

**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

**Assessment of Species Composition and Ecology
of Eye-Seeking Flies (Diptera: Muscidae) in
Relation to Trachoma Transmission in South
Gondar, North West Ethiopia.**

**By
Gashu Fentie**

*A Thesis Presented to the School of Graduate Studies of the Addis Ababa
University in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology*

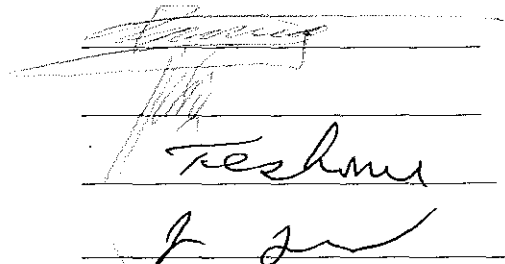
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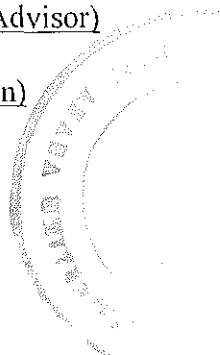
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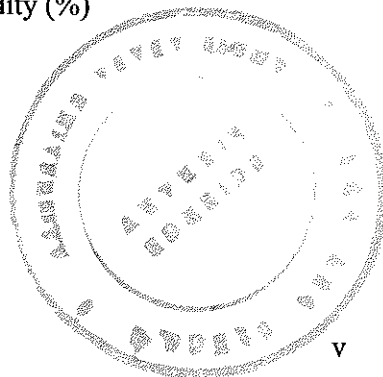
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ABSTRACT

The study was conducted in South Gondar Zone, Derra Woreda, Zara-Jigina kebele and Melefena village from December 2002 to March 2003. Forty households were selected at random from the village and one child between 2-10 years old was recruited for the study.

Attempts to quantify flies swarming the faces of children were made by simply counting and collecting flies using hepa-filter aspirator. To determine the general trend of the diurnal fly activity, the sum of the number of flies counted/collected from each child during each hour of the day was averaged and plotted against time of observation.

Data were also obtained from the heads of households for trachoma risk factor analysis using a prepared questionnaire. Different animal excreta including human faeces and house garbage were investigated to determine the breeding sites of eye-seeking flies under natural conditions in the field using funnel cage exit traps. Finally an ophthalmic nurse for the presence or absence of trachoma screened the study children by everting the upper eyelids and visually examining the tarsal plate with a torch and a 2.5 X magnifying binocular loupe. Eyes were graded according to Thylefors *et al.* (1987) simplified scale. All the trachoma positive children were given 1 % tetracycline eye ointment and health education was given to the community.

The study showed that most (about 94.5 %) of the flies captured on the faces of children were found to be *Musca sorbens*. *Musca domestica* accounted for 2.2 % and the remaining (3.3 %) were unidentified *Musca* species. The main breeding habitat for *M. sorbens* was found to be human and dog faeces. The peak density of flies on the faces of children was observed in the morning between 09:00 – 11:00 hours. Temperature and relative humidity were found to be the two important environmental factors governing the diurnal activity of flies. The highest fly density was observed in a temperature range of 23 to 27°C, and 32 to 35 % relative humidity. During the four-month study, the lowest density of flies was observed in

December 2002 and highest in February 2003. Logistic regression analysis on the risk factors of trachoma have shown that trachoma is directly related with sex, age, education, household size, number of children below the age of 10 years, cohabitation with cattle, fly density, and face drying. Seventy-five percent of the study children were infected with trachoma.

1. INTRODUCTION

It is now becoming accepted that eye-seeking flies are the mechanical vectors of trachoma and are partly responsible for blindness (Taylor, 1988; Brechner *et al.*, 1992; Emerson *et al.*, 2001). *Musca sorbens* is the most likely insect vector in The Gambia Emerson *et al.*, (2000). *M. sorbens* utilizes variety of animal excrement including human faeces for breeding Emerson *et al.*, (2001).

Muscoid flies are commonly found swarming the unclean eyes and noses of children in trachoma endemic areas (Taylor, 1988). This is an observable fact in the present study area even if the type of flies and the measure of fly density that could be related to an increased risk of trachoma is unknown. Laboratory experiments have shown that eye-seeking flies can mechanically carry and transmit *Chlamydia trachomatis* from infected guinea pigs to uninfected guinea pigs (Forsey and Darougar, 1981). The eyes of children with active trachoma are believed to be the principal reservoir of the disease in endemic areas (Emerson *et al.*, 2000).

Trachoma is the leading cause of infectious blindness worldwide and is the case in various parts of Ethiopia, including Northern Ethiopia. About six million people are blind in the world due to trachoma accounting for 15 % of blindness. Elsewhere, several studies have implicated flies as mechanical vectors of trachoma, and presumed to be the case in Ethiopia, although the Epidemiology of the disease in the country is not well studied.

South Gondar has been selected to be the study area because of the high prevalence of trachoma. A study made by Neatherlin (2001) in four 'Woredas' of South Gondar namely Derra, Iste, Ebinat and Simada, located in the lowland areas, identified as having potentially high levels of trachoma prevalence. These 'Woredas' have shown 91.9 % sign of all forms of trachoma and 66.5 % of active trachoma in the study population.

This study was carried out to provide baseline entomological information on the species of flies usually seen swarming around the faces of children, learn more on the ecology of these flies and their density and their association with trachoma in Melefena village of Zara-Jigina 'Kebele' (Derra 'Woreda') in South Gondar Zone where trachoma is highly prevalent.

1.1. Objectives of the study

The general objective of the study was to identify the species composition of eye-seeking flies, presumed vectors of trachoma and to investigate aspects of their ecology in South Gondar, Northwest Ethiopia. The specific objectives were:

- i) to study the species composition of eye-seeking flies;
- ii) to determine the density and diurnal activity of eye-seeking flies;
- iii) to study the breeding habitats of eye-seeking flies, and
- iv) to identify the risk factors of trachoma in the study area.

2. LITERATURE REVIEW

2.1. Vectors of trachoma

Trachoma transmission occurs by several routes, including flies that pass on a disease to the eyes directly, person to person from clothing used to wipe children's faces and by hand to face contact (Dolin *et al.*, 1997). From epidemiological studies, the presence of flies was one of the earliest risk factors observed (Taylor, 1988; Brechner *et al.*, 1992). Flies can be physical vectors, for the transmission of *C. trachomatis* and their control may be followed by a significant reduction in trachoma prevalence. In Tanzania (Taylor *et al.*, 1989; Brechner *et al.*, 1992) and The Gambia (Emerson *et al.*, 1999), the presence of flies on the faces of children was consistently associated with increased risk of trachoma.

The presence of cattle has been associated with trachoma in Ethiopia and Somalia (De Sole, 1987). It is argued that the accumulation of garbage and the presence of cattle inside the yard could attract and increase the abundance of flies and subsequently the prevalence of trachoma. A study in Mali by Schemann *et al.* (2002), however, showed that the presence of stables or cow ownership seems to have a protective effect.

Musca domestica Linnaeus and *Musca sorbens* Wiedemann were the most common vectors maintaining the transmission of trachoma in trachoma endemic areas. Usually the eye-fly contact in infants and preschool children, especially when there is a nasal and/or ocular discharge, has been found to be very high in hyperendemic areas. Eye-seeking flies were also seen feeding on the discharges aggressively and these discharges from children could be the main reservoirs of the infectious agent (Emerson *et al.*, 2000).

Laboratory experiments demonstrated that houseflies carried infectious *C. trachomatis* on their legs and proboscises and in their intestines for up to 6 hours (Forsey and Darougar, 1981). The frequent landing habit of flies on the face of children and the regurgitation habit of flies leads to the rapid transmission of ocular pathogens from one eye to the other.

It has been shown that the secretions of the eyes of infected persons contain the infective agent of *C. trachomatis* (Darougar *et al.*, 1979). The transmission of *C. trachomatis* by the housefly, *M. domestica* and the face fly, *M. sorbens* is possible under laboratory conditions (Forsey and Darougar, 1981). Forsey and Darougar (1981) have isolated the causative agent *C. trachomatis* from flies feeding at the eyes of children but the flies were not identified. Emerson *et al.* (1999) had shown through polymerase chain reaction (PCR) that the most likely insect vector was *M. sorbens*. These findings indicate that flies are the potential vectors involved in trachoma transmission.

Due to the high prevalence of infectious trachoma in preschool children in trachoma endemic areas, the bacteria probably are transmitted from child to child or child to care giver. Possible routes of eye to eye transmission include spread from direct contact during playing or sleeping, transmission on fingers, from contaminated handkerchiefs, towels or clothing used to wipe eyes, or from eye-seeking flies (Dolin *et al.*, 1997; Munoz and West, 1997; Emerson *et al.*, 1999; Emerson *et al.*, 2000 and Emerson *et al.*, 2001).

2.1.1. Classifications and distribution of eye-seeking flies

Musca sorbens and *M. domestica* are members of the family Muscidae (Order: Diptera) to which several other synanthropic species belong. They are usually small to medium sized (6 to 9 mm) and dull coloured with well-developed squamae. The mouthparts (proboscis) of these flies are specially adapted for sucking up fluid or semi-fluid foods

(Service, 1996). Muscid flies undergo complex metamorphosis, i.e., egg, larva (maggot), pupa and adult or fully winged insect. Under warm summer temperatures, the housefly can complete its life cycle in ten days (James and Harwood, 1969) and according to Emerson *et al.* (2001) *M. sorbens* can complete its life cycle in nine days.

Within the genus *Musca*, there are seventy-four species including sub species, according to West (1951) but only a few are synanthropic. The best studied is *M. domestica*, on which vast amounts of literature exists. Over 100 pathogens and parasites have been isolated from this species (Greenberg, 1971). At least three subspecies of *M. domestica* exist; the subspecies *M. d. domestica* occurs worldwide, but is the least abundant in Africa. The two other subspecies, *M. d. curviforceps* and *M. d. calleva* are limited to Africa (Crosskey and Lane, 1993). Of the eye-seeking *M. domestica* isolated, it is unknown to which subspecies they belong. One could suspect *M. d. calleva* because of its exophilic nature (Crosskey and Lane, 1993).

