HOST, TRAP AND ODOUR BAIT PREFERENCE DETERMINATION OF TSETSE FLIES (Glossina morsitans submorsitans) IN THE UPPER DIDESSA RIVER VALLEY - SOUTH-WESTERN ETHIOPIA

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by

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TO BANTAYMOLU AYELE AND MY MOTHER

A C K N O W L E D G E M E N T S

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ABSTRACT

Investigations were carried out in the upper Didessa river valley to determine the preference of Glossina morsitans submoritans towards available hosts, trap design, and odour baits. Biconical trap baited with either acetone, octenol or cow urine or all the three put together in different container was more effective in catching Glossina morsitans submorsians than the Ngu trap under the same experimental setup. Among odour attractants tested separately,acetone appeared to be more potent in attracting tsetse flies than octenol and cow urine. On the other hand, Ngu trap was superior than biconical trap in catching tsetse flies which had residual blood in their gut. Cow urine kept in a container for some days increased the catches of the flies when compared with the freshly collected urine. A low infection rate of 2.6% was detected in Glossina morsitans submorsitans of the upper Disease river valley. On the other hand the prevalence of animal trypanosomiasis on the edge of the escarpment was very high (42.6%). Trypanosoma congolense was the dominant species identified in both the tsetse flies and in the cattle of the locality. Enzyme Linked Immunosorbent Assay (ELISA) test was employed to see the rate of digestion of blood proteins ingested by teneral and non-teneral laboratory reared G.m. submorsitans at different time intervals after feeding. This test showed that the non-teneral tsetse digested the species distinguishing bloodmeal components faster than the tenerals- at 48hr post feeding, the bloodmeal donor was identifiable in 87.5% of the teneral tsetse and 55.5% of non-teneral tsetse flies. The source of the bloodmeals from 160 fed G.m. submorsitans captured in the valley were identified by ELISA test. Accordingly warthog

accounted for 28.8% of meals, whereas human and buffalo blood accounted for 11.9% and 6.9% of the meals, respectively. Others like the giraffe, goat, cattle and elephant accounted for a very low percentage of flies' bloodmeal. Thus the warthog appeared to be the major maintenance host for G.m. submorsitans in the study area. Sex composition of the catches and the efficiency of each bait system as well as the results of the blood meal analysis are discussed.

INTRODUCTION

Tsetse flies and trypanosomes,in partnership, are responsible for the death of tens of thousands of Africans from sleeping sickness, and of hundreds of thousands of domestic animals from a similar disease called 'Nagana'.Nagana known to have made some 11 million square kilometres of potential grazing lands inhospitable for livestock in Africa (Nash, 1969; Jordan, 1986). The literature on tsetse and trypanosomiasis is voluminous, however, efferts have been made to select the most relevant ones.

Tsetse are blood-sucking flies, which are found only in tropical Africa. They are vectors of trypanosomiasis, one of the major tropical diseases in Africa.Trypanosomiasis is not only a serious public health problem causing sleeping sickness, a fatal disease in humans but also is a disease of animals affecting cattle. As it occurs across more than one third of Africa,it is arguably the most important livestock disease in the continent (ILRAD, 1990). The wide occurrence of this disease in people and their livestock is a great constraint to agricultural and economic development of the continent (Wilson et.al, 1963).

Trypanosomes belong to the subkingdom of microscopic unicellular parasites, the Protozoa. They live as parasites in many kinds of vertebrates: fish, reptiles, birds and mammals.But the most economically important are the African species which occur in man, his domestic animals, and game, and which undergo a developmental cycle in tsetse fly.

The tsetse feed solely on blood and are always harmless when they hatch from the pupa; but if they feed on an infected host they can transmit the trypanosome organisms to man or to his domestic animals (Youdeowei & Service, 1983).

The first knowledge of tsetse flies and Nagana comes from South Africa in the middle of the nineteenth century. In 1850 the word "tsetse" was introduced to the English language, and it comes from a Botswana word which refers to 'a fly destructive of cattle' (Nash, 1969). Clues to the understanding of the nagana problem were available for the first time 1880, but it was not until 1895 that those partners in crime-the tsetse and trypanosome-became incriminated (Ford, 1971).

In Zulu land, in 1884, Major Bruce of the British Army Medical Service detected a trypanosome, later named T.brucei, in the blood of cattle suffering from nagana (Nash, 1969). After his successive work Bruce proved that trypanosomes are the cause of the disease, that the game forms a reservoir of the disease, and the tsetse fly transmits it. He then introduced the name "nagana," to the disease. It is a Zulu word which signifies a state of depressed spirits (Nash; 1969, Ford, 1971).

The discovery of the role of Glossina in the transmission of trypanosomiasis drew widespread attention towards tsetse fly. The official view was clearly stated by the east African

Commission in 1925, saying 'the ravage of the tsetse fly are the greatest menace to the development of tropical Africa and constitute one of its most serious problems (Ford, 1971). Much has been written by scientists of many disciplines about this complex involving man, domestic animals, tsetse flies and trypanosomes since Bruce discovered the links between tsetse flies, trypanosome and the disease.

There are 22 species of Glossina and their distribution extends over approximately 11 million km^2 of Africa between latitudes 15°N and 22°S (Youdeowei and Service, 1983). It's northern limit extends across the continent from Senegal in the west to southern Somalia in the east. The limits are determined by climate often through its effect on vegetation (Jordan, 1986). In South Eastern Africa where the rainfall is high and there are no deserts, the limit of Glossina is related to seasonal low temperature. Lower temperature may affect the adults, which are inactive below about 16°C and also prolong hatching period (Brusell, 1960).

Tsetse occupy a variety of vegetation types and transmit different trypanosomes of socio-economic importance: T.b.gambiense to man in Western Africa, T.b.rhodesiense to man in eastern Africa; and T.congolense , T.vivax , T.b.brucei T.simiae cause serious forms of trypanosomiasis in cattle, sheep goats, pigs and horses (Schimidt and Robert, 1981).

T.evansi , a species not transmitted by tsetse flies but by other biting flies cause disease in camels. Increasing

evidence suggests that in large areas of Asia and South America T.evansi possess a threat to other livestock especially cattle, domestic buffalo and pigs (ILRAD, 1990). T.vivax , although usually transmitted by tsetse flies, also exists in the absence of this vector. Although it is potentially a serious threat to livestock in many parts of the world, the extent of the problem of non-tsetse transmitted trypanosomiasis is not clear (Vaucel et.al; 1963, ILRAD, 1990).

In Africa, most trypanosome parasites are transmitted by tsetse flies which occur across more than a third of Africa i.e, in 37 countries (Wilson et.al., 1963). The risk of trypanosomiasis in much of this area precludes farmers from keeping cattle and small ruminants, and this fact largely accounts for Africa's low livestock productivity (Youdeowei and Service, 1983).

The impact of trypanosomiasis must even be greater than these figures suggest, since, many of the areas inhabited by tsetse flies are potentially the most agriculturally productive ,in Africa. For example, thirty percent of African's cattle population, which totals 147 million as well as comparable numbers of small ruminants, are at risk from the disease (Youdeowei & Service, 1983). An estimated annual loss in meat production of $ 5 billion was also reported (ILRAD, 1990). This economic deprivation is exacerbated by losses in milk yields, tractive power, waste products that provide natural fuel and fertilizers; and products such as hides (Youdeowei & Service, 1983).

The trypanosomes that cause disease in livestock and humans also infect some wild life species, which serves as a reservoir of infection for flies that may then in turn infect domestic animals and people (Nash, 1969). Many wild animals carry trypanosomes with no apparent ill effect. In humans and most domestic livestock, however, where such harmless relationship with trypanosomes and their vectors has not evolved, the pathogenic effects of infection are severe (Ford, 1971).

For several days following infection with trypanosomes, animals show no signs of disease. One to two weeks later susceptible animals develop intermittent fever and anaemia. In most endemic areas of Africa, cattle repeatedly bitten by tsetse flies, carry different kinds of trypanosomes. In addition, in most regions livestock must daily forage for food and walk long distances for water, a stressful condition under which infected animals often continue to deteriorate for months before dying.

The life cycle of the single-celled trypanosome parasite is complex. In both the tsetse fly and mammalian host, trypanosomes undergo a series of transformations into different forms. The tsetse fly ingests trypanosomes when it feeds on an animal infected by the parasite. In the fly the trypanosomes differentiate into several forms maturing into the metacyclic, which is able to infect mammalian hosts (Jordan, 1986).

When the infected fly feeds next , these metacyclic trypanosomes are injected into the skin along with tsetse saliva. In the animal, the parasites differentiate into a blood-stream

and they multiply by binary fission and enter the animals lymphatic and blood circulation. As flies feed on animals infected with the parasite, they take up blood containing trypanosomes.

Tsetse flies belong to the Phylum Arthropoda,Class Insecta. Within the Insecta they are grouped within the order Diptera and the genus Glossina. Different species of Glossina have preference towards ecological requirements. Moreover, the flies react to a wide range of visual, olfactory as well as temperature and humidity stimuli during their daily life (Vale, 1974).

The eco-distribution of tsetse is determined by environmental factors (eg, altitude, climate, vegetation, etc) and different species have a preference of different ecotype (Warner, 1985). They are absent from high altitudes and from areas where the vegetation cannot support the development and maintenance of the fly (Snow, 1974).

All species of Morsitans group are restricted to the savanna woodlands surrounding two blocks of lowland rain forest. In the wetter areas the flies roam widely over the woodland, but in the drier parts of their range, such as the Sudan Savanna of west Africa, they are centred on the mesophytic vegetation of water courses, particularly during the severe dry season (Jordan, 1986). G.morsitans , in Pan African terms, is the most important species of Glossina. It infests an enormous area, and it is a vector of sleeping sickness in Eastern and Southern Africa, and is a major vector of animal trypanosomiasis (Ford, 1971).

Although tsetse flies have existed in Ethiopia for a very long time there is little precise information about them or their distribution (Langridge, 1976). The early historical reference to tsetse flies in Ethiopia comes from early travellers and explorers. Nash (1963) indicated that in 1770 James Bruce in his 'Travels to discover the source of the Nile' describes a biting insect called 'Zimb' which he found in Western Ethiopia and which could have been a tsetse.

According to Langridge (1976), one of the earliest records was made by Donaldson Smith in 1895, who in his account of his journey through Southern Ethiopia mentions the Gendi fly (tsetse) attacking his transport animals and causing a disease called Gendi from which many animals died.

Bannister (1957) wrote about the heavy mortality of cattle in Wollo Province due to trypanosomiasis and Peck (1959) also noted that a large part of western Ethiopia was unsuitable for livestock because of tsetse flies and trypanosomiasis. The report of Ott (1961) indicated that Nagana was getting more serious in Ethiopia and he suggested that the disease should be combatted by the control of tsetse flies.

Although several explorers showed an interest in tsetse and trypanosomiasis identification in Ethiopia, it was not until Balis and Bergeon (cited by Langridge, 1976), published their 'preliminary note of the tsetse fly study in Ethiopia' in 1966 that there was some realization of the extent of tsetse infestation and trypanosomiasis problem. This was prompted by

the very serious out break of human sleeping sickness, due to the Rhodesian form, in 1966 that reached epidemic level in Gambella. This out break has been fully covered and reported by Hutchinson (1971).

From ever increasing reports received by the veterinary service, it become very obvious that Nagana of domestic stock was a disease of major importance which had become a serious constraint on the development of the livestock industry in several regions of Ethiopia.

It was in 1976 that a well documented work was released by Langridge which presents the results of tsetse and trypanosomiasis survey in Ethiopia undertaken between September, 1971 and June, 1976. The terms of the reference of the work was 'To organize a survey of the distribution of tsetse fly and the incidence of trypanosomiasis in Ethiopia'. The results of the survey showed that five species of tsetse flies were found in Ethiopia. Namely Glossina tachinoides , G.longipennis , G.morsitans submorsitans , G.pallidipes and G.fuscipes fuscipes. These tsetse flies are confined to the Southern and Western regions between longitude 33°and 38°E and latitude 5°and 12°N. The infested areas altogether amount to 97,855 km^2 (Langridge, 1976).

