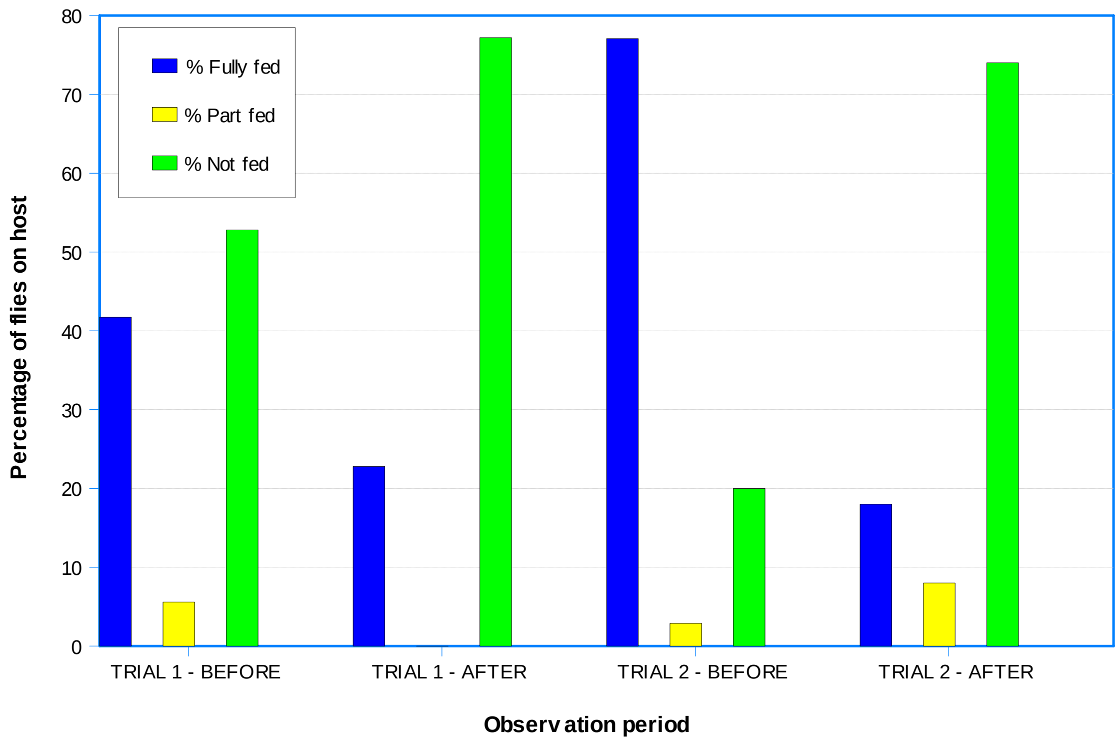


Figure 23: Changes in time or durations of tabanid activities associated with feeding behaviour on cattle.