Musca sorbens is an important nuisance fly that settles around the eyes, suppurating sores, and body secretions of humans and has much the same potential to transmit pathogens as *M. domestica* (James and Harwood, 1969). They breed in human and animal faeces, are rarely found indoors, prefer light to shaded areas and are more attracted to the human body than *M. domestica* (Service, 1996). *M. sorbens* s.s. belongs to the *M. sorbens* complex, which also includes *M. biseta* and *M. vetustissima*. These species are almost morphologically indistinguishable although they are genetically distinct species. *Musca sorbens* and *M. biseta* are equally widespread in Africa and have similar habits. In Australia only one species, *M. vetustissima*, is present, but this species overlaps with *M. sorbens* in eastern Asia, Oriental region, Melanesia and Polynesia (Crosskey and Lane, 1993). In the literature, only *M.*

sorbens has been indicated in the transmission of *C. trachomatis* in Africa, but it remains unclear whether all members of the complex are potential vectors.

2.1.2. Public health importance of eye-seeking flies

Flies are major risk to health in many countries (Meegan *et al.*, 1997). *Musca domestica* has a very significant public health importance. It is both a nuisance and mechanical transmitter of diseases. Houseflies have been reported to be a mechanical carrier and reservoir of pathogenic organisms, including bacteria, viruses, protozoan cysts, and helminth eggs which cause diseases in humans (Tan *et al.*, 1997). Some of these are the causative organisms of amoebic and bacillary dysentery, typhoid fever, poliomyelitis and infectious hepatitis (James and Harwood, 1969). Among these, bacteria are the most common pathogen associated with flies (Sukontason *et al.*, 2000). A study conducted in Chiang Mai province, North Thailand on *M. domestica* showed that 61 % of specimens collected were found to carry bacteria with each harboring 1-5 bacterial species (Sukontason *et al.*, 2000).

In Malaysia, the housefly *M. domestica*, the face fly *M. sorbens*, and blowflies such as *Chrysomya megalcephala* Fabricious are commonly found feeding on or breeding in garbage and other filthy areas. Since they also infect food, they are important mechanical transmitters of pathogens causing human disease (Greenberg, 1973). The dangers of houseflies face flies and blowflies as carriers of disease are aggravated by their feeding habits. They feed on faeces, then feed on human food, and regurgitate for pre-digestion. In the process, they may also mechanically transfer pathogens, such as protozoal cysts, human helminthes, bacteria and viruses to food that is later consumed by humans (Sulaiman *et al.*, 1999).

According to Sulaiman *et al.* (1999) enteropathogenic bacteria were isolated from the body surface and gut contents of *M. domestica* indicating that *M. domestica* is involved in

mechanical transmission. Enteropathogenic bacteria occur in soil, dairy products, water, and sewage and in the intestine of man and animals. *Enterobacter agglomerans* were isolated from *M. domestica*, *M. sorbens* and *Chrysomya megaloccephala*, indicating that these fly species could be involved in disease transmission to man and animals.

The maggots of flies also cause myiasis in humans and other animals (Nazni *et al.*, 1998). Myiasis is the infestation of living human or animal tissue with fly larvae. The larvae may feed on the hosts living or dead tissue. Forms of myiasis include enteric, rectal, urogenital, auricular, cutaneous and nasopharyngeal. Larvae of houseflies have occasionally been recorded in cases of urogenital and traumatic myiasis, and more rarely in auro and nasopharyngeal myiasis. There is, however, no true intestinal myiasis (Service, 1996; Coler *et al.*, 1993).

The role of flies in disease transmission in most cases is incidental; for example, seasonal increase of fly abundance is often correlated with outbreak of diarrheal diseases (Service, 1996) and trachoma transmission (Mabey and Bailey, 1999). Watt and Lindsay (1948) and Cohen *et al.* (1991) have shown a strong correlation between filth fly populations and *Shigella* rates in humans.

One of the striking features of trachoma endemic areas is the presence of high number of muscoid flies around the eyes and noses of children especially when they are not clean (Taylor, 1988). In addition, high household density of flies has been considered a risk factor for clinical trachoma at the family level (Brechner *et al.*, 1992). A recent randomized trial using insecticide spraying to control fly populations in a village provides the best evidence that flies are important in trachoma transmission. In the intervention village, three months of insecticide spraying reduced the fly population by 75%, the number of eye-seeking flies collected on the faces of children was reduced by 96 % and the point prevalence of trachoma

in the community decreased by 61% (Emerson *et al.*, 1999). There are similar findings in acute diarrheal diseases, caused by *Shigella* and *Salmonella* (Lindsey *et al.*, 1953 cited in Crosskey and Lane, 1993).

Studies in Egypt (Hafez and Attia, 1958 cited in Emerson *et al.*, 2000) and The Gambia (Emerson *et al.*, 1999; Emerson *et al.*, 2000) have shown that *M. sorbens* is the predominant eye-seeking fly, but *M. domestica* is also present. Several laboratory experiments have provided evidence that these two species of eye-seeking flies can act as mechanical vectors of trachoma. One of the first experiments demonstrated that *M. sorbens* can rapidly spread fluorescein stained eye secretions from one person to another (Jones, 1975). *Musca domestica* can transmit *C. psittaci* (guinea pig conjunctivitis) from infected guinea pigs to uninfected ones (Taylor, 1988). In addition, the presence of *Chlamydia* DNA has been demonstrated by PCR on washes of *M. sorbens* collected from the eyes of infected children (Emerson *et al.*, 2000). These findings indicate that these flies are probably involved in trachoma transmission. *Musca domestica* is of public health importance as a proven vector of diarrhea (Watt and Lindsay, 1948; Cohen *et al.*, 1991). *Musca domestica* could potentially be a trachoma vector however; it is a generalist feeder and does not specifically seek eyes in preference to other food sources. It is doubtful whether sufficient numbers of this species would effectively transfer the trachoma organism to be an important vector of the disease. A study in The Gambia has also shown low percentage of *M. domestica*-eye contacts and no *Chlamydia* DNA was detected in the washes of eye-seeking *M. domestica* (Emerson *et al.*, 1999; Emerson *et al.*, 2000). Several species of *Musca* settle on bodily secretions and sores (Crosskey and Lane, 1993), and it is possible that other species of flies may be involved; the relative importance of each may vary according to regions. Furthermore, the efficacy of flies in transmitting infectious *Chlamydia* may depend on factors such as concentration and

infectivity of chlamydial agents in contaminated material, feeding habits of flies, exposure of *Chlamydia* to environmental factors during transmission (Forsey and Darougar, 1981).

2.2. History of trachoma problem

The earliest known documentation of trachoma dates back to the 27th century BC in China and the features of the disease have been described by ancient Egyptians and Greek physicians, including Hippocrates (West and Munoz, 1998). In the 19th and early 20th century, the disease was endemic in Europe. As a result, emigrants arriving in the U.S.A. at Ellis Island, New York, were screened for trachoma by everting and examining upper eyelid, which is very similar to the modern way of clinical diagnosis. Those with follicles, which are the characteristic of trachoma, were put on the next boat back to Europe (Mabey and Bailey, 1999). Trachoma has now disappeared from Europe and North America because of improved living standards, but unfortunately, there are many countries in the world where living standards have not improved, and do not appear likely to improve in the near future. Today trachoma is found in underprivileged communities with poor living conditions, where there is little hope of rapid economic development. Trachoma is the major cause of preventable blindness (Thylefors *et al.*, 1995) and the second leading cause of blindness worldwide (West *et al.*, 1996). The main causes of blindness in developing countries are generally associated with poverty and illiteracy and is most commonly found in rural remote areas (Tiliksew Teshome, 2002). Current estimates suggest that about 150 million people are affected in 48 countries and 6 million are blind (Mabey and Bailey, 1999).

2.2.1. The disease

2.2.1.1. Trachoma

Trachoma is an infection of the eye caused by *Chlamydia trachomatis*, which may result in blindness after repeated infections. In Africa and South East Asia, the condition is holoendemic and it is expected that all infants would be infected by the age of two years (Schachter, 1985). Usually the disease starts as an acute, self-limiting conjunctivitis, characterized by the presence of follicles on the upper tarsal conjunctivae. If repeated infections take place, chronic conjunctivitis with a more severe inflammatory response develops. Repeated infection during childhood and young adulthood can result in continued scarring of the inner eyelids. These scars, which form dense bands across the tarsal plate, can eventually result in a distortion of the eyelid, causing entropion of the lid margin, and trichiasis; the potentially sequela of trachoma. Trichiasis is defined as having at least one eyelash in contact with the globe, resulting in the painful and potentially damaging abrasion of the cornea by the inturned eyelashes. Irreversible blindness from trachoma usually occurs as a result of corneal opacification (Daniell and Taylor, 1997).

In hyperendemic areas most children acquire trachoma infection and after the age of 15 years, less than 10 % of them develop entropion and trichiasis. Trichiasis is generally a late complication of trachoma, but it is also a common phenomenon in children of some hyperendemic areas (West *et al.*, 1993).

According to Thylefors *et al.* (1987), active cases of trachoma have been classified into two stages: Trachomatous inflammation follicular (TF) characterized by the presence of five or more follicles in the upper tarsal conjunctiva (the size of follicles must be at least 0.05 mm in diameter) and trachomatous inflammation intense (TI) characterized by the development of pronounced inflammatory thickening of the tarsal conjunctiva, obscuring half of the normal deep tarsal vessels. Cicatricial trachoma is generally regarded as a complication of previous

episodes of active trachoma and tends to be seen in older people, and has been classified into three stages (Thylefors *et al.*, 1987):

- 1) Trachomatous conjunctival scarring (TS) where scars could be seen on the tarsal conjunctiva as white lines, bands or sheets in the tarsal conjunctiva;
- 2) Trachomatous trichiasis (TT) where the eye ball is rubbed at least by one eyelash or the existence of history or signs of epilation; and
- 3) Corneal opacity (CO) easily visible corneal opacity over the pupil (so dense that at least part of the pupil margin is blurred when viewed through the opacity).