The trypanosome species identified during the survey made by Langridge (1976) include Trypanosoma vivax as one of the commonest parasites found in all provinces. T.congolense was next to T.vivax and T.evansi . T.evansi is the cause of 'surra'

in camels and is a non-tsetse transmitted parasite, common in the southern and eastern regions. Others, T.brucei , T.b.rhodesiense, and T.evansi also occur in Ethiopia but are not so common.

Fuller (1978) also conducted a study of the distribution of Glossina in South Western Ethiopia, reporting the presence of four types of tsetse species i.e., G.pallidipes , G.f.fuscipes, G.m.submorsitans and G.longipennis . Moreover he also indicated the severity of animal trypanosomiasis along the Omo river valley.

A systematic work which was accomplished by Getachew and Teferi (1984) on the study of tsetse flies of Fincha Valley has suggested that tsetse flies tend to extend in thier range to a higher altitude up to 2000 m, which is beyond the previously reported height. Several reports presented by the National Tsetse and Trypanosomiasis Investigation Centre (1982/83, 1988/89) also reported that Nagana (trypanosomiasis) excludes cattle from many potentially productive areas of Ethiopia and is a major constraint on agricultural development.

Thus there is adequate evidence to show that the presence of tsetse in many areas of Ethiopia and the disease Nagana (local vernacular, "Gendi") which they transmit, have been responsible for large areas of the country being left undeveloped, and for the huge loss of livestock products.

With regard to the distribution of tsetse in Ethiopia, Ford (1971) stresses that the distribution of many species of tsetse

flies is still far from adequately mapped. The need for more detailed studies on the distribution of Glossina was made even more apparent because efficient vectors of human as well as animal trypanosomiases were reported within 200 km of Addis Ababa and cattle trypanosomiasis is known to extend into Shoa region (Langridge,1976). The distribution of the following species of Glossina in Ethiopia are reported by several authors.

Glossina morsitans submorsitans Newstead 1924. It has an east to west distribution from Ethiopia to Senegal (Jordan, 1986). Relict isolated fly belts survive in the valley of western Ethiopia (Ford,1971). It is incriminated as the most important vector of animal trypanosomes in Ethiopia. Hatchinson (1971) reported the species along Mui river. Balis & Bergeon (1970) and, Langridge (1976) found it around lake Abaya in the Rift Valley, in Didessa Valley, Metekel, Angar, Baro and Gilo river. It is wide spread in Upper Sobat and it Blue Nile drainage area (Hatchinson, 1971; McConnell & Baker, 1974). Fuller (1978) reported the species on the northern and the southern banks of the Akobo river. McConnell et. al. (1970) also found that G.m.submorsitans was an important vector of the cases of human sleeping sickness diagnosed in the Akobo-Gilo river area.

Glossina pallidipes Austen 1903. Balis and Bergeon (1970) and Langridge (1976) recorded it from the lower Omo river, Baro river and from Gamogofa province. G.pallidipes was the most widely distributed species collected during the survey made by Fuller (1978). It was particularly common along the Omo river

where it was traced in a continuous band from 20 km above lake Rudolf to the Jimma - Addis Ababa road. It was found along the southern edge of the Ethiopian plateau from Lake Chamo in the Rift valley to the Sobat and Blue Nile drainage system (Fuller, 1978). Hatchinson (1971) also reported it along Gok forest in Gambella. To the south and east of the Maji Mountains the predominant species away from the gallery forest was G.pallidipes (McConnell et. al., 1970).

Glossina tachinoides Westwood 1850. This has been implicated as a major vector in human sleeping sickness in Ethiopia (McConnell et. al.,1970; McConnell and Baker, 1974). Fuller (1978) did not find it in the Omo Basin, during the survey. It has, however, been previously collected in the Sobat basin along the Gilo, Baro and Akobo rivers as well as along the Didessa river in the Blue Nile Basin (McConnell et. al.,1970; Hatchinson, 1971). It has also been collected just east of Lake Abaya in the Rift Valley (Balis & Bergeon, 1970).

Glossina longipennis Corti 1895. It was found in the savanna from the bases of the Maji Mountains across Mui Game Park and along the Omo river to the base of Hammar plateau east of Kerre (Langridge,1976; Fuller,1978). G.longipennis has been collected across southern Ethiopia from Somalia to the Sudan, although its distribution appears to be patchy (Balis & Bergeon, 1970; Hatchinson,1971).

Glossina fuscipes fuscipes Newstead 1910. This was the dominant species found on the Omo river from the Jimma - Addis

Ababa road to within 20 km of Lake Rudolf (Langridge, 1976; Fuller, 1978). It was also found along the tributaries of the Omo, such as the Mago, the Gogeb and the Mui rivers (Balis & Bergeon, 1970; Fuller, 1978); in the Baro river (Langridge (1976) and in the forest along rivers draining the western escarpment in the southern half of Ethiopia (Fuller, 1978).

Fuller (1978) also described that G.fuscipes was much more abundant than the second most frequently found species, G.pallidipes , along the Omo River Valley. He also noticed that a drop in the number of G.fuscipes corresponded more closely with the drop in the number of hippopotamus and suggested that there was a greater dependence on the hippopotamus than on the crocodile, as food source.

The tsetse fly has caused extensive cultural and social repercussions among the group living in and around the infested area of South western Ethiopia. The Anuak, for instance, have moved villages along the Akobo to avoid sleeping sickness, although, ironically, the fly may have also provided an effective defence against the expansion of their cattle-raising neighbours, the Nuer, who have raided them in the past (Ellman, 1972).

The Kerre and Suri people have lost most of their cattle and have been unable to re-establish their herds. Their herds may have been lost originally due to rinderpest, but the tsetse fly now inhabits much of their territory, making re-introduction of herds exceedingly difficult (Fuller, 1978). Even the Kembatta

and Hadiya people in Shoa province avoid the Omo river for watering cattle, although they must occasionally use the river during the dry season (Langridge, 1976; Fuller, 1978). Glossina plays a continuing role in the lives and economies of the people of south-western Ethiopia.

Following the demonstration that host odours can attract large numbers of Glossina spp. to stationary baits (Vale, 1974; Vale & Hargrove, 1978) attempts have been made to identify the effective components of host odours with the view to improving the economy and effectiveness of baits used to sample or control populations of tsetse.Carbon dioxide, acetone and certain ketones and aldehydes are attractive in the field (Vale, 1980) and it has been found also that 1-octen-3-ol (termed octenol) is an olfactory stimulant capable of inducing Up Wind Flight (Hall et. al., 1984; Brusell, 1960).

Some species of flies though apparently using certain species of host preferentially, nevertheless feed on a wide range of hosts of all main groups of animals except birds and reptiles. Others are distinctly more selective choosing only a few hosts (Challier,1982).

The game animals of Africa are the mammalian hosts of trypanosomes and are also rather loosely referred to as the 'hosts', providing the bloodmeals of Glossina spp. The most detailed review of the feeding habit of Glossina spp. was based on the identification of over 22,600 bloodmeals collected by various workers from many parts of Africa (Weitz,1963). Although

further bloodmeals have been collected and identified since that time, the general conclusions reached have not been affected. Most species of Glossina have specific feeding habits,feeding predominantly on only some available host species, although the economically important species of palpalis group (G.palpalis , G.fuscipes , G.tachinoides) with cosmopolitan feeding habits, are often an exception to this general rule (Jordan, 1986). According to Weitz (1963), for instance, G.m.submorsitans in Africa in general was found to feed preferably upon Suids (warthog 45.6%), Bovids (buffalo, bushbuck and others 22%) and man (18.4%).

Therefore, wildlife plays an important role in maintaining tsetse fly and trypanosome populations. Consequently epidemiological studies of trypanosomiasis have to take into account the relationship between tsetse and their hosts (Weitz,1963). The ability to identify natural hosts of blood-sucking insects is an integral part of many ecological investigations, and it becomes of paramount importance in epidemiological studies when the insects are of medical or veterinary importance (Rurangirwa et. al., 1986).

Accurate identification of bloodmeals in haematophagous arthropods such as the tsetse fly is,therefore, an important aspect of the epidemiology of vector borne disease and investigation of host-vector interactions. (Rurangirwa et. al.,1986; WHO, 1987). It is important to know the kinds of animals on which the different species of tsetse depend for food. However, for many years there was no accurate knowledge on this

subject which has proved to be too difficult to investigate.

The early literature contains records by observers who had caught flies containing fresh blood,from an area resided by troops of baboons and had assumed that it came from the baboons. It was latter shown that such belief that the flies were feeding on the commonest host seen in the area was the most dangerous assumption of all (Nash,1969).

In 1924, Lloyd and Johnson (cited by Nash 1969) improved on all crude methods previously used by using the diameter of the red blood cells for all possible hosts. By measuring the diameter of the blood cells found in a recently fed tsetse they could then determine to what group the host might have belonged.Although an improvement was seen over the previous methods, it was not sufficiently precise.

One of the variables, encountered with bloodmeal identification is that the size of the bloodmeal ingested by the insect can vary greatly. This small amount demands sensitive microtechniques for host identification. Another factor is that tests are usually performed on bloodmeals that have deteriorated at least to some extent, antigenically, due to digestion processes.

The break through in bloodmeal source identification came after the improved techniques for the identification of blood meals was done by Weitz (1952) that devised a precipitin test involving inhibition of agglutination of tanned red cells. The test which have yielded precise and reliable information about

the source of food of most species of Glossina. The introduction of the precipitin test for the determination of the host , from bloodmeals, opened a further possibility for a better understanding of tsetse - host relationships.

A more specific precipitin test was later on introduced carried out by Weitz & Jackson (1957) and Weitz (1956).This test for the first time, indicated that Glossina spp. showed a selective feeding patterns. Later, studies based on the identification of some 23,000 meals, taken from 15 species and subspecies of Glossina collected from different localities (Weitz, 1963; Allosopp et. al., 1972) revealed that although a wide variety of potential sources of food were available, the flies were highly selective in their choice of host; feeding on small number of species of game animals present in the area.

Several, serological methods can be used to identify bloodmeals, e.g., passive haemagglutination inhibition tests, the latex agglutination test and fluorescent antibody techniques. However, the most widely used procedure remains to be the precipitin test usually performed as the precipitin ring test in small-bore glass tubes. The precipitin test , although relatively simple and inexpensive, lacks sensitivity and specificity, and can be some what time consuming (Service et. al., 1986). The advantages and limitations of these techniques have been well reviewed by Washino and Templis (1983).

None of these tests described above satisfies the requirements of a simple, yet sensitive, test which can be

considered as an alternative to the precipitin test. The need for blood identification method that is both specific to at least the generic level and sensitive enough to test for many possible hosts led to the adaptation of the Enzyme Linked Immunosorbent Assay (ELISA) for the purpose of bloodmeal identification (Burkot et. al., 1981; Rurangirwa,1986; Wirtz et. al. 1989). The test proved both sensitive and specific. By this method very small quantities of fresh blood (about 0.02 ml) can be detected (Service et. al.,1986) even after 24 hr post-ingestion by Anopheline mosquitoes (Edrissian & Hafizi, 1982).

Removal of the most favourable hosts from the habitats of tsetse seems to result in adaptation to another available host species. Vale & Cumming (1976) removed the warthog, which was the predominant host of G.m.morsitans in two areas of Zimbabwe. This had no drastic effects on the number and nutritional status of the flies. The elephant became a dominant host and when removed in turn,bovids formed about 90% of the tsetse diet. This showed that there is much local variation in the fauna of Africa,if the most favoured host in one locality is absent, the same species of tsetse may show a different preference; the choice depending on the availability of host species (Nash, 1969).

It is due to this tsetse (Glossina) adaptability in their feeding habit that bloodmeal analysis for the host range determination needs to be done in a specific area and situation. Although some bloodmeal analysis on tsetse (G.m.ugandesis) were done in the past in Ethiopia (Getachew, 1983), this was carried out in restricted areas and in a limited magnitude.

The present study is aimed at determining the host range of G.m.submorsitans in the upper Didessa river valley where this Glossina species is dominant and at the same time responsible for transmitting 'Nagana' in the area. Data were also collected on such aspects as odour attraction, trap efficiency, as well as on the rate of digestion of bloodmeal in the flies in order to standardize a trapping and bloodmeal identification methodologies.