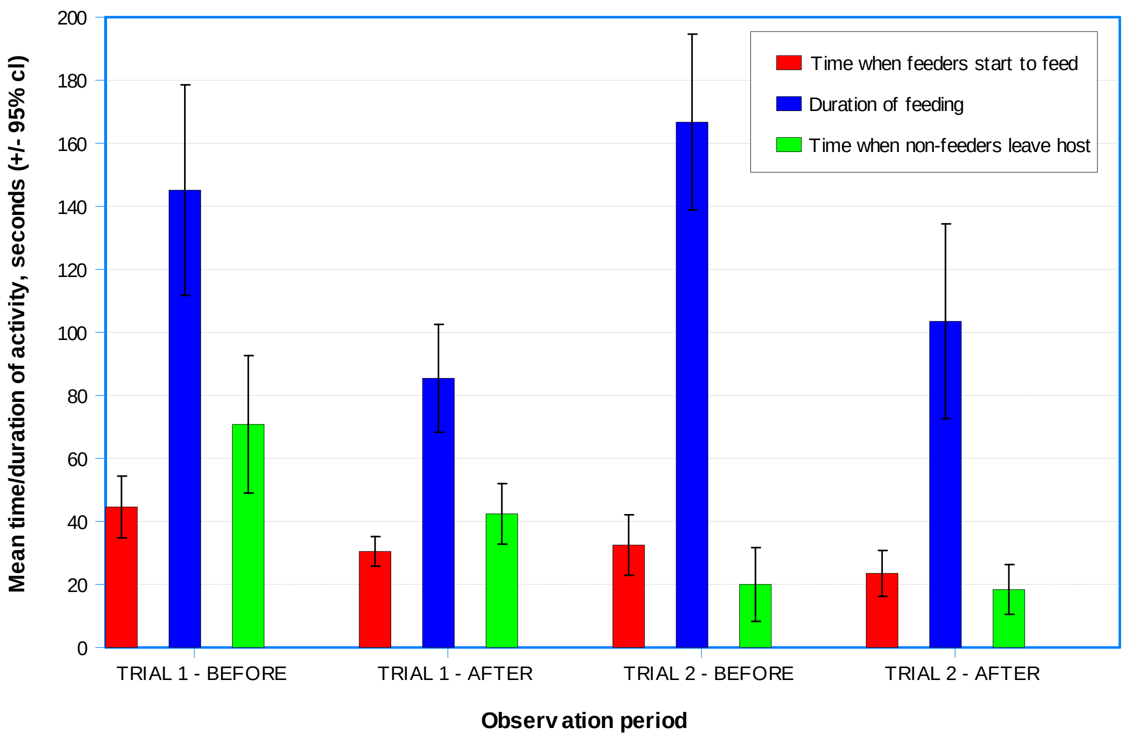


Figure 24: Change in feeding activity of tabanid flies alighting on a cow across a period before and after two applications of pour-on, at times 0 and 71 hours as indicated.

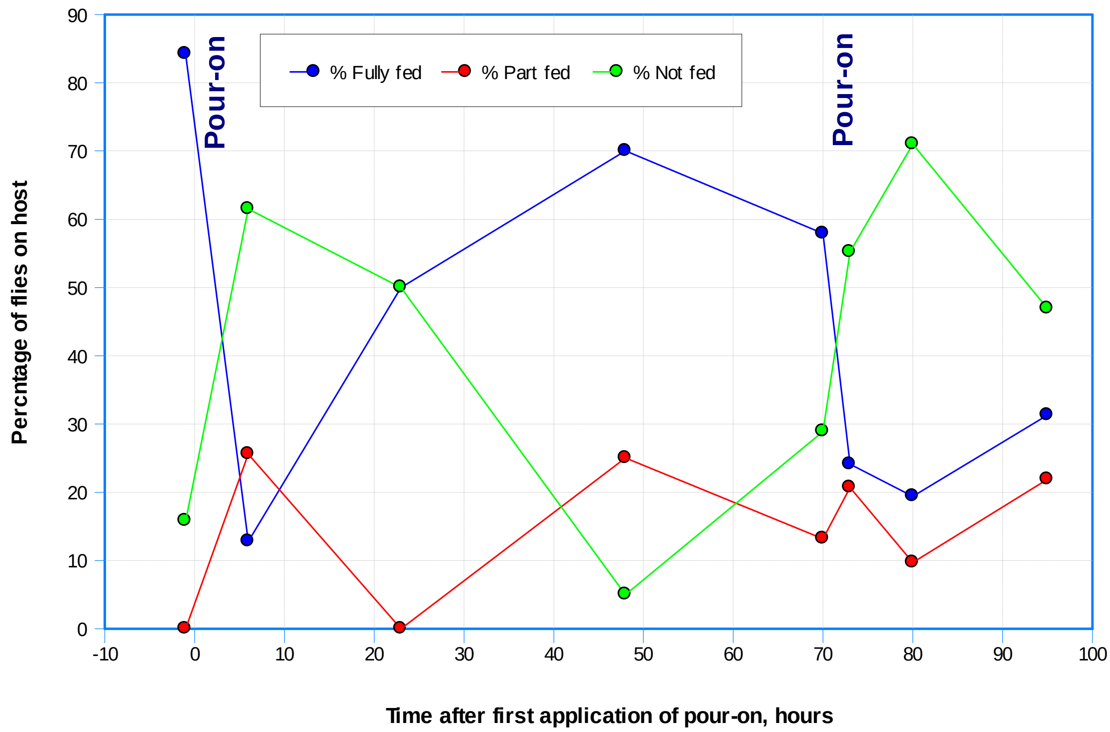


Figure 25: Change in duration of feeding of tabanid flies alighting on a cow across a period before and after two applications of pour-on, at times 0 and 71 hours as indicated.