In trachoma endemic areas the disease shows different patterns at different age groups. Active trachoma infections are indicators of current infection with *C. trachomatis*, which are commonly seen in children. Trachomatous follicular and intense follicular trachoma is seen in young children among which up to 60 % of them show clinical signs. After the age of 15 years, active trachoma starts to decrease progressively and conjunctival scarring begins to rise up. Most of scarred individuals will develop entropion and trichiasis which later could lead them to high risk of blinding corneal opacification (West *et al.*, 1991). Specific factors associated with the transitions from active disease to scarring and then to trichiasis are not well understood. However, the transitions takes place in most of the children who have repeated infection of active trachoma, but a smaller percentage go on to severe scarring and trichiasis and an even smaller percentage progress to blinding corneal opacity.

The association of trachoma problem and subsequent mortality in children has not yet been established, but it was suggested that severe clinical manifestations of trachoma aggravate the death of young children through other infectious processes. In addition, severe inflammatory trachoma could be an indicator of poor socio-economic status (Hsieh *et al.*, 2000). High mortality among very young children in sub-Saharan countries is still a very

important health problem. Factors associated with childhood mortality, such as socio-economic status and poor water sources, are also related to the presence of trachoma (Grayston *et al.*, 1985). Blindness from trachoma is linked to poverty and is more likely to occur in women than men (West *et al.*, 1991). It causes disability, dependency, and poverty and is a barrier to development.

2.2.1.2. The pathogen: *Chlamydia*

Chlamydia trachomatis, the causative agent of trachoma, is an obligate intracellular bacterium with no known animal reservoir. *Chlamydia trachomatis* can be further separated into several serovars. The primary serovars responsible for trachoma are A, B, Ba and C. The serovars D-K are associated with genital infections, and L1-L3 are the lymphogranuloma venereum serovars (West and Munoz, 1998).

Chlamydia has a unique developmental cycle distinguished by two specialized forms. The elementary body is the metabolically inert, but infectious particle, which, through endocytosis, infects susceptible host cells. Endocytosis is followed by transformation of the elementary body into a reticulate body, which is metabolically active and multiplies rapidly over the next 15 hours. The reticulate bodies are non infectious, and is unable to survive in extra cellular environments; but approximately 20 hours after infection, they divide by binary fission and transform into elementary bodies. The cell then dies and the elementary bodies are released to initiate infection in other susceptible cells. The reticulate bodies and the elementary bodies are enclosed in an inclusion body, which can occupy up to 90% of the cell cytoplasm (West and Munoz, 1998). With rupture of the cell, infection of other cells by the newly transformed elementary bodies starts. The trachoma serovars target columnar and squamo columnar epithelial cells, and thus the conjunctiva, genital, respiratory and intestinal tissue can become infected. Characteristics of the developmental cycle of *Chlamydia* may

well explain some features of the infection, such as its possible persistence (West and Munoz, 1998). *Chlamydia trachomatis* is not only an infectious agent of the eye, but also it is an important sexually transmitted disease causing infertility both in developing and developed countries (Schachter, 1985).

2.2.1.3. Geographical distribution

Trachoma is found mainly in remote rural areas of most African countries, in some Eastern Mediterranean countries and in certain parts of Central and South America and several Asian countries (Negrel and Mariotti, 1998). Current estimates suggest that about 150 million people have active infection, most of which are children (Dolin *et al.*, 1997). It has been estimated that as many as 500 million people are at risk of active trachoma infection (Thylefors *et al.*, 1995). As stated earlier, worldwide, there are about 6 million visually disabled people (Thylefors *et al.*, 1995) and about 10 million people have trichiasis and are at risk of blindness (WHO 1997 cited in Bowman *et al.*, 2001).

Most of the world's blind people live in developing countries (Potter, 1991; Thylefors *et al.*, 1995). Cataract is the leading causes of blindness in the world (Budden, 1981; Wondu Alemayehu *et al.*, 1995). The majority of blindness in developing countries is due to either preventable or curable causes (Thylefors *et al.*, 1995).

In some regions, trachoma specific control programmes have led to large reduction in the prevalence of the disease. However, in most developing countries, especially in sub-Saharan countries, trachoma continues to be a major public health problem as these countries do not have the advantage of good living standards or effective trachoma control programmes (Dolin *et al.*, 1997).

The overall prevalence of active trachoma varies widely from region to region, but the age distribution is fairly equal in both sexes in children while this distribution changes at old ages and the severe forms such as trichiasis develop almost four times greater in women than men (West *et al.*, 1991).

2.2.2. Risk factors in the transmission of trachoma

Trachoma is one of the priority diseases targeted by the WHO. The alliance for the Global Elimination of Trachoma has been established with the goal of eliminating blinding trachoma before the year 2020 (GET 2020) Mabey and Bailey (1999). For the success of this goal WHO has recommended the SAFE strategy which is composed of Surgery for trichiasis; Antibiotics for the treatment of active (inflammatory) trachoma and for the elimination of the reservoir of infection; Facial cleanliness to reduce transmission between children and Environmental improvement to reduce fly population (Mabey and Bailey, 1999). In order to design more effective activities to address particularly the F and E components of the SAFE strategy, understanding the risk factors for the disease has been considered very important (Schemann *et al.*, 2002). Many studies have produced important data on the distribution and transmission of trachoma, but the specific risk factors for blinding trachoma are still not adequately understood. For instance, the relationship between availability of water and its use for children's hygiene appears to be complex, and is true also for other environmental or socio-economic factor (Prost and Negrel, 1989; Bailey *et al.*, 1991).

Important risk factors for trachoma include lack of facial cleanliness, poor access to water supplies, lack of latrines and a high number of flies (Taylor *et al.*, 1989; Courtright *et al.*, 1991). The disease is known to occur within households and family groups. The prevalence is disproportionately high in women and children, and people in poor rural communities (Dolin *et al.*, 1997).

An improved access to water source had shown a protective effect on trachoma in Mali (Tielsch *et al.*, 1988), while Negussie Zerihun (1997) showed a decreased prevalence of trachoma with increasing distance of water source from the household in Ethiopia. People living within 15 minutes walking distance had the highest trachoma prevalence. Hence, people living closer to the water source may not necessarily have better utilization than those living further away. Another study in Tanzania had non-significant result (West *et al.*, 1996).

West *et al.* (1989) showed an association between prevalence of trachoma and distance to the water source. However, the estimated amount of water brought into the household did not appear to influence either the prevalence of trachoma or the prevalence of unclean faces, and it was suggested that a behavioural factor influencing water use was implicated. It is an advantage to have a water source in the household whatever the nature of this source, traditional well or piped water (Schemann *et al.*, 2002). In Brazil Luna *et al.* (1992) found a large reduction in trachoma prevalence when water was piped into the house. Quality of water does not appear to be a determinant factor. However, a study in Mali showed higher prevalence among children using unclean water and this was explained by the greater distance to the source rather than the quality (Schemann *et al.*, 2002).

Pit latrines have been shown to be protective against trachoma, at household level, in risk factor analysis in Egypt (Courtright *et al.*, 1991), Malawi (Tielsch *et al.*, 1988), Ethiopia (Negussie Zerihun, 1997) but not in Tanzania (Taylor *et al.*, 1989). Collection of human faecal material into pit latrines may reduce the density of *M. sorbens* because they like to breed in faecal materials on the ground and have not been found to breed in latrines where the faecal material rapidly liquefies (Emerson *et al.*, 2000). Although Schemann *et al.* (2002) failed to demonstrate a harmful effect from lack of latrines; data from the majority of studies

strongly suggest that emphasis should be given to the use of latrine along with the making latrines available.

Garbage collection made in the households of Ethiopia had reduced trachoma prevalence (Luna *et al.*, 1992). Schemann *et al.* (2002) in Mali showed no differences in trachoma prevalence between households with or without garbage. A number of studies have shown that a decreased fly score on the faces of children resulted in a decreased effect on trachoma prevalence (Brechner *et al.*, 1992; Taylor, 1988; West *et al.*, 1991). In addition facial cleanliness has been associated inversely with the prevalence of trachoma in children (Taylor *et al.*, 1989; West *et al.*, 1991; 1995; 1996). Nevertheless, the frequency of face washing was not significantly associated with trachoma prevalence (Tielsch *et al.*, 1988; Luna *et al.*, 1992). In addition, receiving at least one bath per a day had shown a significant protective effect (West *et al.*, 1995)

Crowded living conditions increase risk of trachoma. However, the prevalence of active (TF/TI) or intense (TI) trachoma was not associated with the number of children within a household indicating that trachoma may be more prevalent in the smallest household units. This could be due to accidental contact with an infected person (Schemann *et al.*, 2002) when the room is smaller.

Active inflammatory disease peaks in preschool children (3-5 years old). Prevalence and duration of trachoma in children appears to decrease with age (West *et al.*, 1991; Courtright *et al.*, 1989). Reasons for this decline are not well understood. There may be less exposure to the pathogen with increasing age because of improved hygiene and less exposure to young children (Hsieh *et al.*, 2000).

Trachoma is not uniformly distributed over a given area. A study has shown a pronounced clustering at the sub village level (West *et al.*, 1991). It has been also found finer clustering within the households and particularly within bedrooms (Bailey *et al.*, 1989).

2.2.3. Control of eye-seeking flies and trachoma

2.2.3.1. Environmental and personal hygiene improvement

Removal of human faeces from the environment, through the provision of basic sanitation, is the most reliable and sustainable way to reduce fly density, and this again reduces fly-eye contact and hence trachoma transmission. But the presence of other animal faeces is a threat as *M. sorbens* have alternative breeding sources (Emerson *et al.*, 2001). The breeding habitat for *M. sorbens* was found to be the faeces of different animals, but the best larval medium preferred by this species was human faeces (Emerson *et al.*, 2001; Hafez and Attia, 1958 cited in Emerson *et al.*, 2001). In addition, *M. sorbens* uses only human excreta available on the ground and any of the immature stage have not been found to emerge from latrine as a result adults have not been collected emerging from latrines (Curtis and Hawkins, 1982). Since latrines effectively remove the larval habitat from the environment, they may be a suitable method to control *M. sorbens* and hence reduce trachoma transmission. To bring an observable and tangible change at wider range it is preferable to construct latrines at the community level rather than focal household coverage since this would have more profound impact on the density of the apparent vector. Providing latrines to communities where they do not have existed previously without creating awareness ahead of time may not have a powerful effect in reducing faecal contamination of the environment.