MATERIALS AND METHODS

1. STUDY AREA

The study was conducted in the Upper Didessa River Valley, in South-Western Ethiopia, situated at 8°40'N and 36°20'E (Fig.1) where cases of animal trypanosomiasis is considered to be very high (Plate 3). The area is infested with both Glossina morsitans submorsitans and G.tachinoides where the latter is riverine, restricted only along the course of Didessa river (Plate 2).

1.1. CLIMATE

The climate is marked with wet and dry seasons; most of the rain falling between June and September. The year round data for the annual rain fall as well as the maximum and minimum temperatures of the study region are shown on Figure 2. It has an average total annual rain fall of 1946 mm and mean monthly max. and min. temperatures of 25°C and 12.5°C, respectively. The altitude of the valley is in the range of 1250-1900m above sea level.

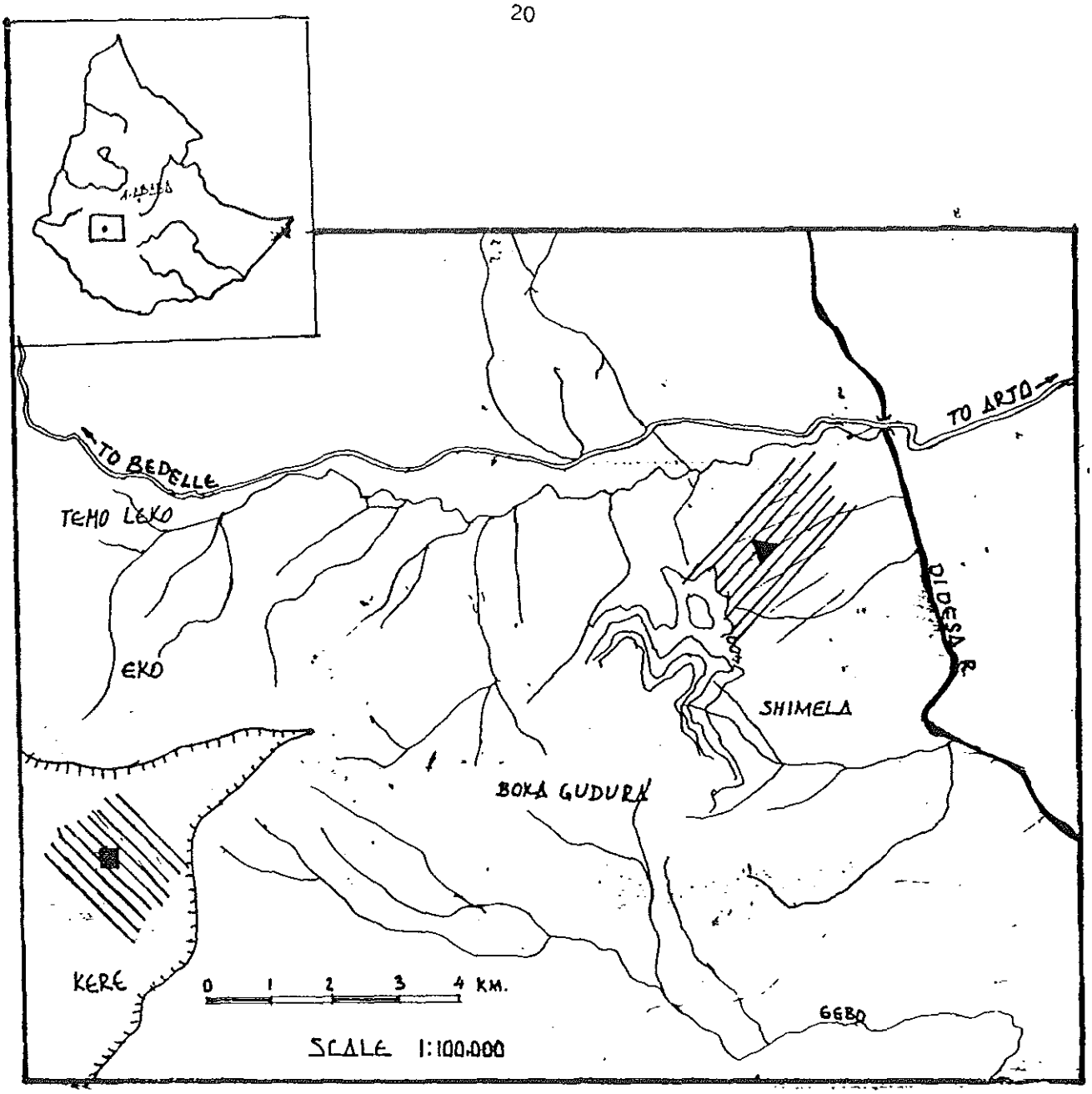

Fig.1. Map of the study area in the Upper Didessa River Valley.

LEGEND

- RIVER
- TRIBUTARIES
- ESCARPMENT
- ROAD
- BRIDGE
- FLY TRAPING AREA
- CATTLE BLEEDING AREA

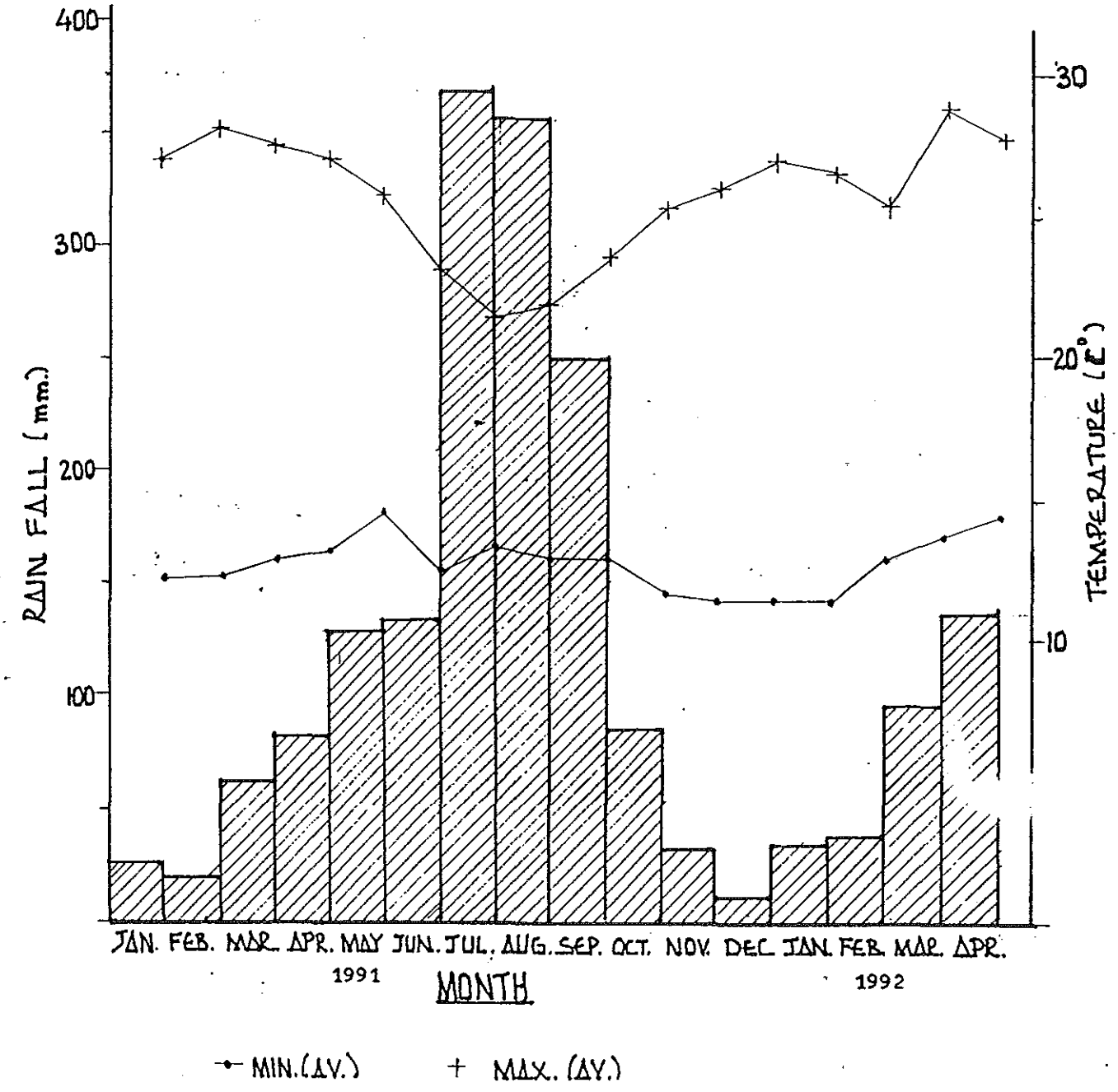

Fig 2. Monthly total rainfall as well as average maximum and minimum temperatures of Bedelle area as obtained from Bedelle Meteorological Station.

1.2. FLORA AND FAUNA

The vegetation of the area is characterized by wooded Savanna land where different grass species of Hyperrhenia can grow a height of up to two metres. Tree like Terminalia spp. and Combretum spp. are dominant(Plate 1). During the dry season most of the trees and grass except those found along the water course of the river, become leafless as result of annual burning.This forces morsitans to seek refuge in the unburned strips of vegetation along water courses or to the isolated evergreen tickets.

Among species of wild animals believed to be present are Phacochoerus aethiopicus (Warthog),Hippotragus equinus (roan antelope), Patamochoerus porcus (bush pig), Colobus polykomos (monkey), Papio anubis (baboon), other primates, Crocuta crocuta (Hyaena), Dipodina spp. (rabbit), Hippopotamus amphibuis (hippopotamus). Ungulates such as Syncerus caffer (buffalo) and Tragelaphus scriptus (bush buck) are rare(personal comminication and my own observation).

There are virtually no cattle or other domestic animals in the valley.

Plate 1. Habitat of Glossina morsitans submorsitans in the upper Didessa river valley.

plate 2. View of the Didessa river

2. ODOUR BAITS

The efficiency of odour attractants namely acetone, octenol, cow urine and their combinations, i.e., acetone + octenol, acetone + octenol + cow urine and unbaited (control) were tested using 6 x 6 latin square design by replicating with NGU (NG2G) (Plate 5) as well as the Biconical (Challier-Laveisser) (plate 4) traps. Each odour bait, was put in standard dispensers with traps through out the day and collection of the flies were made at the end of the day. The number and sex composition of the flies caught were determined.

3. COLLECTION OF BLOOD MEAL

The blood-fed flies were captured using NGU as well as Biconical traps . The stomach of flies containing visible blood, or partially digested blood were squashed on azid coated Whatman No I filter paper. The filter paper was then dried in the air, labelled and kept in a desiccator jar over silica gel, and stored at -20 c until analysed.

4. DETERMINATION OF INFECTION RATE

4.1 TSETSE

After the fly was killed with chloroform, fly dissection for determination of trypanosome infections were made using Lioyd and

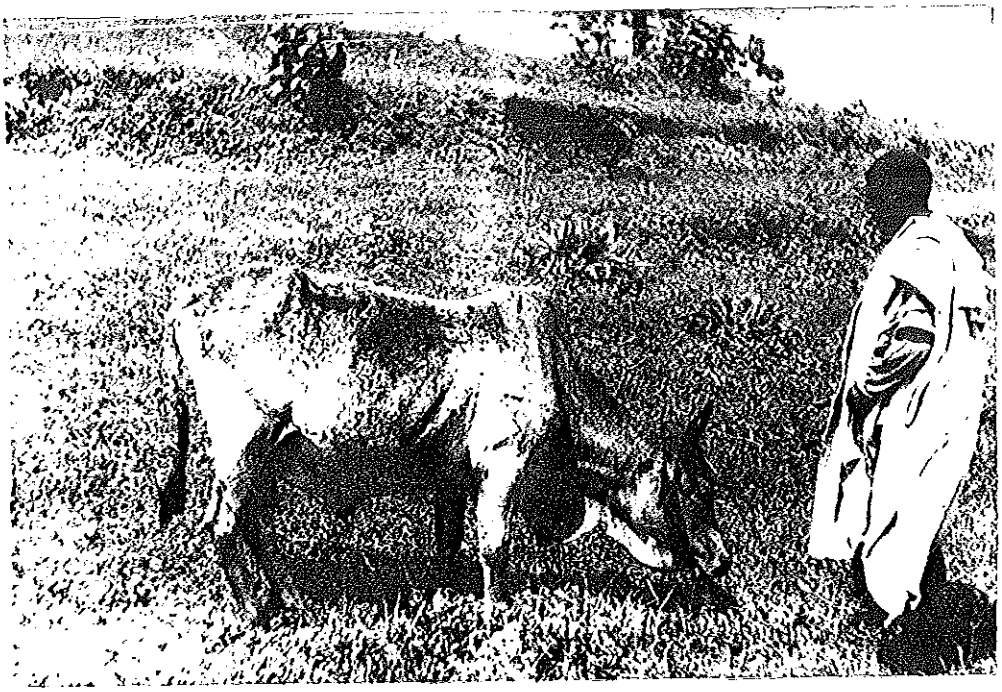

plate 3. An ox from Bedelle escarpment infected with trypanosome and showing typical emaciated condition.