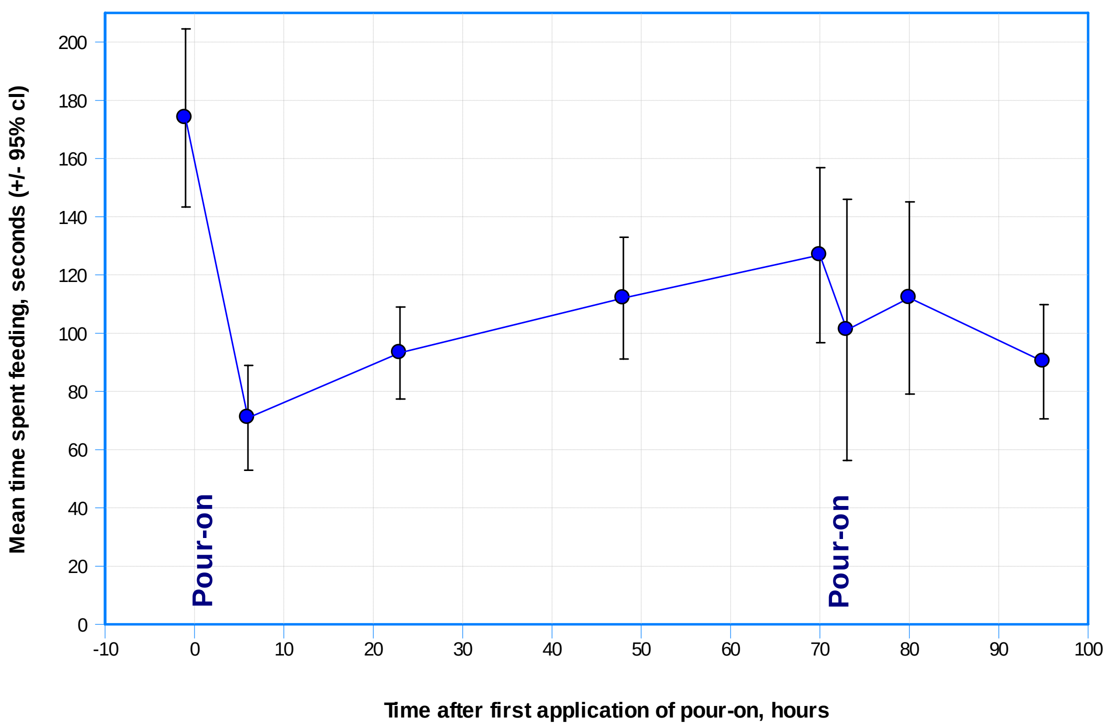


Figure 26: Changes in survival of *Tabanus occidentalis* following capture on cattle either not treated (controls) or treated with cypermethrin pour-on.

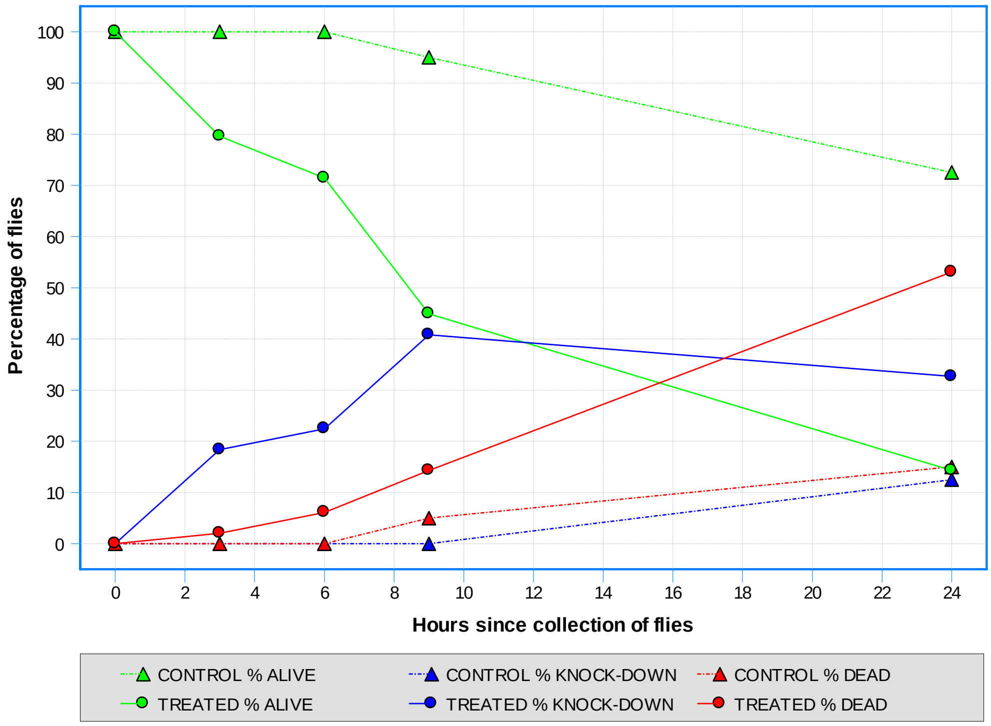


Figure 27: Changes in survival of *Tabanus occidentalis* following capture on cattle either not treated (controls) or treated with fipronil pour-on.