Several methods are being employed for the control of housefly. It can be managed to some extent by sanitation measures that reduce the accumulation of organic wastes that are

serving as suitable breeding sites. Peck and Anderson (1970) have suggested mechanical removal of the livestock dung to control houseflies.

Water availability and improved hygiene is associated with lower rates of trachoma (Thylefors, 1999). In Mozambique, the prevalence of trachoma in a village without a water supply showed twice more prevalence of trachoma than a nearby village with water (Cairncross and Cliff, 1987). Epidemiological study on eye infection found that access to water was significantly associated with a lower rate of active trachoma (Prost and Negrel, 1989). Environmental sanitation intervention program in The Gambia had showed strong evidence that facial cleanliness and sanitation are the two important tools to reduce blinding trachoma (Pruss and Mariotti, 2000). A longitudinal study in The Gambia had showed a dramatic fall in trachoma prevalence through improvements in sanitation, water supply, education and access to health care in the village without any trachoma specific interventions (Dolin *et al.*, 1997).

Active trachoma has been associated with unclean faces in Tanzanian preschool children (Taylor *et al.*, 1989). In the same study, the use of handkerchief and towel appeared to be protective against trachoma. Studies from Brazil (Luna *et al.*, 1992) and Malawi (Tielsch *et al.*, 1988) failed to show an association between frequency of face washing and prevalence of active trachoma, but these were based on self reporting and may have been biased.

Trachoma was once wide spread throughout the world but disappeared from many countries before the development of antibiotics, as a result of general improvements made on sanitation (Schachter and Dawson, 1981 cited in Lynch *et al.*, 1994). This indicates that trachoma may be prevented by bringing behavioral changes in personal hygiene and general improvement on sanitation without waiting for enormous economic development so that it is

possible to reduce blindness and disabilities through sanitation measures (West and Taylor, 1988).

Despite the fact that antibiotics known to be effective against trachoma have been used widely during the last half century, they have not been uniformly effective in reducing the magnitude of the disease. Studies from The Gambia have shown that provision of medical services and antibiotics alone have little impact on trachoma in the community (Mabey *et al.*, 1991). In central Tanzania treatment with 1% tetracycline coupled with intensive educational intervention to encourage face washing have significantly reduced the prevalence of intense trachoma compared to treatment alone (West *et al.*, 1994). The availability of effective oral treatment combined with fly control; community mobilization, health education, and provision of surgical services could have a major impact on the prevalence of trachoma (Mabey and Bailey, 1999). As early as 1920 recommendations have been made for a multi-level approach to control trachoma that included fly controls (Elliot, 1920 cited in West and Munoz, 1998). According to West and Munoz (1998) the best approach to control trachoma is to treat the disease as a disease of the entire community rather than considering it as a series of cases.

Thylefors (1996) identified four major constraints to trachoma control in the course of a review held on international efforts to eliminate the disease as a public health problem. These include lack of socio-economic development in some parts of the world, poor patient compliance with antibiotic eye ointment, lack of, and reluctance to access services for trichiasis and cost of providing services to isolated communities. A major problem in the use of tetracycline eye ointment for active trachoma in the community appears to be the expected long duration of treatment (Bailey *et al.*, 1993), physical handling of the ointment and lack of mental connection between childhood eye infection and blindness (Ajewole *et al.*, 2001),

suggesting that health education is very crucial before the provision of antibiotics within the community. It has been also indicated that poor compliance and cost has also prevented the wide spread use of tetracycline.

The identification of feasible and effective methods for trachoma control led the WHO to launch, a new initiative in 1997 for the global elimination of trachoma by the year 2020 based on the "SAFE" strategy (GET 2020). Thus the global alliance has adopted the "SAFE" strategy consisting of four components to give a combined medical, behavioral and environmental approach.

For valid operational reasons, national control programmes may put initial emphasis, on the S and A part of the strategy. However, the ultimate sustainability of trachoma control relies on reducing the frequency of infection in addition to the provision of surgery and medication (Emerson *et al.*, 2000).

2.2.3.2. Chemical control of eye-seeking flies

Pyrethroid chemical groups have promising results in controlling flies. According to Nazni *et al.* (1998) lambdacyhalothrin and bendiocarp seems to be the most effective insecticides for fly control. Lim and Visvalingan (1990), also showed that lambdacyhalothrin can be used at extremely low dosage in thermal fogging for effective control of houseflies and mosquitoes. Similarly, bendiocarp is effective for fly control but it is not widely used in public health due to its toxic effects to humans (Kenaga and Morgan, 1978 cited in Nazni *et al.*, 1998).

The use of chemical insecticides provides the most effective control of flies within a short period of time (Bull and Xu, 1995). However, resistance to nearly all major groups of insecticides especially by houseflies has been an increasing problem worldwide (Nazni *et al.*,

1998; Scott *et al.*, 1989). Hence, it is important to reevaluate the susceptibility status of flies to insecticides before control measures can be undertaken.

According to Freeman and Pinniger (1992), because of the ineffectiveness of contact insecticides due to resistance, there is an increasing interest in the use of toxic baits to control flies. Despite the high utilization of baits, the levels of *M. domestica* resistance to active ingredients remained relatively low in the U.K. for some years indicating that baits are more effective in delaying development of resistance (Barson, 1987).

There is a need to maintain the efficacy of baits and this will depend on their ability to attract flies from distant area to take up a dose, which is sufficient to cause knock down and kill. Most housefly baits therefore have some form of attractant and arrestant, such as food, colour, or odour to improve their efficiency (Freeman and Pinniger, 1992). The effectiveness of such attractants is dependent on the right location of baits in relation to the surrounding environment. Barson (1987) found suspended baits were less efficient than baits attached to the wall/floor junction. The mobility of the flies was considered to be important, since flies with a reduced tendency to fly did not come into contact with suspended bait.

Because of the development of resistance, effect on non-target insects, cost and sustainability problems, fly control based on insecticide spray is not a suitable option as a long-term community based control measure; hence pesticides should be used only as a backup to sanitation and exclusion.

2.2.3.3. Biological control of eye-seeking flies

The releases of Hymenopteran parasitoids such as *Pteromalid* species have been considered to be important biological control agents (Morgan *et al.*, 1981; Patterson and Rutz, 1986). Activities of these parasitoids have been evaluated through numerous surveys and

parasitoid release studies, but lack of sufficient detailed data on many aspects of their biology and behavior hinder their use as biological control agents (Mann *et al.*, 1990).

The parasitic wasps *Spalangia nigroaenea* and *Muscidifurax zaraptor* have been demonstrated penetrating the deeper layers of *M. domestica* breeding habitat and parasitizing the pupae. The adaptability of these two parasitoid species in the stable has been found acceptable and *S. nigroaenea* showed greater migration activity after it has been released to the stable (Muska, 1992)

Nine species of parasitoids were found parasitizing the pupae of filth flies breeding in refuse dumps and poultry farms in Malaysia. *Spalangia* were the most common. The parasitized fly hosts included *M. domestica*, *Chrysomya megalocephala*, *Fannia* species and *Ophyra* species. *Spalangia endius* has been the dominant parasitoid attacking *M. domestica* in refuse dumps and poultry farms (Sulaiman *et al.*, 1990).

Two commercial formulations of *Bacillus thurengiensis* as a wettable powder and a liquid concentrate have resulted in a significant reduction in maggot numbers, pupal development and adult emergence in faeces. The liquid concentrate has shown a higher larvicidal effect than dry powder formulation (Labib and Rady, 2001). In addition contaminated faeces from *Bacillus thurengiensis* orally fed chickens have shown a significant toxic effect to the breeding maggots, reaching maximum larvicidal activity till the 4th day post feeding faeces and continued in less activity till the 6th day. This indicates the importance of adding *Bacillus thurengiensis* in chicken food in poultry breeding for housefly control (Labib and Rady, 2001).

Toxic isolates have also been discovered from *Bacillus thurengiensis* for controlling the housefly *M. domestica* as well as other Diptera and Lepidoptera. Crystal delta-endotoxins

have been purified from this isolate and have been effective in killing 50 % of *Musca* larvae at 10.2 microgramms/ml concentrations (Hodgman *et al.*, 1993).

Laboratory evaluation of six species of entomopathogenic fungi for the control of adult and larval stages of the housefly *M. domestica* have shown that *Metarhizium anisopliae* and *Tolypodadium cylindrosporium* were the most virulent, pathogenic to the larvae of *M. domestica*. Adults were susceptible to aqueous suspensions of conidia of all species tested; however; *M. anisopliae* was the most pathogenic resulting in 100 % mortality in 6 days (Barson *et al.*, 1994). In addition linseed and soya been oil have been found to be the most effective carriers giving a complete mortality within 3 days, reducing the time by half compared to the equivalent aqueous suspensions of conidia (Barson *et al.*, 1994).

Biological control organisms are usually effective only in one or a few breeding habitats, and their activities are limited under certain climatic conditions. For example, the effect of natural enemies on houseflies breeding in garbage is very low as compared to those breeding in accumulated animal excrement (Legner *et al.*, 1974). The main purpose of biological control is to locate and establish natural enemies performing effectively in different breeding habitats.

2.3. The status of trachoma in Ethiopia

Trachoma is endemic in Ethiopia (Negussie Zerihun, 1997). About 350,000 people have lost their vision due to trachoma. Around 10 million children have active trachoma and about 1 million trichiasis patients are looking for the lid surgery. It was also found that 56.8% of the total minor operations performed in 1994 were for trichiasis corrections, indicating the public health significance of trachoma in Ethiopia (Foster, 1986; MOH, 1996; Spath, 1999; Tewodros Assefa *et al.*, 2001). Both active and cicatricial trachoma were significantly

associated with the female gender, living in rural areas, where parental illiteracy and absence of latrine predominates (Negussie Zerihun, 1997).