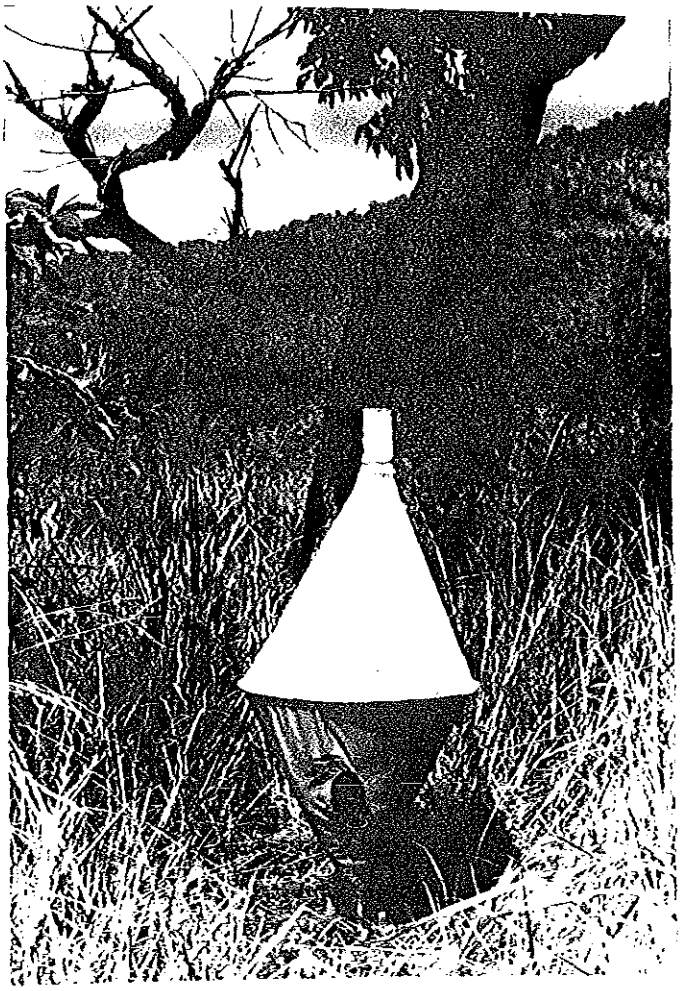

plate 4. Biconical trap at work

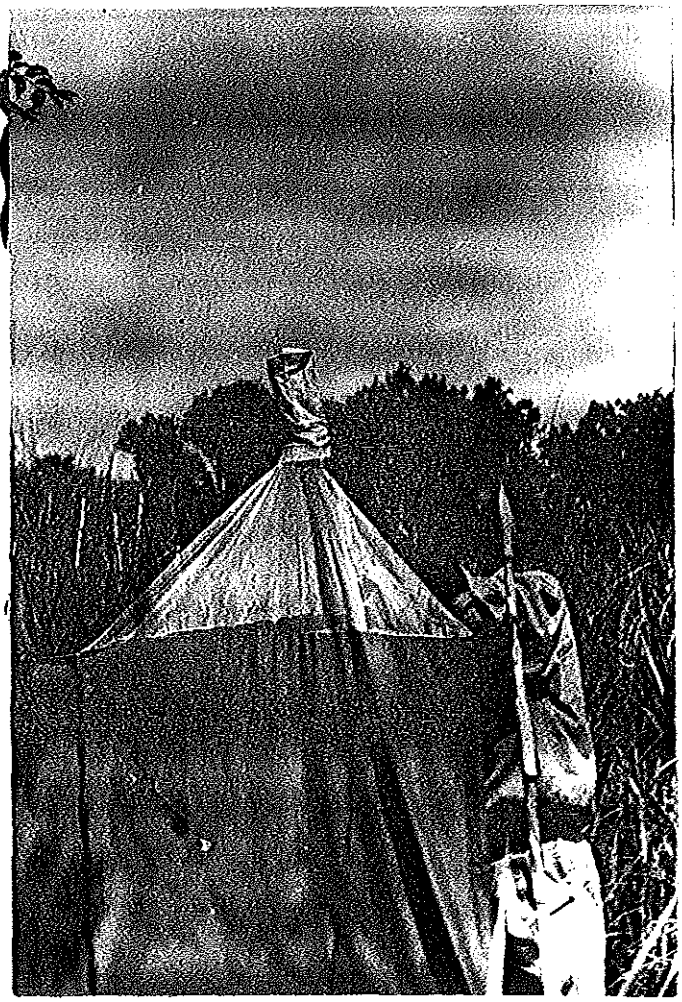

plate 5. Ngu trap at work.

Johnson (1927) method,i.e. the forms of trypanosoms in tsetse fly can be identified based on the sites of development within fly. First the wings and legs were pulled off. The proboscis, midgut and salivary glands were the organs examined. Salivary glands were withdrawn from the body by pulling off the head, the proboscis was next removed; finally the tip of the abdomen was cut off and the gut content and other viscera squashed out. These parts were then examined under the microscope using 0.9% saline solution for the presence of trypanosome parasites. The trypanosome infection rate is therefore the percentage of flies having mature (fully developed) trypanosome infections in the gut, proboscis or salivary gland.

4.2. **CATTLE**

From the cattle population that reside along the Southern part of the upper Didessa river valley escarpment (Bedelle escarpment) , 54 animals were randomly selected and then examined for the presence of trypanosomes in their blood. The ears of animals were pierced using lancet and veinous blood sample was taken using heparinized haematocrit capillaries. After centrifuging the blood specimens collected, wet smear was prepared and the presence of trypanosomes was checked under the microscope with a magnification of 400X.The species of the trypanosomes found was determined based on their morphological characteristics. PCV (packed cell volume) the proportion of red blood cells in blood which is used to estimate anaemia , in cattle were also measured.

5. DETERMINATION OF BLOODMEAL DETECTION TIME FOLLOWING FEEDING

About 2000 pupae of G.m.submorsitans were obtained from ICIPE (Nairobi, Kenya). The pupae were then kept in the insectary at National Research Institute of Health, Addis Ababa. Flies were maintained at 25°C and 60-70%RH. Samples of newly emerged flies (teneral) as well as previously fed flies (non-teneral) which had been starved for 72h were taken and allowed to feed on rabbit for 10 minutes. Finally flies are squashed on to azid coated whatman filter paper No 1 at 1hr, 6hr, 24hr, 48hr, 72hr, 96hr and 120hr, post-feeding. The filter papers were then labelled and put in desiccator until analysis is done, using anti-rabbit conjugate with ELISA test.

6. BLOODMEAL ANALYSIS OF FIELD COLLECTED SAMPLES

The analysis was done at Nairobi University with the kind collaboration of Dr E.K.Kange'the according to the agreement made with ICIPE. The method of the bloodmeal analysis used was as follows.

The dried spots of the contents of the flies on the filter papers were cut out as small paper discs and each disc placed in a small sample well. Elution of the spots was done with PBS (Phosphate buffer saline, Nacl 8.0g/l,Kcl 0.020g/l,Na2HPo4 1.15g/l ,KH2po4 0.20g/lh) PH 7.2 500µl per elution. After dilution of the sample (1:1000) using coating buffer (Naco3 1.59g/l, NaHco3 2.93g/l in 100ml distilled water), pH.9.6

100µl/well of each dilution was added in flat bottomed wells of microtitere plate in duplicates. Wells with out blood were used as conjugate and substrate controls (negative) and two wells were also used as positive controls in the same plate. The plates were then incubated overnight at room temperature, to allow coating of blood meal components. Washing of plates were made by flicking off the buffer followed by three washes with PBS-Tween 20 (0.05M phosphate, 0.5M NaCl, 0.5% Tween 20 , pH 8) at 5 minutes intervals. One hundred microlitres of 1:1000 concentration of conjugate peroxidase (labelled anti-species IgG) were added and left for 45 min. after which the above washing procedure was repeated.

One hundred microlitres of substrate (0.1% ortho-phenylene diamine, sigma) in substrate diluent(citric acid buffer PH 4.0(9g/l), 0.05% (v/v) Tween-20 in PBS 500ml, 30 % H_2O_2 5ml, 1l dist.water) were added to each well and left at room temperature for 1h in the dark. Colour development was read visually or with a micro-ELISA auto reader at 492 nm. Any optical density (O.D.) reading that was 0.100 and above was considered positive.

7. **DATA ANALYSIS:**

The statistical methods used for the present data analysis were F test(two factor variance analysis) and the student t test.

RESULTS

1. ODOUR BAIT SYSTEM

Trials were carried out to test the effectiveness of bait systems towards G.m.submorsitans . The result (Table 1) revealed that the catching efficiency of Biconical trap for G.m.submorsitans is superior to that of Ngu trap in all odour baits. Among odour attractants assessed, Acetone + octenol + cow urine combination followed by acetone+octenol combination showed marked increase in mean catches of tsetse flies(Fig.3).

Comparison between types of traps indicated that the difference of the mean number of flies caught using Biconical and Ngu traps were highly significant ($P < 0.005$) at all odour baits. Concerning the efficacy of odour attractants tested, each of the described odour attractants and their combinations (Table 1) showed significant performance ($p < 0.005$) in both traps. Among odours tested separately, acetone was a better attractant than octenol or cow urine (Fig.3)

Biconical trap baited with cow urine had increased the catch size by 2.73 times when compared with the control (unbaited). Similarly, the same odour attractant with NGU trap was 2.48 times more attractive than unbaited. It was also possible to recognize that a cow urine sample kept in container for some days, was more attractive for tsetse flies than the fresh sample (Fig.6).

Table 1. Mean catches of flies (G.m.submorsitans) with Biconical and NGU traps using various odour attractants.