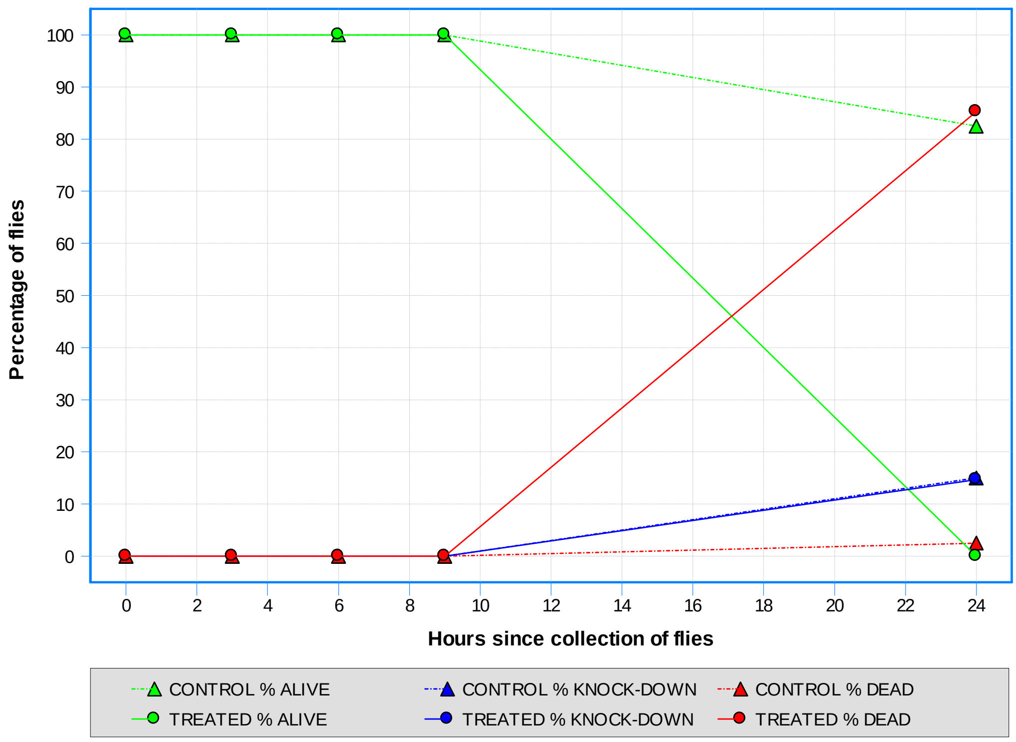


Figure 28: Fly species caught on cattle during the study period for blood squashes