Trachoma associated lesions are major causes of blindness in Ethiopia (Wondu Alemayehu and Assefa Cherinet, 1993; Tiliksew Teshome, 2002) while cataract accounted only 11.4 % (Tiliksew Teshome, 2002). Trachoma was also the major cause of ocular morbidity in school children in southern Ethiopia (Yoseph Worku and Samson Bayu, 2002). According to Budden (1981) the main causes of blindness in Ethiopia was found to be trachoma (42 %) and cataract (29 %). A similar study in Butajira (Ethiopia) found trachoma to be the leading cause of blindness accounting for 20-35% of cases and showed an overall blindness prevalence of 1.1 % (Wondu Alemayehu *et al.*, 1995). However, hospital statistics showed cataract as the commonest cause of blindness, demonstrating the selective nature of hospital based eye patient populations (Budden, 1981).

Of the total people who responded to questions about the circumstances related to their blindness, in a cross sectional community based survey, the majority (47.8 %) did not know the conditions related to their blindness. About 17.4 % related their blindness to inturning of eyelashes, 23.5 % to eye infection, and 5.2 % to trachoma (Tiliksew Teshome, 2002).

The estimated prevalence of blindness in Ethiopia is 1.2% - 1.5% (approximately 720,000-900,000 people). Thus, the World Health Organization and other international organizations have identified Ethiopia as one of 16 priority countries targeted for trachoma control activities (Spath, 1999).

An epidemiological study of trachoma in rural highland communities in Gondar region showed hyperendemicity (Wondu Alemayehu and Assefa Cherinet, 1993). The study also reported the highest prevalence in Ethiopia in children less than 10 years of age group.

Preliminary studies have shown that four districts (Woredas) of South Gondar Zone have a very high prevalence of trachoma (95.5% in Ebinat, 93.7% in Iste, 89.7% in Derra and 88.6% in Simada) Neatherlin (2001). About sixty seven percent of the study population had active trachoma and among children 1-10 years old, the prevalence was 87.9 % (Neatherlin, 2001). According to WHO, a prevalence rate of 20 % TF/TI or a prevalence of more than 5 % TI in children below the age of 10 years has been considered a severe health risk (Neatherlin, 2001). Thus, in the South Gondar region, the problem is more than serious and has been targeted for immediate and long-term intervention activities for trachoma control.

3. MATERIALS AND METHODS

3.1. Study area

The study took place in Amhara National Regional State, South Gondar Zone, Derra 'Woreda' (district). South Gondar Zone is one of the eleven Administrative Zones of the Region. It is divided into ten administrative districts and has a total area of 14,338 sq. kms, with an estimated population of 2,095,018 (Amhara National Regional State Plan and Economy, 2002). Of these, about 90% of the population lives in rural areas where subsistent farming is the main means of livelihood. South Gondar Zone includes both lowland and highland regions.

Derra 'Woreda' is located 35 km from Bahir-Dar, the capital of the region and has a total surface area of 1590.78 hectare. About 95 % of the area is in the lowland with 29 kebeles where 26 of them are rural and has total population of 248,652. The health coverage of the 'Woreda' is 34.9 % with one health center, five health stations, and six health posts (Derra Woreda Health office). According to Neatherlin (2001) the prevalence of TF/TI was 81.2 % among children between the ages of 1-10 years. The areas in which the studies were carried out ranges from 1880 meters to 1900 meters above sea level.

There are two main seasons. The rainy season is short ranging from June to September while the rest are dry seasons. The study was done for four months from December 2002 to March 2003.

3.2. Selection of sampling site

The Zara-Jigna 'Kebele' in the Derra 'Woreda' was selected based on its accessibility. Different parts of the 'Kebele' were surveyed together with a health worker (a sanitarian in

the nearest health station), and then selected Melefena village as representative of the 'Kebele' where houses were relatively less scattered and children were observed with flies swarming around their eyes. This selection was made since the nature of the study requires continuous observation.

3.3. Assessment of prevalence of trachoma and its possible risk factors

Forty scattered households were selected at random from Melefena village for the study. Forty children (21 males and 19 females) between the ages of 2-10 years old were selected at random, one from each household for the study of trachoma prevalence and potential risk factors of trachoma such as eye secretion, fly density educational status, waste disposal, age, sex, etc.

For the prevalence of trachoma, the study subjects (40 children) were screened by an ophthalmic nurse for the presence or absence of trachoma by everting the upper eyelids and examining the tarsal plate using 2.5 X magnifying binocular loupe and a torch. Eyes were graded according to Thylefors *et al.* (1987) simplified scale. All the trachoma positive children were given 1 % tetracycline eye ointment and health education was given to the community. For socio-demographic risk factors other than fly density, data were obtained from the head of the households for trachoma risk factor analysis. Such data were collected using a prepared questionnaire (see Appendix II).

3.4. Assessment of population density and diurnal activity of flies

In the selected village, the density of eye-seeking flies was determined weekly (for four months, 40 children) by scoring the number of flies landing on the faces of 10 children. The average number of total flies counted from the faces of children was plotted against the weeks

of the study period. For each point in the graph, the sum of the fly counts for each day (three one minute counts X 12 hours) for each of 10 children were averaged and the precisions were represented with confidence intervals.

For this purpose, two field assistants were recruited in the study village and were trained for two days in the field before commencing the entomological study. The number of flies landing on the face of each child was counted every week at one-minute intervals for three minutes every hour between 06:00 and 18:00 hours. At the end of each minute flies were waved away with a notebook and landing flies were then counted. The average densities of eye-seeking flies were calculated as flies per person per minute (Brechner *et al.*, 1992). The counts were performed outside the house of each child. Finally the average number of flies counted on each child for a total of three minutes was plotted against the hours of the day.

During the diurnal fly count and collection of flies every week during the study period (December 2002 –March 2003), air temperature (minimum and maximum, °C) and relative humidity (RH) were measured using a digital thermometer/hygrometer (Fisher Scientific). Readings were made every hour from 06:00 to 18:00 hours. The average of the maximum and minimum readings was calculated for both variables for each observation hour of the study period.

3.5. Determination of the species composition of eye-seeking flies

To determine the species composition of eye-seeking flies, flies were sampled using hepa filter aspirator (Fig. 1) from the faces of children (Emerson *et al.*, 1999; 2000). This aspirator is made up of a hollow rubber tube. One end of the tube is transparent and is used to pick flies from the study subject and the other end is non transparent but flexible and is used for sucking by the collector. At the middle, a filter possessing globular structure measuring

5.5 cm in diameter prevents flies and other particles passing to the collector. The transparent part is 10 cm in diameter and the other end is 0.7 cm.

Flies were collected on a second day of the week from the same ten children in the same village as for the assessment of the density and diurnal activity of flies. Collections were carried out hourly from 6:00 to 18:00 hours. During collections a child was allowed to sit outside the house and the flies that touched the eyes were collected for 5 minutes every hour using the aspirator. The tip of the aspirator was rinsed with ethanol after collection of flies from each child. All collected flies were transferred to holding tubes and killed with chloroform. The dead flies were further transferred to labeled micro-tubes for later identification. Anhydrous CaSO₄ (silica gel) was added to the micro tubes to absorb the moisture from the flies. In addition, the presence of ocular and nasal discharge record has been made. Identification was done using a dissecting microscope and a taxonomic key (Crosskey and Lane, 1993). Their sexes were also determined based on the position of the compound eyes (dichoptic in females, and holoptic in males).

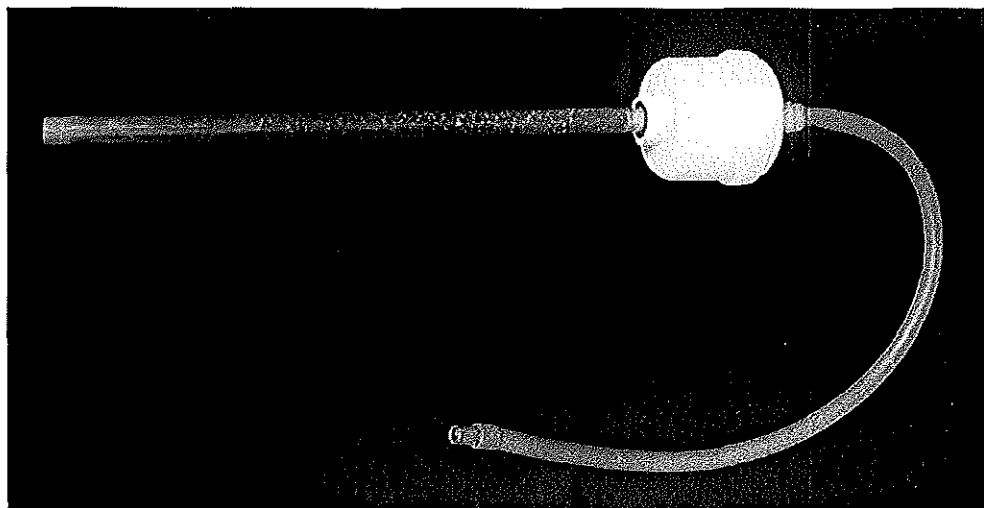


Fig. 1. Hepa filter aspirator.

3.6. Investigation on the breeding habitat of eye-seeking flies

Different animal excreta including human faeces and house garbage were investigated to determine the breeding sites of eye-seeking flies under natural conditions in the field. Funnel cage exit traps (Fig. 2) were deployed on the faecal materials of different animals. The traps were white in colour, and have a conical shape. It is open both on the upper and lower surface. The upper side has a string, which is used to knot when the trap is deployed. This is to prevent the emerging flies from escaping out of the trap. The string is also used to attach it with a stand so that it will not collapse while deployed. During fly collection, one can untie and insert aspirator to remove flies. The lower side is much wider than the upper side and a circular wire is attached on the lower side to make it heavier so as to fit with the ground during deploying the traps. It has a diameter of 90 cm on the lower side while it is 35 cm in the upper side. On the average, the height of the trap is 1-meter.