Odour baits	Flies Per trap/day (Mean ± S.D.)		Index of Increase (Biconical over NGU)
	Biconical	N G U	
Unbaited (Control)	13 ± 8	9.9 ± 3.53	1.31X
Acetone	45.6 ± 23.3	27.6 ± 5.65	1.65X
Octenol	31.2 ± 17.96	11. ± 1.41	2.48X
Acetone + Octenol	53.7 ± 2.82	45.2 ± 6.36	1.19X
Acetone + Octenol + Cow Urine	81.9 ± 10.6	67.2 ± 2.12	1.22X
Cow Urine	35.5 ± 10.5	23.9 ± 2.11	1.49X

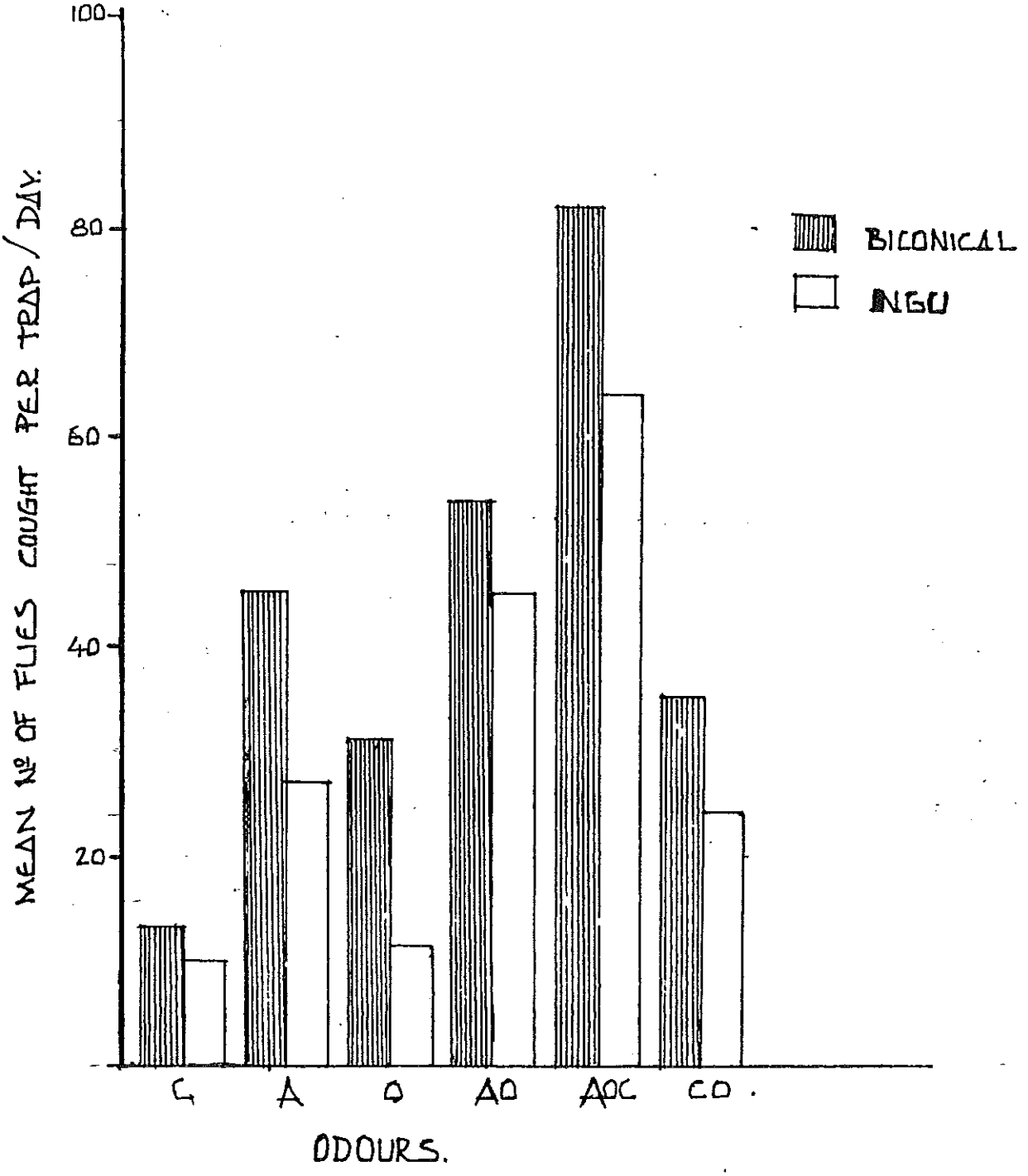

Fig.3 Distribution of the mean number of tsetse (G.m.submorsitans) caught with Biconical and Ngu traps using different odours as attractants.(C=unbaited, A=Acetone, O=Octenol,AO=Acetone+Octenol;AOC=Acetone+Octenol+Cow urine,CO=cow urine)

In general both types of traps (Ngu & Biconical) tested had caught more female G.m.submorsitans than males in all the odour used, except octenol in Biconical trap, although the percentage of female flies in Ngu trap was superior to Biconical trap (Fig.4 and Fig. 5).

The percentage of male and female flies trapped and the daily catches of both sexes per trap per day are shown in Table 2. A relatively higher number of females were trapped by the Ngu trap than by the Biconical trap and the differences is statistically significant ($F = 11$, $P < 0.05$). This suggested that Ngu traps were more effective (by a factor of 1.18) in catching the females than the males. The result revealed that the differences in catches using cow urine odour stayed from one to six days had been statistically highly significant ($F = 53.29$ $P < 0.05$) in both traps. Significant difference was also observed between the efficiency of NGU and Biconical traps $F = 11.66$, $P < 0.05$) in catching flies using the same odour and method.

The ratio of the number of flies caught by using acetone, cow urine, and octenol as odour attractants when compared to the unbaited condition is in the order of 3.52: 2.73: 2.40: 1 for Biconical trap and 2.79: 2.4:1.1: 1 for Ngu trap.

Table 2. Comparisons of sex composition of G.m.submorsitans caught with Biconical and NGU traps using different odour attractants (C = Unbaited, A = Acetone, O=Octenol AO = Acetone + Octenol, AOC = Acetone + Octenol + Cow urine, CO= Cow urine M=male,F=female).

Type of trap	Sex	No. of flies /trap /day O D O U R S C	A	O	AO	AOC	CO
Biconical	Male	6.1	15.8	16.1	19.2	32.3	16.1
	Female	6.9	29.8	15.1	34.5	49.6	19.4
	Density	13.0	45.6	31.2	53.7	81.9	35.5
	M:F	1.13	1.89	0.94	1.80	1.54	1.21
	% Female	53	65	48	64	61	55
	Index	1X	3.5X	2.4X	4.14X	6.3X	2.73X
NGU	Male	3.6	7.2	3.2	16.5	20.9	8.9
	Female	6.3	20.4	7.8	28.8	46.3	15.0
	Density	9.9	27.6	11.0	45.2	67.2	23.9
	M:F	1.75	2.83	2.44	1.75	2.22	1.69
	% Female	64	74	70	64	69	63
	Index	1X	2.79X	1.11X	4.56X	6.78X	2.48X

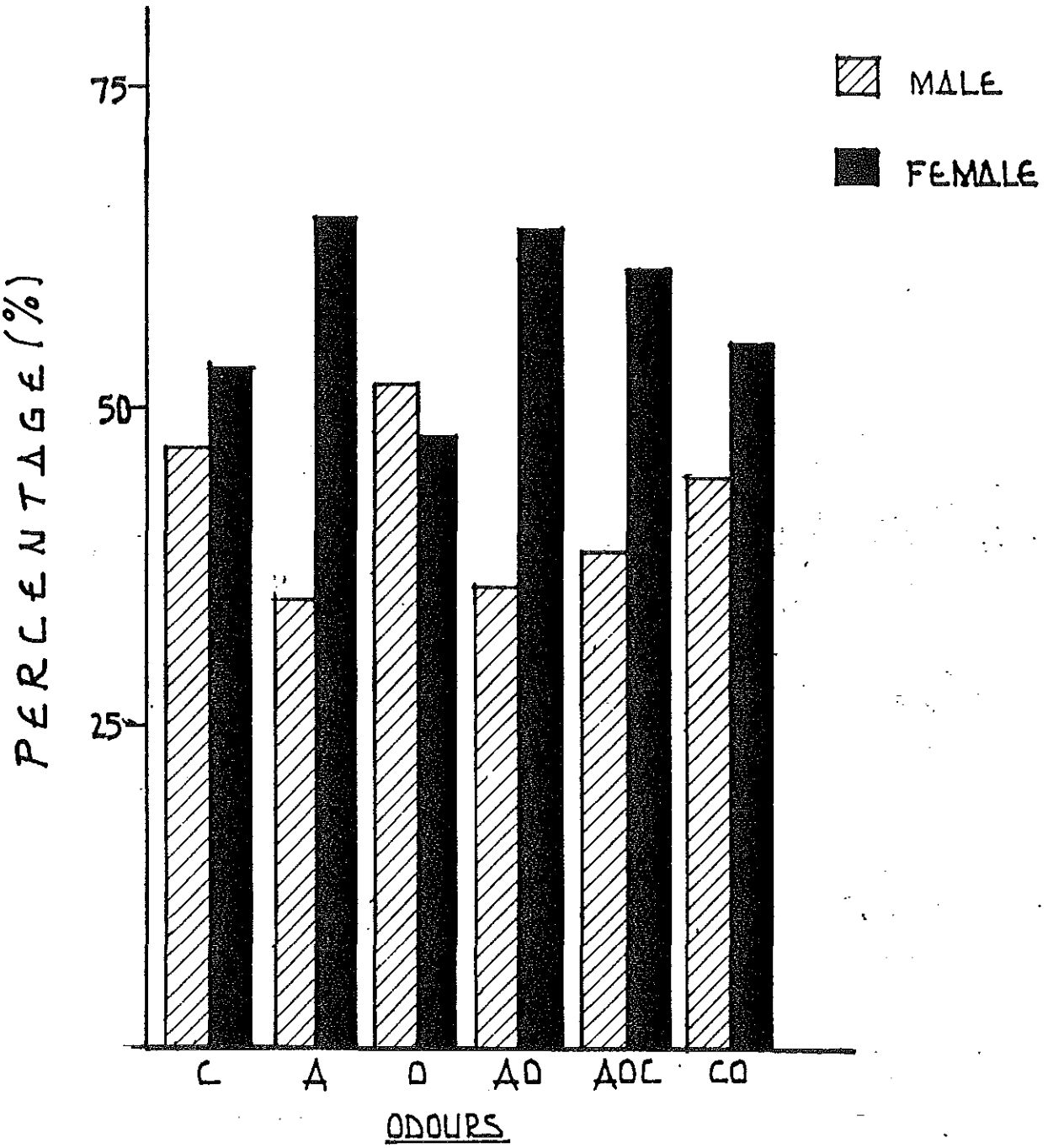

Fig. 4. Frequency distribution of the percentage of male and female tsetse (G.m. submorsitans) Caught with Biconical trap using different types of odour attractants. (C=unbaited, A=acetone, O=octenol, Ao= Acetone + octenol, Aoc=Acetone + octenol +cow urine, Co = cow urine)

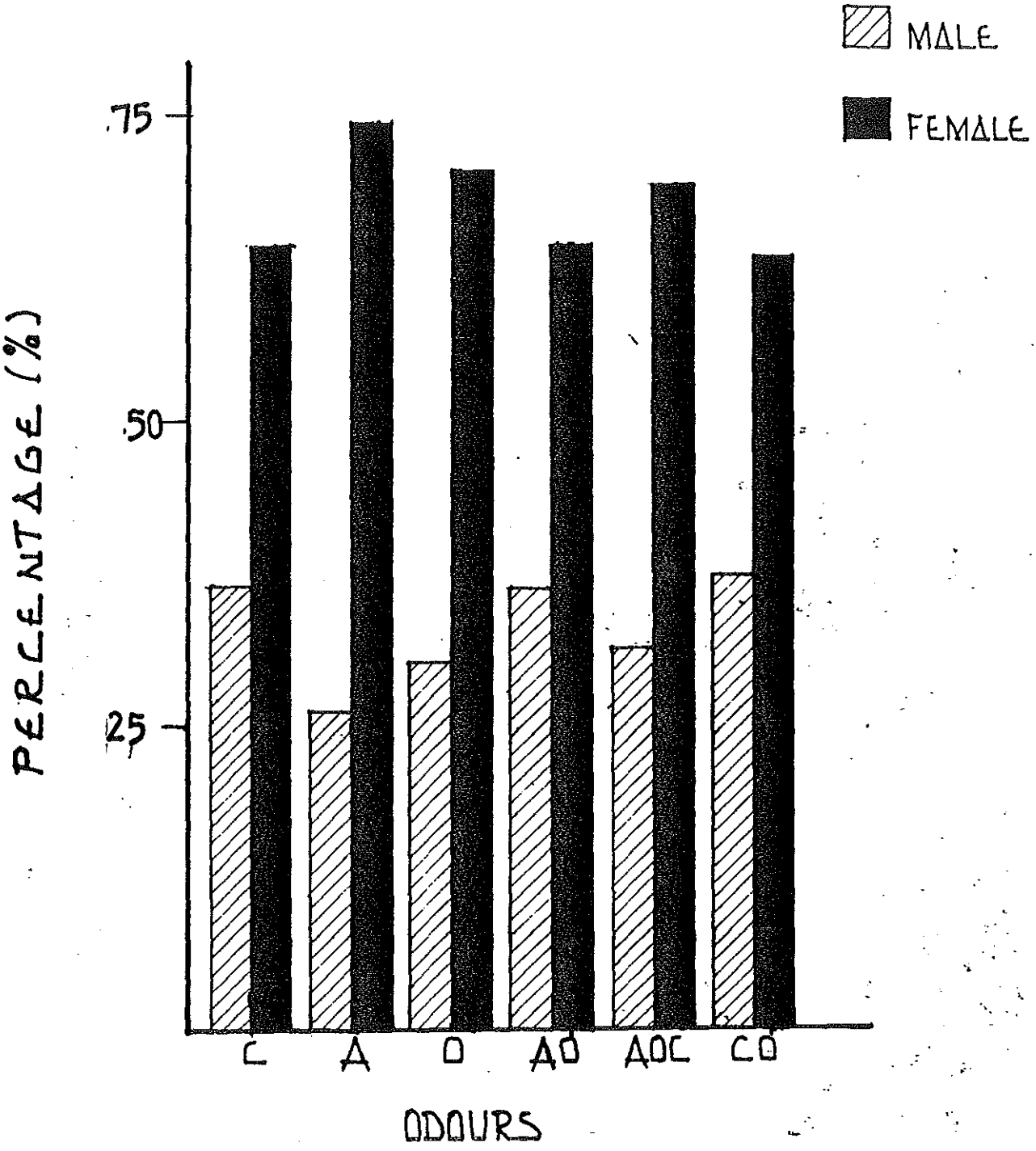

Fig. 5. Percentage distribution of male and female tsetse (G.m.submorsitans) caught with Ngu trap using different odour attractants. (C=unbaited, A=Acetone, O=octenol, Ao=Acetone+octenol, Aoc=Acetone+ octenol+ cow urine, Co=Cow urine)