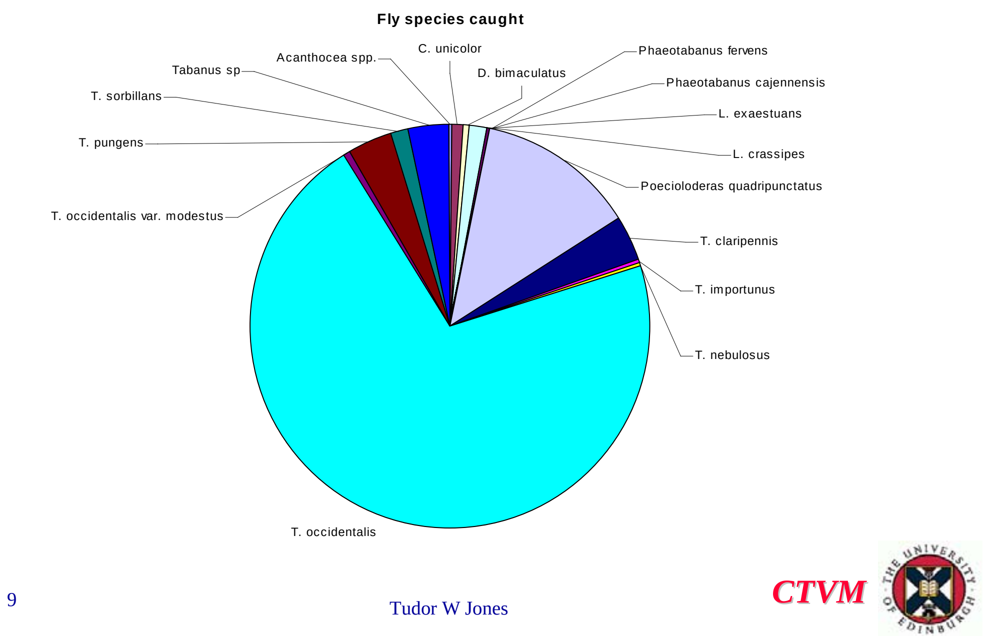


Figure 29: Flies caught each month from cattle for blood squashes

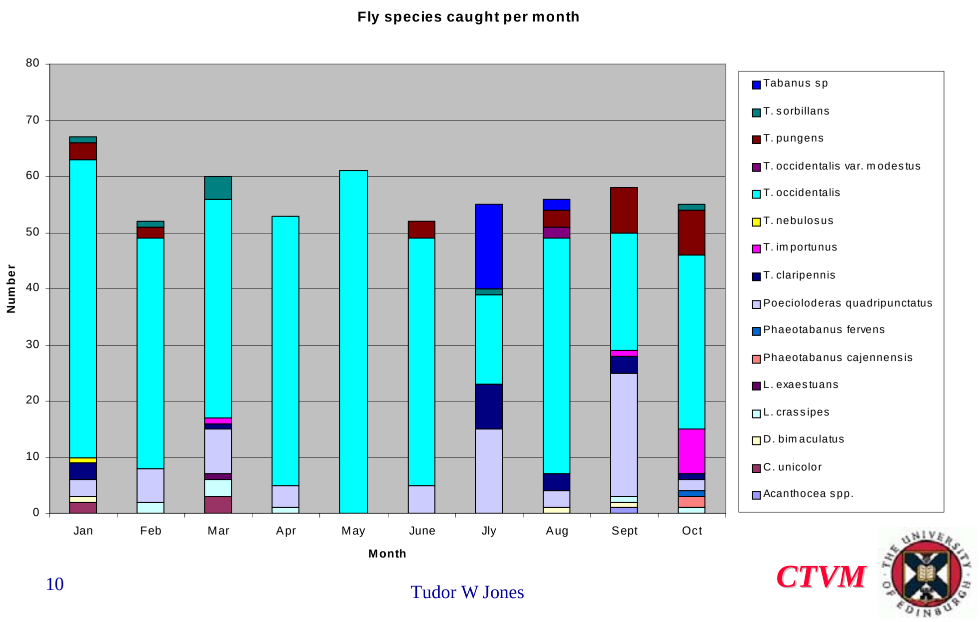


Figure 30: Fly species in which trypanosome DNA was detected by PCR (taken from presentation made at end-of-project seminar, March 2001)

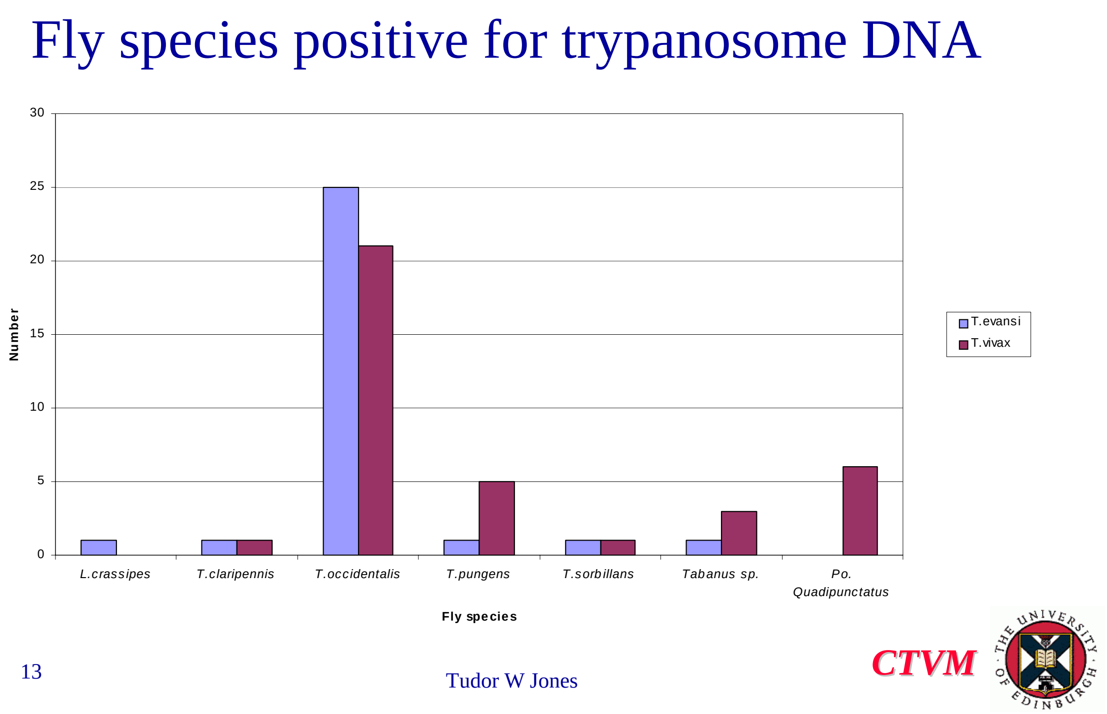
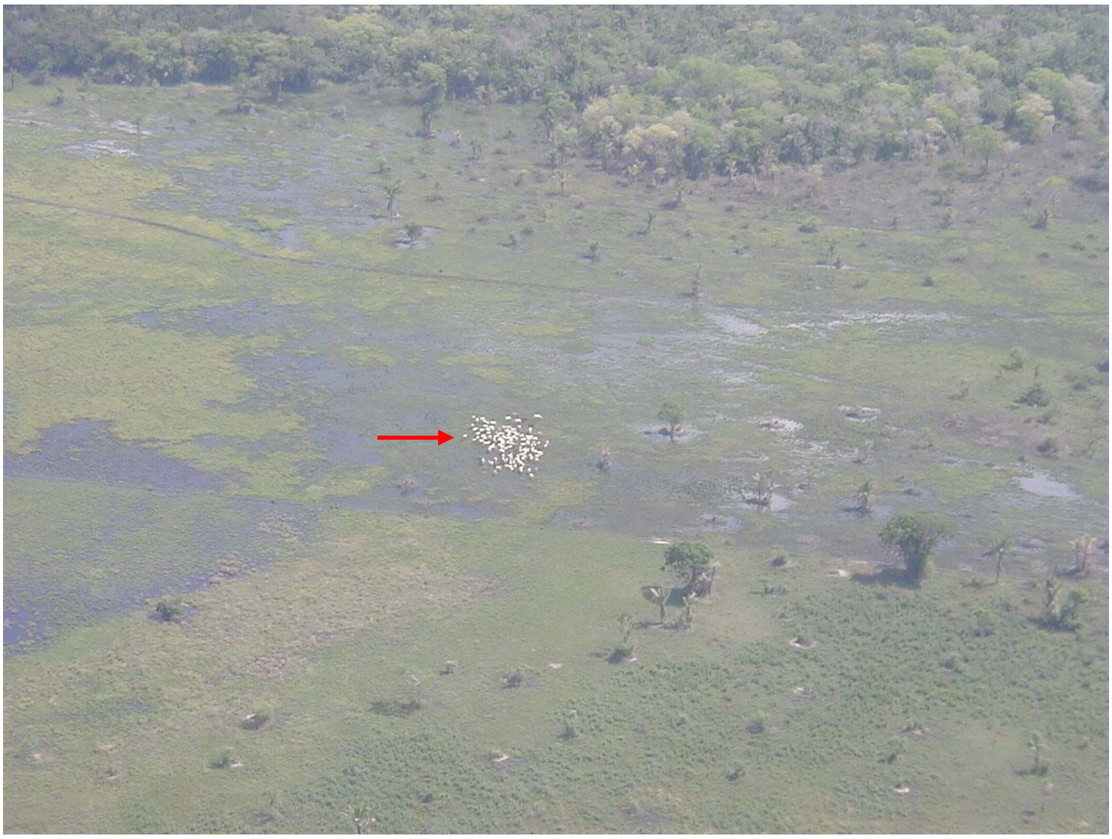