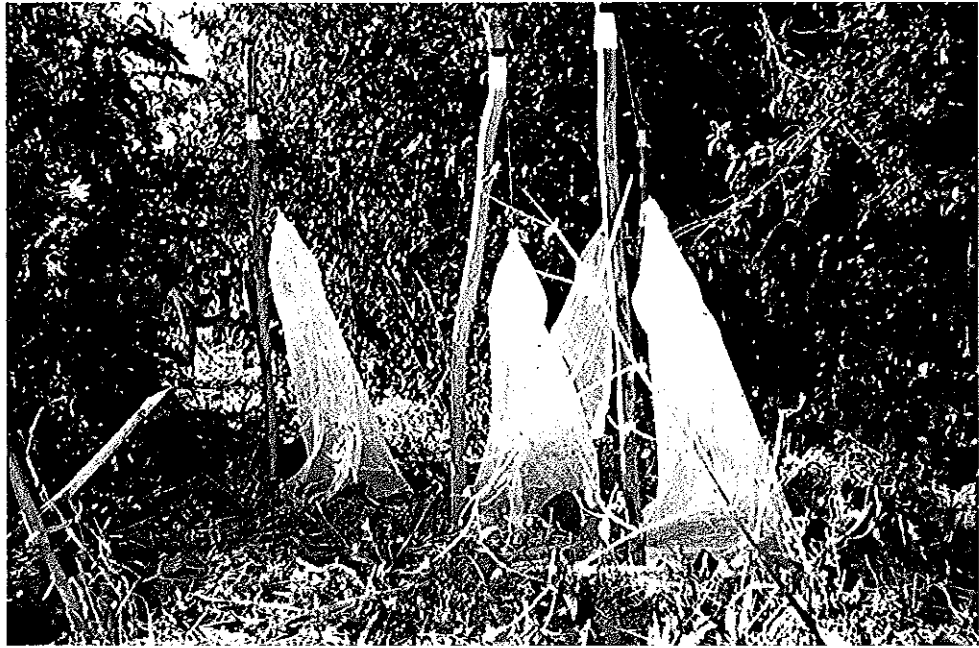


Fig. 2. Conical exit traps.

A selected area was fenced to prevent animals affecting the traps. Villagers were asked to defecate in an assigned spot in the testing area after 6:00 in the evening. Early the following morning, the cow, calf, goat and donkey faeces were collected from homes or fenced enclosures and placed in the testing area. Homes and fenced enclosures were cleaned daily, therefore the animal dropping were from the previous evening. One free ground was used as a control for each trial to justify that the soil did not contain fly pupae. On the next day around 18:00 hours in the evening after the faecal materials have been exposed to fly contact for one day, the conical exit traps were placed over the faecal materials. Three replicates of each potential breeding material was run in each trial. Three trials were done but the last trial failed because ants ate the immature stages. For each replicate a code number was written on the traps and on the stand used to deploy the traps. Each faecal matter was covered with a numbered conical exit trap and, every trap used for the particular type of sample was deployed consecutively. Traps were observed daily. All flies emerging from the different

faecal materials were caught using a hepa-filter aspirator and killed with chloroform in holding tubes. Later all flies were identified using a dissecting microscope and transferred to labeled micro- tubes (Emerson *et al.*, 2001).

3.7. Statistical analysis

All data collected were entered into Microsoft Excel 2000 to compute the average of species composition; fly density and diurnal activity of flies. Multiple logistic regression was used to analyze the risk factors of trachoma using SPSS version 10.0 windows. Association of the different risk factors with trachoma was considered at 10% level of significance. SAS (1991) statistical computer software was used to plot the graphs for diurnal activity of flies and weekly fly count variation; and the relation of temperature and relative humidity with the diurnal activity of flies. Comparisons of fly production from different breeding habitat were done using Kruskal-Wallis Several Independent Test and Wilcoxon - Mann-Whitney Test following the methods described in Conover (1980) and using SPSS (1999) statistical computer software at 5% level of significance and exact P-values of the computer out put were used.

4. RESULTS

4.1. Fly counts, density and species composition (identification)

During the study period (December 2002 - March 2003) and based on the weekly observation, a total of 26,673 flies were counted on the eyes of children in the study village (Table 1). The number of flies counted showed variations in each month, the lowest being recorded in December 2002 and the highest in February 2003. Expressed as flies per child per minute or per hour, it can be seen that the man-fly contact was also lowest in December 2002 and highest in February 2003 (Table 1). The average fly density for the study period was 4.63 flies per child per minute or 278.1 per person per hour.

Based on actual collection of flies and their identification, a total of 13,386 flies were collected from the faces of children and were identified (Table 2). Among all flies collected, 12,650 (94.5 %) were identified as *M. sorbens* based on the presence of two broad and dark longitudinal stripes on the scutum. The other 2.2 % were identified as *M. domestica* based on the presence of four dark longitudinal stripes on the scutum and the remaining flies collected were muscid flies that were not identified (3.3%). Female *M. sorbens* were more commonly caught feeding from the eyes of children than males (female to male ratio was 2.5:1) and all the collected *M. domestica* were females only.

Table 1. Density of eye-seeking flies in children in Melefena village, Zara- Jigina kebele
(December 2002 – March 2003).

No	Parameter	December 2002	January 2003	February 2003	March 2003	Overall
1	Total number of flies counted / 10 children	4782	7452	8376	6063	26673
2	Average density of flies/child/minute	3.32	5.2	5.81	4.21	4.63
3	Average density of flies/child/hour	199.2	312	348.6	252.6	278.1

Table 2. Number of flies captured from children in Melefena village, Zara-Jigina Kebele
(December 2002 – March 2003).

Species	Month	Male	Female	Total
<i>Musca sorbens</i>	December	555	1695	2250
	January	1329	2600	3929
	February	1017	2346	3363
	March	719	2389	3108
	Total	3620	9030	12650
<i>Musca domestica</i>	December	0	161	161
	January	0	77	77
	February	0	11	11
	March	0	46	46
	Total	0	295	295
Unidentified <i>Musca</i> species	December	21	50	71
	January	60	113	173
	February	60	83	143
	March	10	44	54
	Total	151	290	441
	Grand total	3771	9615	13386

The number of flies collected varied throughout the study period. The highest number of flies was collected in January 2003 although the highest counts were observed in February and the lowest numbers of flies were observed in December 2002 (Tables 1 and 2). Weekly counts of flies in December 2002 remained low and a sharp increase was noted in the first

week of January when a peak number of flies were observed. As slight decline was noted in next two weeks with the highest peak observed in the second week of February 2003. The numbers decreased until the second week of March 2003 when the numbers began to gradually increase until the end of March (Fig. 3).

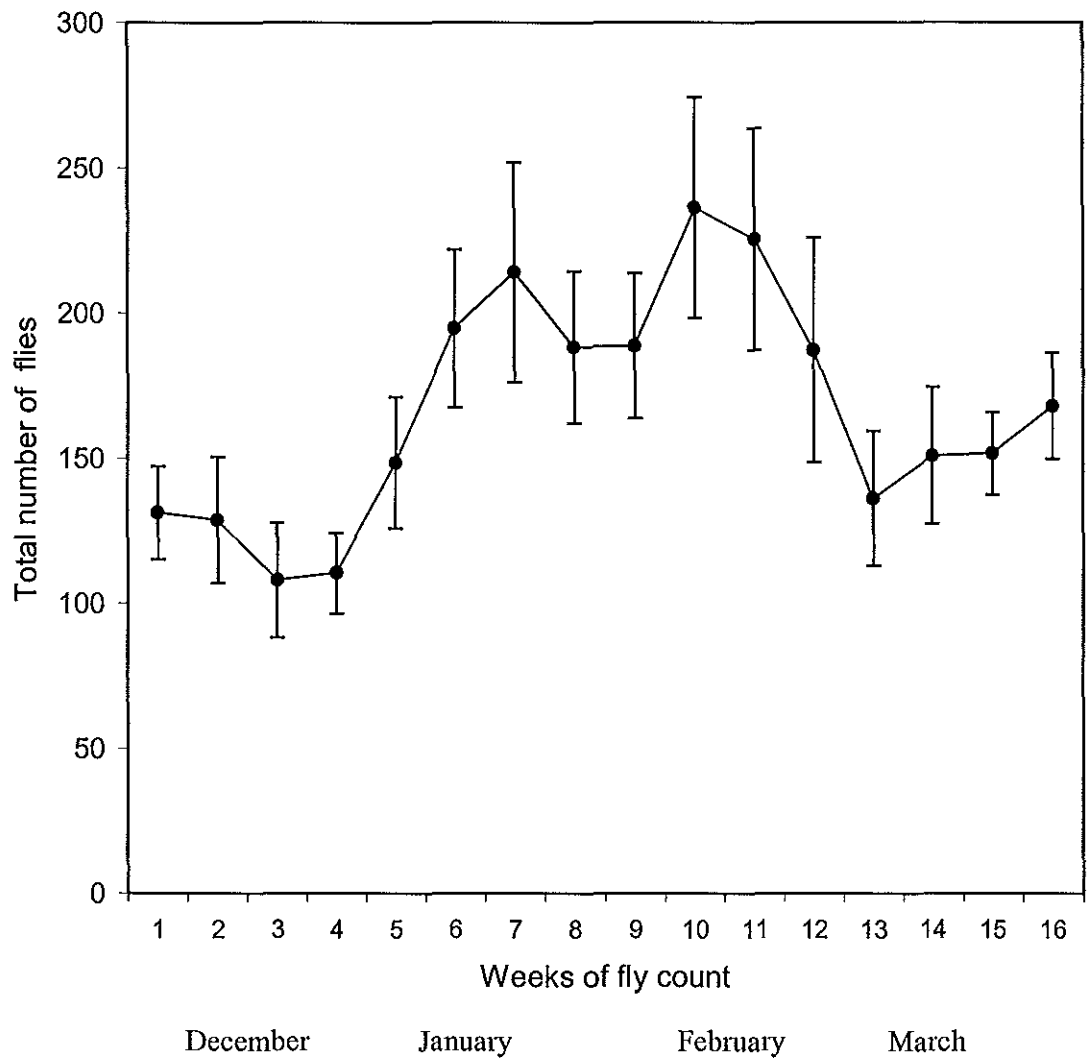


Fig. 3. Average number of total flies counted on the faces of children for each week of the study period (December 2002- March 2003).