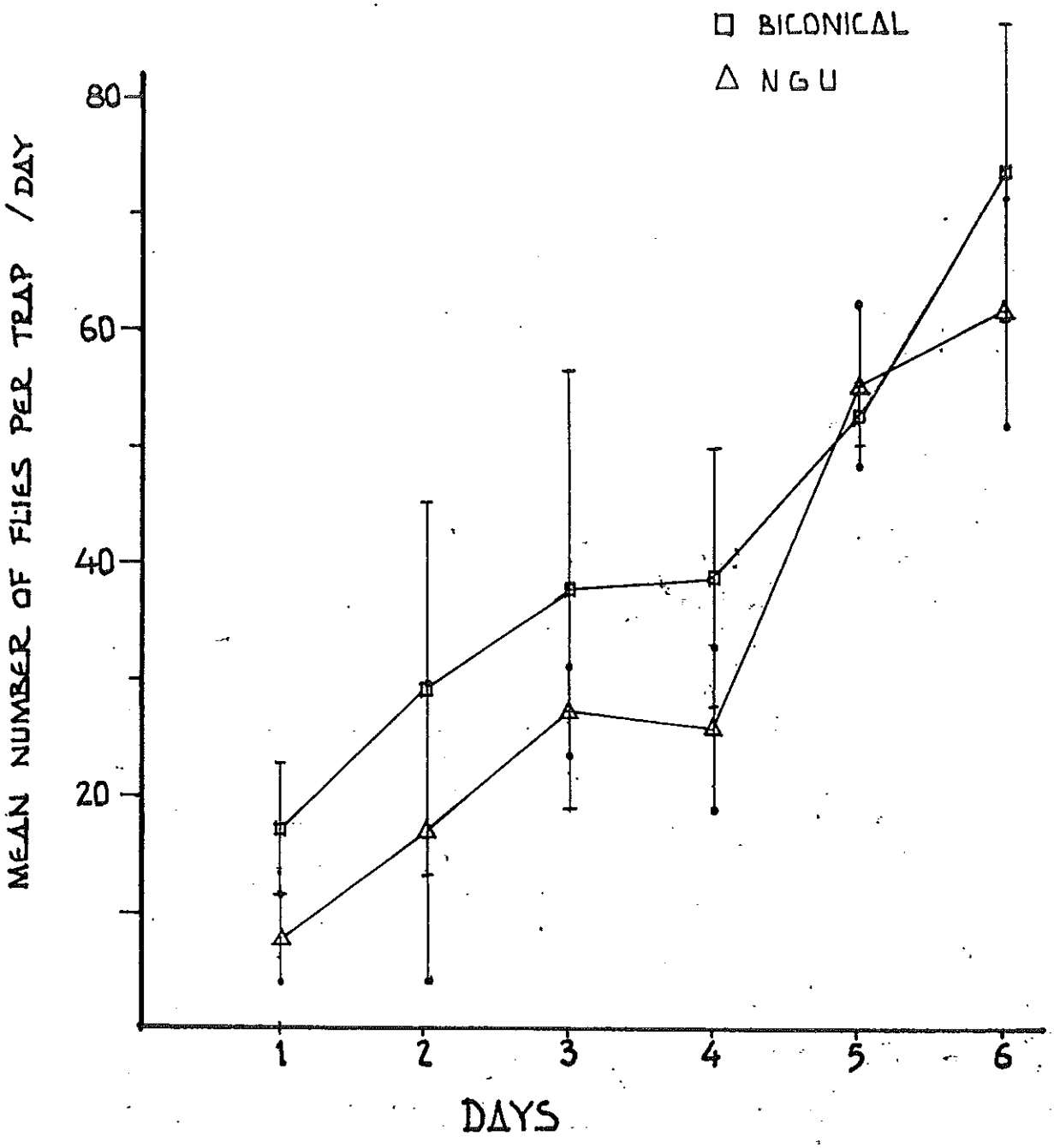

Fig. 6. The relationship between the number of flies (G.m. submorsitans) Caught using Biconical and Ngu traps With cow urine odour as attractant which had been kept in container for different days.

Variations were also observed regarding the catching of fed flies with Biconical and Ngu traps. In this regard Ngu trap was found to be effective in catching a higher proportion of fed flies than the Biconical. The result obtained is given in Table 3.

2. TSETSE INFECTION RATE

Among the 151 G.m.submorsitans (82 females and 69 males) dissected and examined for the presence of trypanosome, only 4 flies were found positive for matured T.congolense and T.vivax . In the subgenus Duttonella (T.vivax) development occurs only in the proboscis, in the Nannomonas (T.congolense) in the midgut and proboscis whereas in the Trypanozoon (T.brucei) it occurs in the midgut and salivary gland (Jordan, 1986) The prevalence of the overall infection for both trypanosome spcies in G.m.submorsitans in the area is therefore 2.65% (Table 4).

Table 3. proportions of fed and unfed Glossina m.submorsitans, caught by NGU as well as Biconical traps during blood meal sample collection.

Traps	Total No of flies Caught	Fed flies			Ratio Efficiency	
		F	M	Total		
NGU	2628	54	14	68	1:38	1.5X
Biconical	5441	60	32	92	1:59	1
Total	8069	114	46	160		

3. TRYPANOSOME INFECTION IN CATTLE.

Survey for the prevalence of animal trypanosomiasis in cattle at about 4km south of southern escarpment of the Upper Didessa River Valley (alt. 1900 m) revealed that the over all rate of infection with pathogenic trypanosome was 42.65% (23 infections in 54 animals)(Table 5). The mean PCV (packed cell volume) of the cattle examined was 22.6% (14-29%).

Table 4. Infection rate of G.m.submorsitans in the upper Didessa river valley.

	No.of Flies	Sex		Inf.(%)
		M	F	
Flies Examined	151	69	82	-
Positive for T.congolense	3	1	2	1.99
Positive for T.vivax	1	1	-	0.66
Total Infection	4	2	2	2.65

Table 5. Prevalence of Trypanosomiasis in cattle examined from the southern part of the escarpment of upper Didessa river valley.

	No.	%
Total Examined	54	-
T.congolense	15	27.8
T.vivax	4	7.4
Mixed (T.C. & T.V)	4	7.4
Total infected	23	42.6
Vivax ratio	8/27	29.0

4. BLOOD MEAL IDENTIFICATION

The capacity of ELISA test to identify the blood meals taken from known host (rabbit) at different time post-feeding, i.e from 1hr up to 120hr, after ingestion were tested and showed that the degradation of the species-specific serum components was slower in teneral than in non-teneral tsetse (Fig.7).

As assessed by ELISA at 48hr post-ingestion, the blood meals were identifiable in 87.5% of fed teneral tsetse as compared to 55.5% in non-teneral tsetse. At 72hr post-feeding, the blood meal was identifiable in 37.5% of teneral 13.33% of non-teneral flies (Fig.7). At 96hr the donor was identifiable in 12.5% ofa teneral compared to 10% of non-teneral tsetse.

The present investigation also indicated that the high rate of digesting serum protein has started after 24hr post-ingestion in non-teneral flies whereas in teneral tsetse, it was after 48hr. (Fig 7). Differences in the rate of blood meal digestion between male and female G.m.submorsitans in the laboratory was not observed .

One hundred and sixty fed flies were collected from the field using biconical and NGU traps and their blood meals were analyzed by the ELISA test. Among the 160 bloodmeal samples collected ,hosts that provided bloodmeal were identified only from 87 samples. The result of these determinations are presented in Table 6.

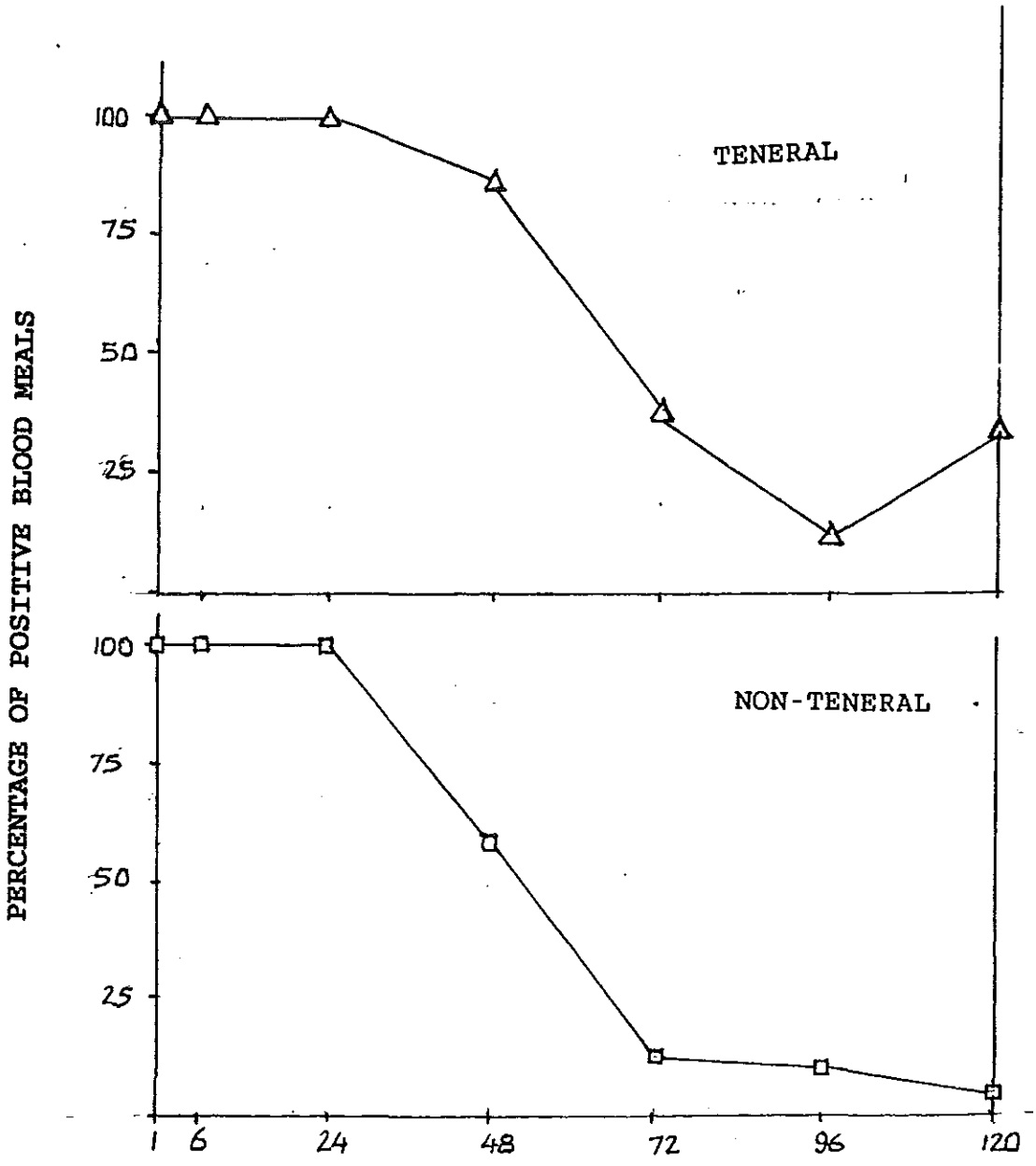

Fig. 7. ELISA results for bloodmeals of teneral and non-teneral G.m.submorsitans, at successive time intervals after feeding on rabbit.