Figure 31: *Tabanus occidentalis* feeding on the foreleg of a cow at Chocolatal, demonstrating the blood loss that can result from large numbers of flies (note the fully engorged fly on the right hand side of the leg, preparing to leave the cow).

Figure 32: Natural selection by cattle (arrowed) in the Beni of a grazing site well away from the edge of a forest margin to minimise attack by tabanid flies.



Contribution of Outputs

Trapping and hand collection of flies from cattle has shown that cattle are exposed to biting flies throughout the year in the Chocolatal area of Guarayos and that the risk of transmission of *T. vivax* and *T. evansi* is likely to be continuous. Mechanical transmission by tabanid flies, especially *Tabanus occidentalis* is likely to be the principal method of transmission for both trypanosome species. Therefore, control regimes based on established fly avoidance techniques could result in a significant reduction in transmission risk in this area and in other areas with a similar fly profile.

The roles in an integrated control programme of the strategic use of insecticides against tabanids and of prophylactics against trypanosomes are complex. In theory, their application should be concentrated during the warm and wet season of greatest fly activity. In the central and southern parts of Santa Cruz Department there is a reasonably clear period of reduced fly activity, during which controls could be relaxed. However, in Guarayos Province tabanids were present all year round and much more work is needed to determine at what level of trap catches the risk of mechanical transmission from tabanids becomes unacceptable, necessitating treatment. A further complicating factor is the potential for induction of resistance in other ectoparasites, such as ticks, if insecticide use is not carefully managed. In addition, the economic cost of the use of chemicals needs to be considered in the decision concerning their use, balanced against the potential benefits.

Further work is needed to select an insecticidal treatment that will be effective against tabanid flies for a substantial period, with minimal risk of increasing the incidence of interrupted feeds. The ideal extremes of feeding that negate the possibilities of mechanical transmission are for no feeding (best for the animal) or for feeding to fullness. Anywhere in-between these extremes presents a risk of mechanical transmission, as flies move from one host to another to complete their bloodmeals. Studies of interrupted feeds are therefore of crucial importance in mechanical transmission and in understanding the epidemiology of disease. In the latter part of this project, bloodmeals were collected and sent for analysis at the Natural Resources Institute in Chatham (Dr S. Torr) as part of a new technique to identify multiple bloodmeals (i.e. evidence of interrupted feeds) by detection and identification of host DNA that can be characterised for individual cattle. This will be a very worthwhile technique for future application in studies of mechanical transmission.

Much more work is need in Bolivia on the actual impact of the disease on livestock. Acute infections have been recorded, but chronic infections with minimal overt symptoms also occur. Although these chronic infections may not present a serious threat to the welfare of the individual carrier, if left untreated they can act as a reservoir of infection that could be spread to naïve cattle with serious consequences. The situation regarding bovine trypanosomiasis in Bolivia is dynamic and what appeared to be a major problem just five years ago appears now to be less so. Data from LIDIVET indicates that diagnosed cases have fallen from the peaks of 1997-1998, but that the disease is being maintained at a low level (Figure 33).