A very similar trend was observed by *M. sorbens* collected from the faces. Peak numbers were observed in the third week of January and second week of February (Fig. 4). However, *M. domestica* showed a different trend with the highest numbers observed in the first week of December followed by a progressive decrease in all weeks of the month except a slight increase in the first week of March (Fig. 5). The unidentified *Musca* species showed almost a similar trend to the total fly count (Fig. 6).

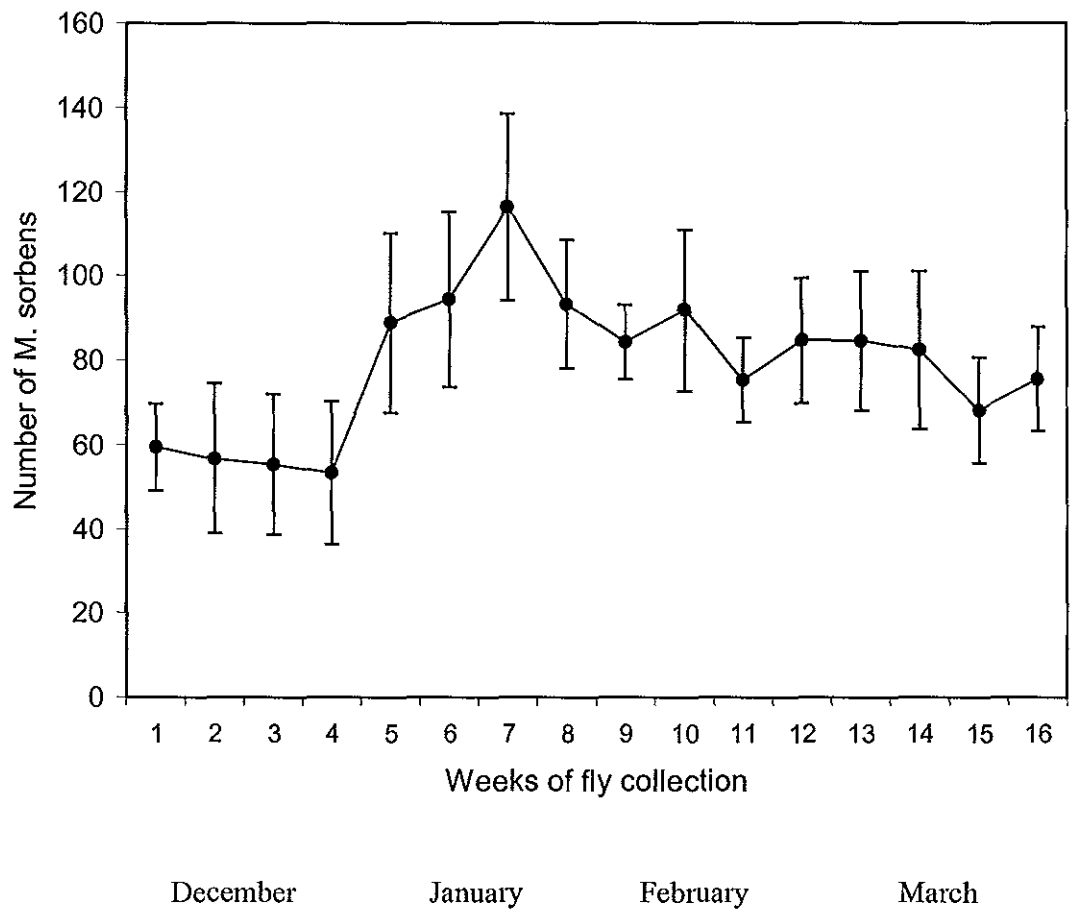


Fig. 4. Average number of total *Musca sorbens* collected from the faces of children for each week of the study period (December 2002- March 2003).

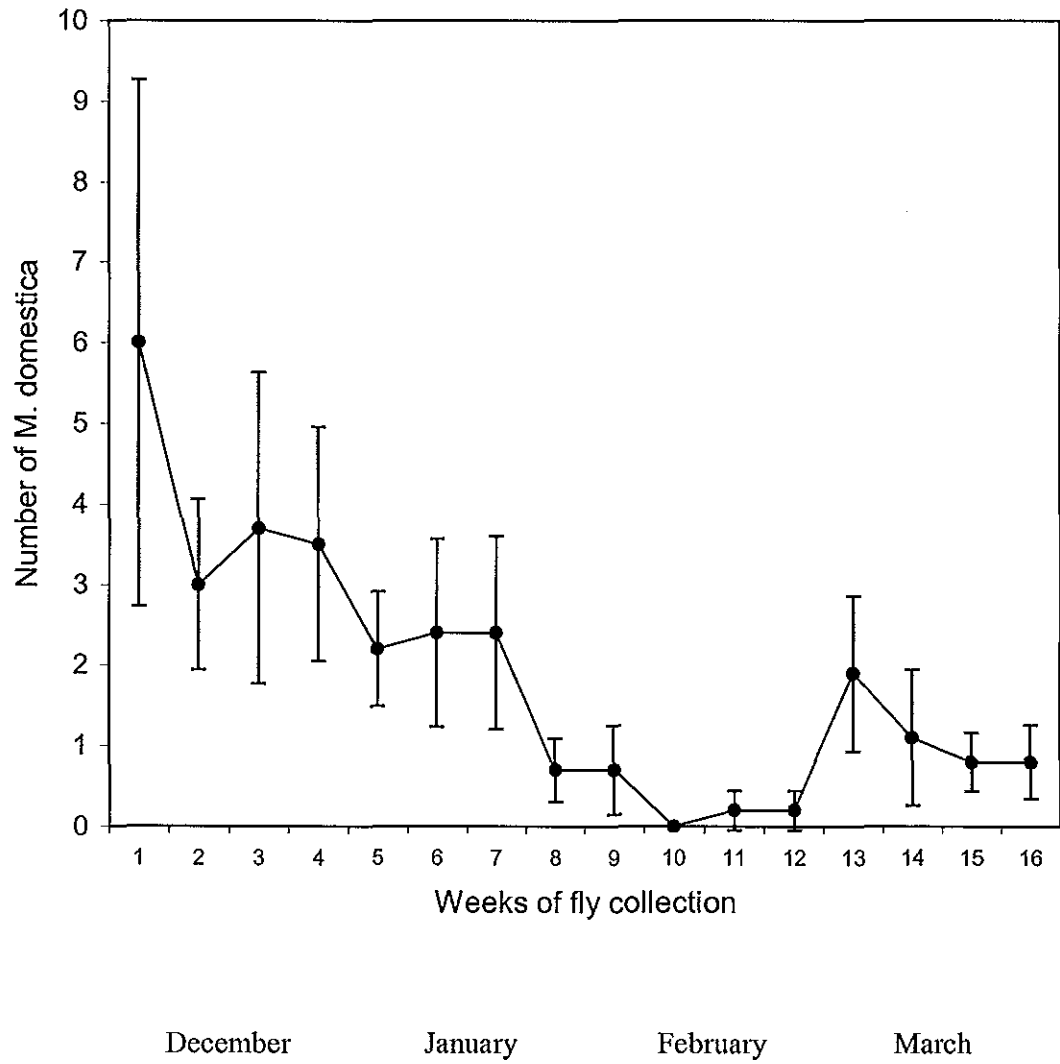


Fig. 5. Average number of total *Musca domestica* collected from the faces of children for each week of the study period (December 2002- March 2003).