Table 6. Identification of blood meal source of G.m.submorsitans from upper Didessa river valley

Bloodmeal source

Flies	Wartho g	Human	Buf- falo	Giraffe	Elephant	Goat	Cattle	Total
Male	16	11	3	2	-	1	-	33
Female	30	8	8	5	1	1	1	54
Total	46	19	11	7	1	2	1	87

At least 28.75% (46/160) of the flies had fed on warthog which indicates that it is the most preferred or available host of G.m.submorsitans in the locality. Next to warthog 11.88% of the meals was derived from human host. Others such as cattle provided much lower percentages of blood meals to the flies (Fig.8) as the livestock population are normally absent in the study area due to the disease, Nagana. Mixed feeding had been also identified in 4 blood meals comprising two meals of human/warthog, one of cattle/ elephant and one of buffalo/giraffe.

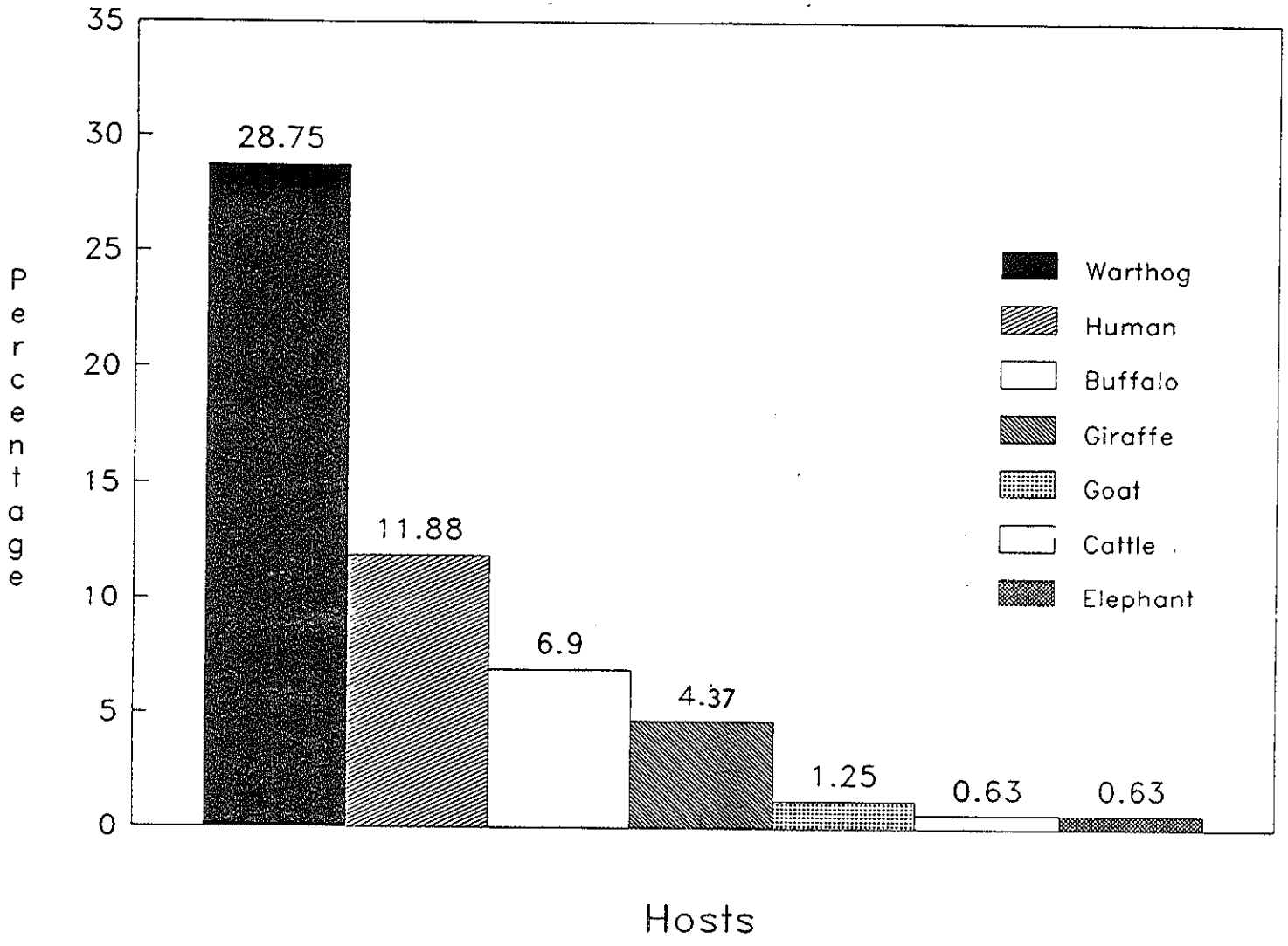

Fig. 8 Frequency distribution of the bloodmeal source of G.m. Submorsitans in the upper Didessa River Vally.

DISCUSSION

Availability of insects for capture is a function of population density and activity. Activities vary according to extrinsic factors such as temperature and humidity and intrinsic elements of the insects e.g. sex, age, and hunger stage (Mary, 1985). Catch size of tsetse also is affected by the range of attraction of a particular trap (Dransfield, 1984) and the odour types used. Hence, trap catches are often biased because of particular behaviour related to these factors.

According to the result of trap catches (Table 1) which is expressed as mean value of flies caught per trap per day, Biconical trap was consistently more effective than similarly baited Ngu (NG2G) trap for G.m.submorsitans . A universal trap and odour bait which is equally efficient in catching all types of Glossina spp. is not yet developed. It has already been proved that each of the species of Glossina has different preference towards various types of trap designs and colours (Flint, 1985). For instance Brightwell et, al. (1987) found that NGU version traps are about 3 times as effective as the biconical trap for catching G.pallidipes which is one of the Morsitans group tsetse. Thus the use of the available traps in this study was justified as the results of lack of an earlier information.

Regarding the sex composition of the catches, statistically significant differences were observed between the Biconical and the NGU traps. NGU traps were found to catch more proportion of female flies than the Biconical. And the presence of heavily

pregnant females , some of which had some blood meal in their gut, in NGU trap, suggested that some females may have been seeking larviposition sites. Its shape and its closeness to the ground might have been responsible for attracting flies which had last instar larva to be deposited. The present study in general indicated that more female flies are caught by both traps.

In the present study the mean sex ratio distortion of wild G.m.submorsitans was 2.11 and 1.42 in the NGU and the biconical traps respectively in all odour baits tested. Similar sex ratio distortion (2.0) was reported by Rawling and Maudline (1984) on this tsetse species and they have suggested that the distortion is caused by distorter males that either passed only x-bearing sperm to their mate or passing non-functional y-bearing sperm. In a field study a composition of 48% of male and 52% female G.m.submorsitans form ugandesis was reported by Getachew and Teferi (1983) from a year round work conducted in the Fincha valley using Biconical trap. It is difficult to determine the true sex ratio in adult tsetse population because the sampling methods used are selective and can produce excess of either sex and because females live longer than males as reported by Buxton, (1955).

For comparison of the odour attractants tested, as the number of odour baits in the cocktail, increases it was observed that the catch size also improves. But among odour attractants tested alone, acetone showed a high index of attraction than others. Electroantennographic (EAG) responses which are used to

detect the potency of olfactory stimulants had shown that the dose-response curve to octenol is 10^6 times more potent than acetone for G.pallidipes and G.m.morsitans (Hall et.el., 1984). Although the potency of octenol to make olfactory sensation towards G.pallidipes and G.m.morsitans in the laboratory was reported to be higher, its efficiency as odour bait was lower than that of acetone and cow urine for G.m submorsitans, in the present study. This indicates, that cow urine can be used as one of the odour baits in preference to the imported octenol for catching G.m.submorsitans , since it is available in large quantity at no direct cost.

Cow urine kept in bottle for some time was found to be more effective in attracting tsetse flies than a newly collected one. This could be due to the bacterial activities going on in the urine sample, the action of which may have been responsible for releasing of compounds like phenols, aldehyde, ketones, etc. which may have been responsible for the attraction of more flies to the traps. The indication that larger catches are possible using cow urine kept for some time in a container suggest the feasibility of employing cow urine baited-traps for control of G.m.submositans.

For the collection of fed flies, NGU trap had caught more number of female fed flies than the Biconical trap. This observation indicated that NGU is much better than the Biconical in catching fed flies when the aim of the study is bloodmeal sample collection. The tendency of fed flies to be captured by the NGU trap than the Biconical may be due to the preference of

fed flies to use the NGU trap as a resting site.

Vale (1980) reported that acetone alone increased up to six times the catches of*G.m.morsitans* and *G.pallidipes* with the Biconical trap.This is more than what is presently recorded for *G.m.submositans*. The index of catch using octenol was almost consistent with the report of Vale & Hall (1985) on *G.m.morsitans* and *G.pallidipes*. The proportion of female and male catches of *G.m.submorsitans* was also affected by odour type. The use of acetone as odour attractant either alone or in cocktail with other odours proved to increase the catch of female flies than males in both traps relative to the other odour baits namely cow urine, octenol and unbaited traps.

In this study the trypanosome infection rate of *G.m.submorsitans* in the upper Didessa river valley was much lower than the rates commonly reported by various researchers. The predominant species of trypanosome identified was *T.congolense* followed by *T.vivax*. The rate of infection with trypanosome species in *Glossina* spp. vary from species to species and from one locality to another. In general, however,the overall infection rates are lower in *fusca* and *palpalis* groups than in the *morsitans* group. Although it is difficult to generalize,the infection rates of 10 - 15 percent in the morsitans group and of about 5 percent in the *palpalis* group can be considered typical (Jordan, 1986).

In the present study, however, the trypanosome infection rate identified from G.m.submorsitans , was 2.65 percent (Table

4), which is much lower than is expected typically. But a variety of factors are known to influence the establishment of infections of salivarian trypanosomes in Glossina. For instance endogenous factors associated with the fly (eg. fly age, host preference, sex etc.); ecological factor (eg.climate,availability of infected hosts, etc.) and parasite and host (eg. parasite number available to the fly, type of parasite, susceptibility etc.) are among the factors playing an important role in the maintenance of infection in tsetse flies. In the present finding factors like host preference, availability of infected hosts, number of parasites available to the fly and the likes could be considered in order to explain the low prevalence of infection in G.m.submorsitans.

Regarding the fauna of the area where this study was conducted it is totally devoid of any type of livestock population. Few settlers are known to be living in restricted resettlement villages in the valley and are using hand tools to cultivate their farm yards. According to Jordan (1965) those species of Glossina which feed primarily on species of wild or domestic bovidae are more likely to be infected with trypanosomes than those species which feed primarily on other hosts. Riordan (1977) also reported that highest trypanosome infection rates in wild tsetse ever reported were in G.m.submorsitans caught close to a route along which heavily infected trade cattle passed regularly.

Thus one of the most likely reasons for the much lower infection rate recorded from G.m.submorsitans in the upper Didessa river valley may be the absence of domestic bovidae in

close contact with the fly. On the other hand although the preferred wild hosts like Suidae (warthog), are usually infectable, trypanosomes are very scanty in their blood (Jordan, 1986) and hence can only present lower infections to the flies.

In another pilot study , under taken in Merochisa and Galle Ferdeyo resettlement areas, which is situated along the Didessa River valley which is about 40km away from the study area where cattle are present the infection rates of G.m.submorsitans was also determined. This comparison was made in order to see the importance of the presence of domestic bovidae for the variations of infection rates in this particular tsetse species. According to the result obtained, the infection rate was 8.3 and 9.1 percent in Merochisa and Galle Ferdeyo resettlement areas, respectively,which is relatively higher and may be due to the presence of cattle which could provide higher infection to the flies fed up on.