One of the original project objectives that was not met was to determine the effect of tabanid numbers on milk production. Unfortunately, it was not possible to carry out this study because of the low numbers of tabanids at San Diego farm. However, a study of the milk production of dairy farms in Santa Cruz Department suggests that the impact of tabanids is negligible, at least on these larger farms (Figure 34 - compare with Figures 13-15). The period of low milk production coincides with the period of lower numbers of tabanids and milk yields rise at the same time as tabanid populations rise. The seasonal trends in milk production are linked to pasture condition on the larger farms rather than to tabanid numbers. Nevertheless, on smallholder farms in areas such as Guarayos, the potential for tabanids to reduce milk production deserves further study.

Following the discovery of trypanosome DNA in the abdominal contents of flies that had no evidence of a bloodmeal, the origin of this DNA deserves further attention, i.e., was it from viable trypanosomes? If trypanosomes can survive in the gut of tabanids for substantial periods, then it increases the chances of their being mechanically transmitted, by regurgitation or even by excretion into the wound made by feeding, and increases the possibilities of a role for biological transmission.

Due to the change in focus of the Animal Health Programme away from South America during the course of this project, the submission of a concept note to extend the project was not carried out. However, plans are being prepared to further the present work on the effects of insecticide treatments of cattle on tabanids and also to study the potential for insecticide-impregnated targets as an element within an integrated control programme.

Figure 33: Numbers of cases of *Trypanosoma vivax* and *T. evansi* diagnosed by LIDIVET (Santa Cruz) as published in their three monthly, "Informe tecnico y economico del Laboratorio de Investigacion y Diagnostico Veterinario, Santa Cruz, LIDIVET".

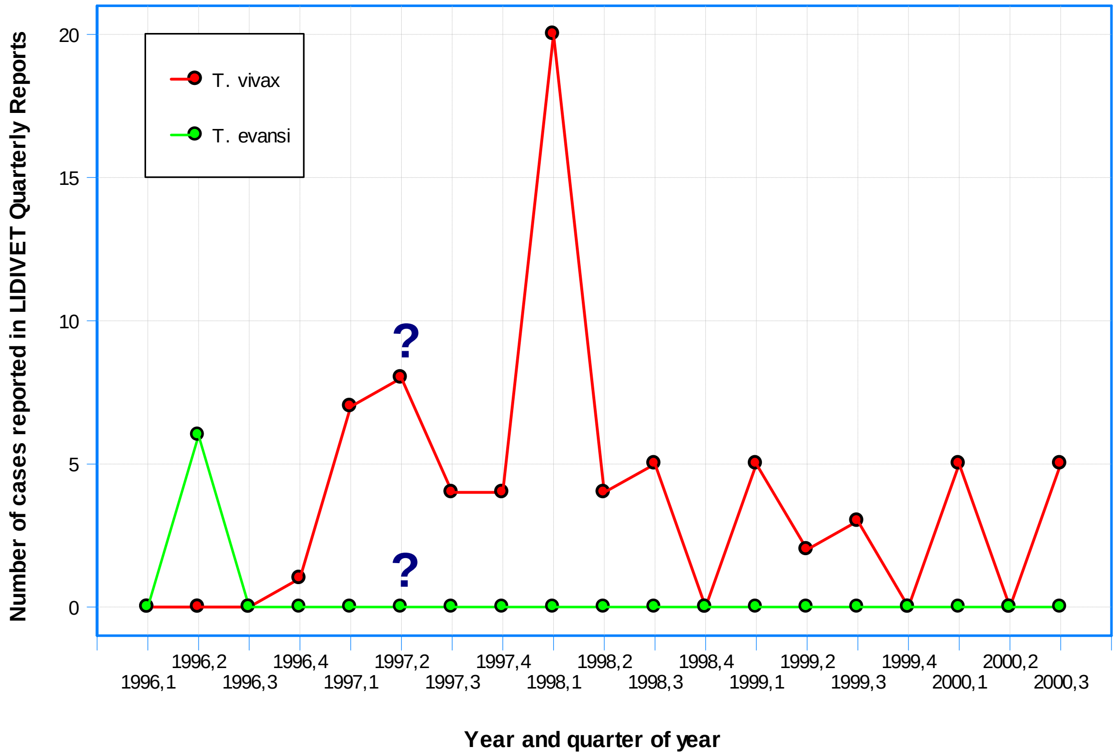
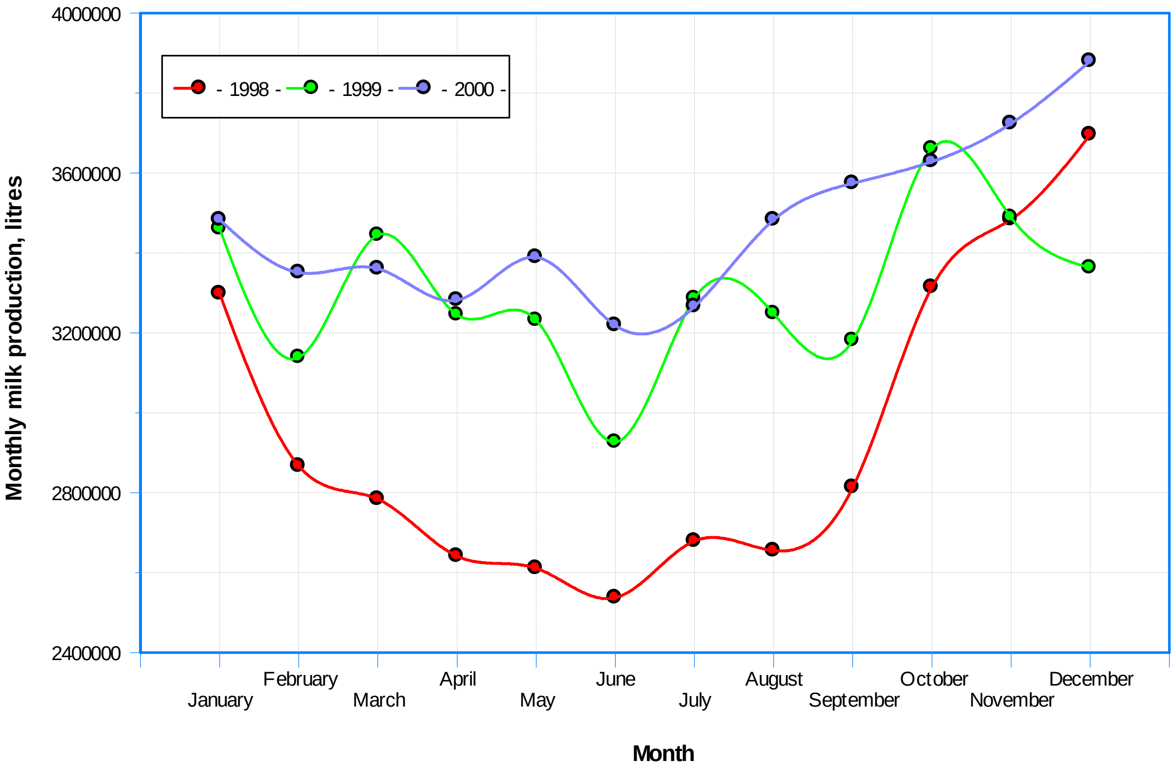


Figure 33: Production of milk by dairy farms in Santa Cruz Department over a three-year period (data supplied by milk producers association, FEDEPLE).



Annex

1) **Programme for seminar held during the final month of the project, in Santa Cruz de la Sierra.**

2) **Information leaflet on bovine trypanosomiasis.**

3) **Information leaflet on tabanid flies, their biology and control.**

CICLO DE CONFERENCIA INTERNACIONAL
TEMA : LOS TRANSMISORES DE LA TRIPANOSOMIASIS BOVINA
14 DE MARZO 2001
LUGAR: AUDITORIO DEL LIDIVET

Av. Ejercito Nacional # 141

Moderador : Dr. Gerardo Méndez

08:30 – 09:00 Palabras de Bienvenida.
Dr. Gerardo Méndez (Director Ejecutivo del LIDIVET)

09:15 – 09:45. Estudios de trampeo, distribución y estacionalidad de los tábanos (Díptera tabanidae), en el Departamento de Santa Cruz.
Ing. José Luis Aramayo B.
Museo de Historia Natural Noel Kempff Mercado U.A.G.R.M.

09:45 – 10:15. Interacciones entre los tábanos (Diptera tabanidae) y sus ocasionales victimas.
Dr. Martin Hall
Museo de Historia Natural de Londres Inglaterra

10:15 – 10:45. Análisis moleculares de contenidos estomacales (***Bloodmeal***) de tábanos (Diptera tabanidae) **¨Antes que nada, agarra tu mosca¨**
Dr. Tudor Jones
Centro de Medicina Tropical Veterinaria de Edimburgo, Escocia.

10:45 – 11:00 REFRIGERIO

11:00 – 11:30 La Tripanosomiasis bovina en Bolivia.
Dr. Hugo Rivera – Dra. Ana Maria Cuellar.
Laboratorio de Investigación y Diagnóstico Veterinario (LIDIVET).

11:30 – 12:00 Investigaciones sobre tábanos (Diptera Tabanidae)
Dr. Foil Lane
Universidad de Lousiana Estados Unidos.

12:00 – 12:30 Estudios sobre tábanos (Diptera tabanidae), en la región del Pantanal Brasil.
Dr. Thadeu Barros
Embrapa – Brasil.

12.30 13:00 .Quién mandará en el futuro ? los tábanos**...........?**
Dr. M.Hall – José Luis Aramayo B.
Museo de Historia Natural de Londres.

13:00 Clausura.
Ing. Nelson Rodriguez Director del Museo.

07 de marzo 2001
Santa Cruz de la Sierra – Bolivia.