The innate capability of wild life to minimize parasitemia in their blood and the severe infection of trypanosomiasis that existed in the body of domestic livestock contribute to the variation of infection rates identified from similar species of Glossina found in different habitats.

In addition to this, the coinciding of the study time with the period of high build up of fly population that is took place just before the rain (May-June) in the area, might have also contributed to the lower rate of trypanosome infection in the flies since the newly emerged flies may have out-numbered the

older and more likely infected flies.

Results obtained from the present investigation showed that the prevalence of animal trypanosomiasis at the edge of the escarpment was very high. Almost half of the cattle population examined was found to be infected either with T.congolense or T.vivax, or both. In East Africa it is generally found that when tsetse of the morsitans groups are the transmitters of T.vivax and T.congolense, the latter is usually the dominant trypanosome (Ford, 1971). Godfrey (1960) also found that whilst congolense was easily transmitted by G.m.submorsitans all attempts to transmit it through G.papalis did not succeed.

According to Jordan (1986) in general, T.congolense is the predominant trypanosome in cattle populations within or close to the infestation of Glossina; but as a distance from known infestations increases, T.vivax becomes more frequent and eventually predominant. Ford (1971) noticed that, in Uganda infection records show that a high vivax ratio is associated with low overall incidence of pathogenic trypanosomes under natural conditions. This occurs either where there are no Glossina at all or only G.fuscipes exist. The proportion of T.vivax infection is also raised by successful attack on the vectors.

It is possible therefore to suggest that the lower 'vivax ratio' recorded among domestic livestock found along the Bedelle escarpment near the upper Didessa river valley, is due to the presence of tsetse-cattle contact in the locality. Although, it was not possible to catch tsetse flies in the area where the

cattle population is residing, the factors that may have contributed to the high transmission of trypanosomiasis in this location may have been, (1) the presence of very low density population of G.m.submorsitans which are notoriously difficult to detect; (2) the presence of a busy all weather road from Lekemete to Bedelle which crosses the G.m.submorsitans infested valley,which may also have facilitated transmission, since tsetse are attracted to moving objects such as vehicles,and will follow them over long distances from their habitats and feed on and infect domestic livestock up in the escarpment before they die.

The pattern of the disease over Bedelle escarpment was not constant. It's distribution and incidence being affected by population density of G.m.submorsitans along the valley. Observations indicate that the building up of G.m.submorsitans population before and after rain i.e., during May - June and September - October were found to coincide with higher peak of the disease among domestic livestock found at the escarpment. Thus it may be suggested that excessive hunger that occur in the newly emerged population of the fly could cause the tsetse flies to go up into the higher altitude out of the valley where domestic bovidae are available. Moreover when the grasses grow both tsetse and game can penetrate further in to the marginal land where cattle are kept. These could componsate for the low density of the fly population in the Bedelle escarpment.

Thus during periods of high fly density, just before and after the rains, the incidence of the disease increases as tsetse

come in close contact with livestock on grazing grounds around the fly belt, on the other hand during periods of fly recession (during dry season when bush fire clears the area and tsetse are restricted along the course of the river) pressure on livestock is relaxed and the incidence of disease declines. The low PCV value of the animals examined i.e; 22.6% is one of the indications of the maintenance of animal trypanosomiasis among the cattle population in the area since anaemia is one of the major symptom of the disease.

The time after ingestion of a bloodmeal at which plasma proteins can be detected by immunological methods, such as ELISA, will depend on both the size of the bloodmeal and the rate of its breakdown due to digestion (Service et, al; 1986). Most workers have recorded the detection time after feeding as a guide to the sensitivity of their technique. The ELISA test was chosen for the present study as it is more sensitive than other immunological tests (Rurangirwa, et.al; 1986). As a test which uses minute quantities of sample it is suited for tsetse bloodmeal analysis. For example, Edrissian and Hafizi (1982) found that ELISA method could identify human blood in Anopheles stephensi for up to 24hr (temperature not stated). Also ELISA is easy to perform and is amenable to large scale use since many samples can be tested simultaneously.

By using the antisera of rabbits, it was possible to identify the bloodmeal in 87.5% of the non-teneral flies 48hr after they had ingested. Rurangirwa et.al (1986) reported that at 40hr post-feeding in G.m.centralis the bloodmeal donor could

still be identified in 100% of the fed teneral and 67.5% of the non-teneral tsetse flies, respectively (temperature and humidity not mentioned). In the present work, however, at 72hr post-ingestion only 37.5% in teneral and 13.33% in non-teneral G.m.submorsitans had been identified. The highest rate of digestion of serum components were therefore observed after 48hr and 72hr post-feeding in non-teneral and teneral tsetse, respectively (Fig.7).

Moreover even after 120hr post ingestion 2 meals out of 6 and 1 meal out of 18 samples were identifiable in teneral and non-teneral flies, respectively.However,the relatively larger sample size used after 120hr post-feeding might have contributed much for the occurrence to one positive bloodmeal sample.Also the higher percentage of identifiable meal obtained after 120hr (33.3%) in teneral flies which is more than that of 96hr (12.5%) could be due to chance during sampling (Fig.7).

Differences observed in the rate of digestion of plasma proteins between teneral and non-teneral tsetse (G.m.submorsitans) are comparable to those reported by Weitz and Buxton(1953) and show that the distinguishing serum components of cattle are degraded faster in non-teneral than teneral tsetse given first bloodmeal. This suggests that the proteolytic enzymes are released at a faster rate in non-teneral than fed teneral tsetse. It is possible that after digestion of the first feed the release of the proteolytic enzymes (in non-teneral) is increased by stages at subsequent feeds (Rurangirwa et. al.1986).

Obviously detection time will depend not only on the size of the bloodmeal but on temperature, which is the greatest environmental factor determining digestion rates (Service et. al; 1986). The speed of digestion also varies between species of Glossina (Langley and Stafford, 1990), with their age (Weitz and Buxton, 1953), amongst individual of the same species (Fig.7), with physiological condition (Moloo, 1976) and type of blood ingested (Rurangirwa et. al. 1986). Regarding the sex factor, in the rate of digestion, significant difference between male and female G.m.submorsitans were not observed, suggesting that males and females of G.m.morsitans digest their blood meal at the same rate (Langley, 1967) which may also be extended to other species.

In the field situation it is difficult to encounter recently engorged flies in their resting places. It is more likely or possible to catch flies using traps with only little residual bloodmeal. It is thus encouraging to note that at up to 48hr post feeding 87.5% and 55.5% of host bloodmeal from fed teneral and non-teneral G.m.submorsitans, respectively, could still be identified. Although the percentage of identifiable blood meals are lower due to individual differences one can even identify the donor host of those particular tsetse species after 120hr of post-feeding if as large as possible bloodmeal samples are collected and analyzed.

It is known that the distribution and abundance of at least some Glossina spp. are closely related to the abundance and habits of wild animals. Vale (1974) suggested that availability of food-seeking flies may be inversely related to the abundance

of commonly utilized hosts. Warthog was found to be an apparently favoured or available host of G.m.submorsitans in the upper Didessa river valley. Similarly 45% feeds on warthog have been noted in a previous report (Weitz, 1963). According to the report of Snow and Borham (1979), 90% feeds taken by G.m.submorsitans in Gambia was also from Warthog.

In Northern Nigeria where wild Bovidae were relatively scarce, warthog feeds made up a greater proportion of G.morsitans feeds than in East Africa, where bovides were abundant (Jordan, et. al. 1962). Warthogs is one of the reliable hosts of tsetse (Nash, 1969) having relatively restricted home ranges in favoured tsetse habitats and being most active in the early morning and late afternoon when flies are usually most actively searching for food. Nash (1969) describes that for different species of tsetse the proportion of bloodmeals arising from different hosts may depend on the availability of host species in the locality, the reliability of the hosts habits, the attractiveness of the host's scent, the thickness and texture of host's skin and host evasive ability.

The position of warthog in the epidemiology of Nagana in Ethiopia has not been investigated, but their abundance may be important for two aspects. First, the results of the blood meal determination shows that they are major hosts of G.m.submorsitans and support large population of this species through out the upper Didessa valley from which domestic animals are normally absent. Secondly, they may be important reservoirs for trypanosomes since Trypanosoma congolense, T.vivax , T.brucei and

T.suis have all been isolated from warthog (Hoare, 1972), and 10% of infection with trypanosome parasites had also been identified in warthog (Jordan, 1986).

Although presently no data on trypanosome infection rates on warthogs in Ethiopia are available, in terms of its importance as a reservoir species reported by others, and also as to the result of blood meal analysis obtained by the present work, it is believed to be the most important host species in the epidemiology of Nagana in the locality.

Thus in the upper Didessa valley, Warthog should be taken as one of the most important maintenance hosts for G.m.submorsitans populations which, as vector of trypanosomiasis, are the major limiting factor on cattle production in the area. Warthog control is carried out in a sporadic and unsystematic way by local people to reduce crop damage and at the same time the beast is taken as edible which may be considerable. Nevertheless P.aethiopicus and G.m.submorsitans co-exist to the detriment of livestock production in the upper Didessa river valley.

Human were found to be the favoured host of the flies next to the Warthog. This has a connection with the existence of the settlers in the valley as well as the presence of the all weather road crossing the valley through which people from Bedelle to Arjo travel. Furthermore, in tsetse fed on a mixture of blood from more than one host, the two host species were also identifiable. For instance two meals of human/warthog , one meal of cattle/elephant and one meal of buffalo/giraffe were

identified by the ELISA test. This is of epidemiological interest since such double feeds have been reported from elsewhere for tsetse caught in the field (Moloo et. al; 1971).

According to the personal communication made with the local people elephant and giraffe are not found in the area. However in the present study 4.37% and 0.63% of meal of the flies were found to be taken from giraffe and elephant,respectively. This suggests that (1) since the Didessa river system has a connection with a western part of the region of Illubabor where these game are normally existing, fed tsetse flies may have migrated from this area, which might openup another field of study concerning the pattern of fly movement;(2)The game may also come closer to the upper Didessa river valley sometimes during the year so that the flies could fed upon.

As shown by Randolph and Rogers (1978), field caught flies digest blood meal slightly faster than flies in captivity.This might be the reason why large number of field collected blood meals remained unidentified which probably are well digested to react with their respective antisera.

C O N C L U S I O N

The present work attempted to standardize the best bait system for catching Glossina morsitans submorsitans. The widely used biconical,and the recently introduced NGU traps were evaluated under the specific ecological conditions. The relatively higher catch obtained by the Biconical trap using cow urine as odour attractant than the more costly chemical attractant octenol suggests an opportunity to use cow urine as odour attractant for community based tsetse management activities. As cow urine is available in large quantities at a no cost to the rural communities, tsetse control based on trapping has become potentially feasible.

A high prevalence of trypanosomiasis observed among the cattle population residing along the edge of the escarpment requires special attention since the well being of the local population is directly dependent on the productivity of their domestic animals.

Identification of the bloodmeal source of the tsetse flies is an important aspect of the epidemiology of trypanosomiasis. The ability of the ELISA test to identify the bloodmeal source up to 72hr post-feeding has crucial importance in the determination of host-tsetse interaction in animal trypanosomiasis. The ELISA bloodmeal analysis results obtained from the present work showed that warthog is the most Common, if not the favoured host of the tsetse flies in the area. Consequently it could serve as a reservoir host for the parasite. This indicated the need for

special attention that must be given to the distribution and abundance of the warthog in the Control of Nagana in the study area.

The fact that some flies were found positive for human blood indicated a potential for the transmission of human trypanosomiasis if infected individuals or the reservoir game animals are (introduced) into the region. The detection of giraffe and elephant blood in some flies bloodmeal would require further investigations with regard to the pattern and route of fly movement in the area. This is because these two wild animals are not normally seen in the Upper Didessa River Valley.

The work has also shown that a large number of tsetse flies can be trapped by using an appropriate trap and odour attractants. This is a good indication that a low-cost tsetse control strategy run by the local community based on fly trapping combined with low level of chemotherapeutic use can be instituted to control animal trypanosomiasis .

The findings of this study, in general, will contribute 1) to the epidemiology and the study of the disease dynamics of tsetse and trypanosomiasis in the area; 2) to the development of efficient trapping methodology for effective tsetse control program. 3) to mounting a tsetse control program based on the most prefered maitenance host.

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