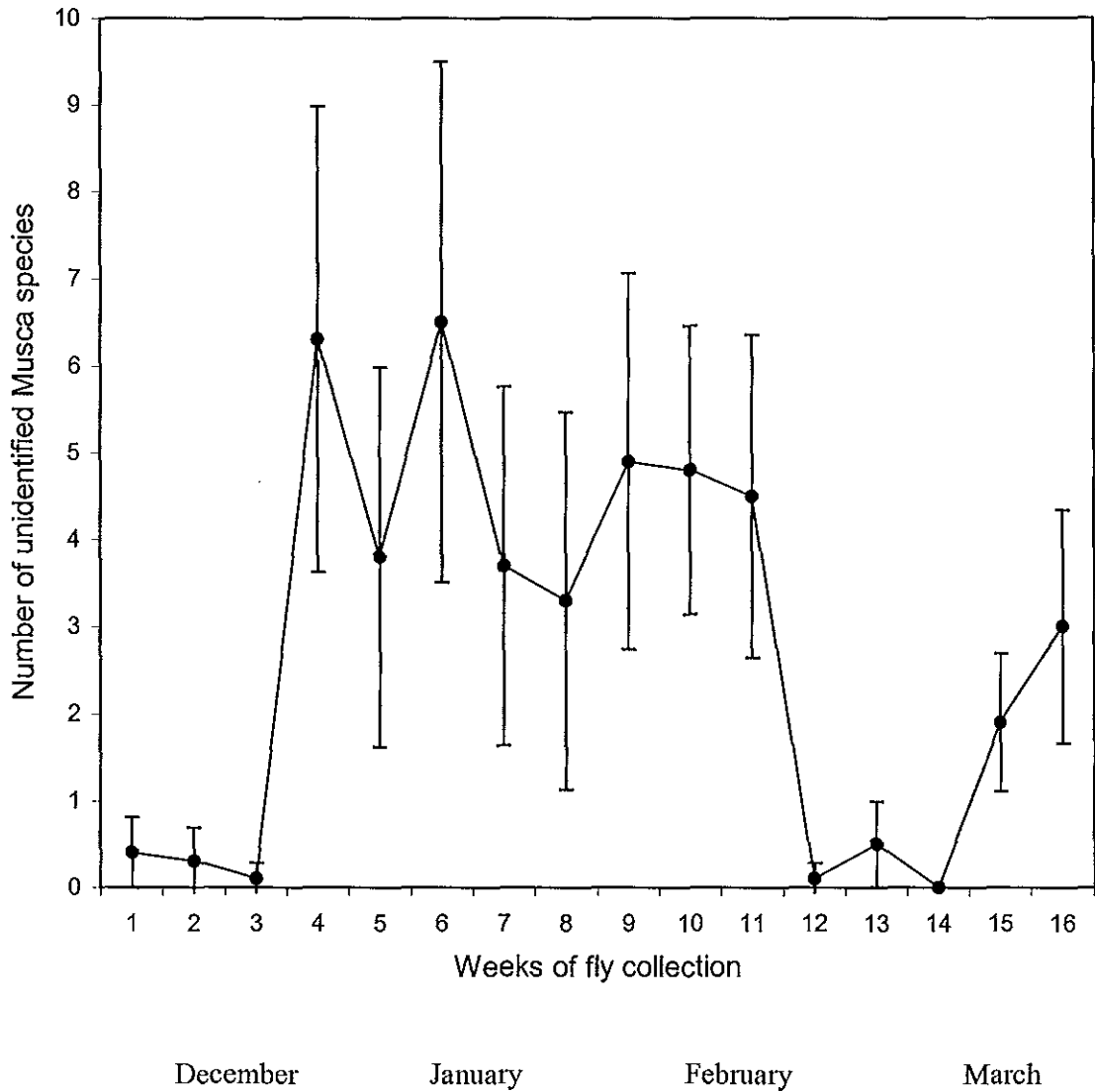


Fig. 6. Average number of total unidentified *Musca* species collected from the faces of children for each week of the study period (December 2002- March 2003).

4.2. Diurnal activity of eye-seeking flies

The general trend of the diurnal fly activity during the study period has indicated that flies were active during most of the day. The diurnal fly activity showed two peaks, one in the morning (9:00 –11:00 hours) and the other in the afternoon (16:00-18:00 hours) but the

former being the most pronounced (fig. 7). This pattern of diurnal fly counts was consistent throughout the study period from December through March.

Temperature and relative humidity were the two environmental factors measured during the study periods. In the mornings as temperatures increased, an increase in fly density was observed, but when temperatures increased above 27°C, fly counts decreased. The optimal temperature appeared to be between 23°C and 27°C (Fig. 7). However, no flies were observed around the eyes at extreme temperatures below 14°C and above 41°C (data not shown). The relative humidity was above 39 % in the early morning hours and steadily decreased throughout the day. The highest fly counts correlated with relative humidity between 32 % and 35 %. However, in the late afternoon when fly activity generally tended to increase, the relative humidity was around 21 % (Fig. 8).

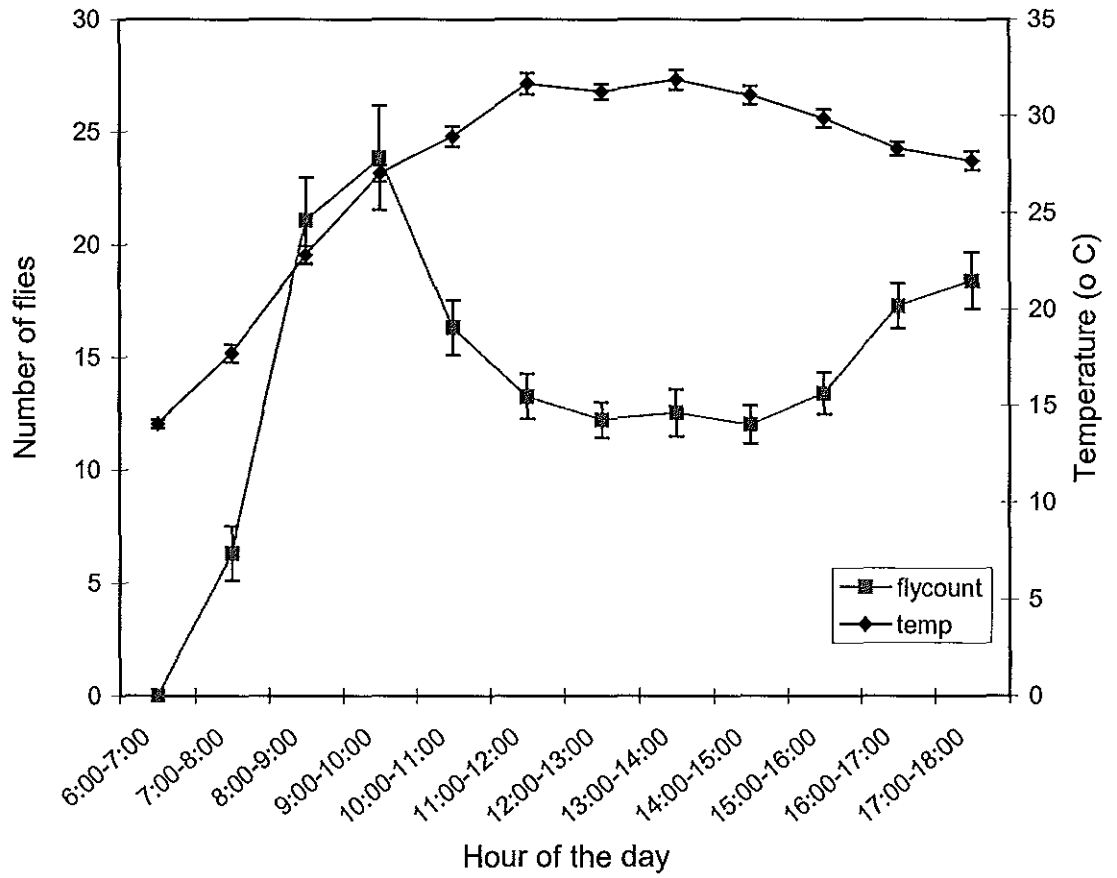


Fig. 7. Diurnal activity of flies compared with temperature (°C).

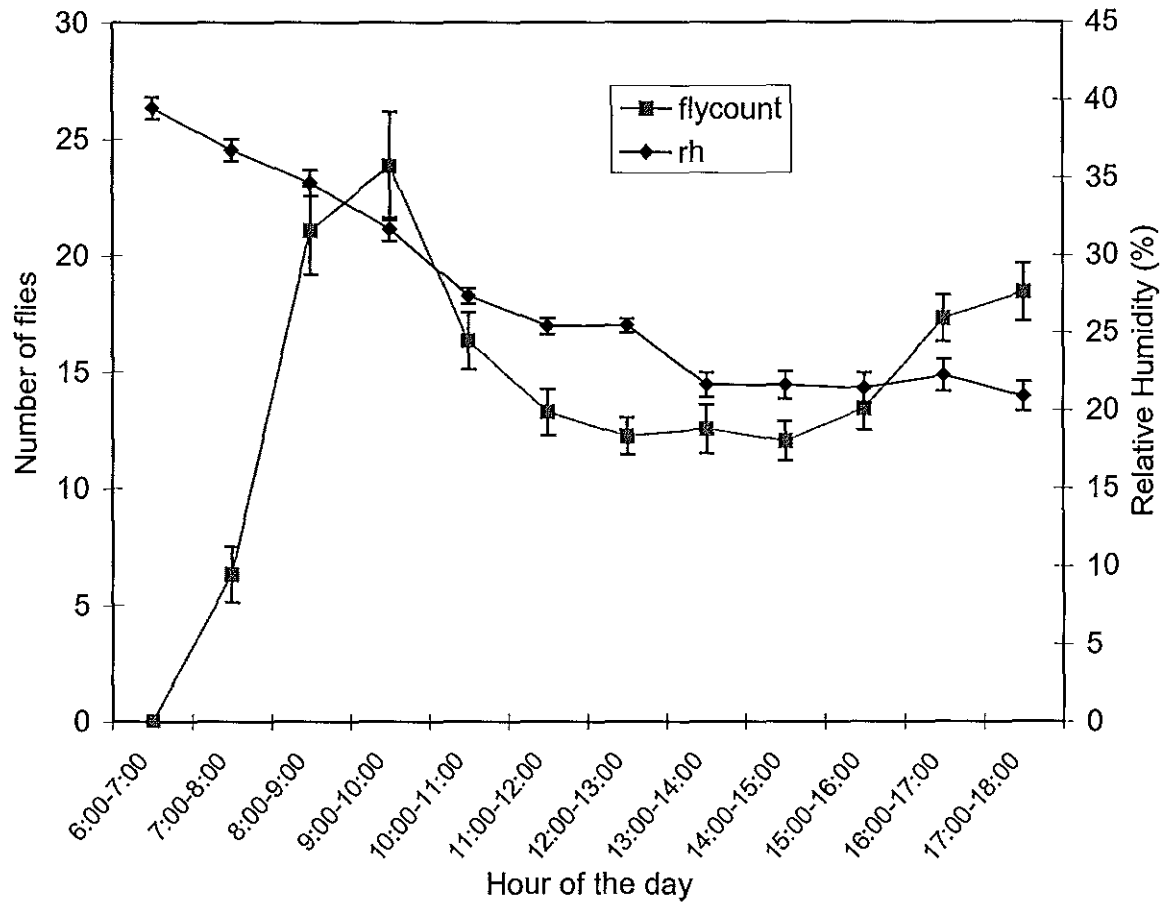


Fig. 8. Diurnal activity of flies compared with relative humidity (rh).

When the diurnal activity is examined by species, a similar trend was observed in the numbers of *M. sorbens* collected from the faces of children with a morning and afternoon peak. The favourable temperatures for the peak activity of this species were between 23 °C and 27 °C in the morning and around 28 °C in the afternoon (Fig. 9). Similarly, the favourable relative humidity was 32 and 35% in the morning and around 22% in the afternoon (Fig. 10). However, *M. domestica* numbers peaked in the morning between 9:00 – 11:00 hours of the day when the temperature and relative humidity were between 23-27°C and 32-35%, respectively (Figs. 11 and 12), but no peak was observed in the afternoon. On the other hand,

the activity of the unidentified *Musca* species followed a similar trend with that of the total fly score trend (Figs. 7, 13 and 14).

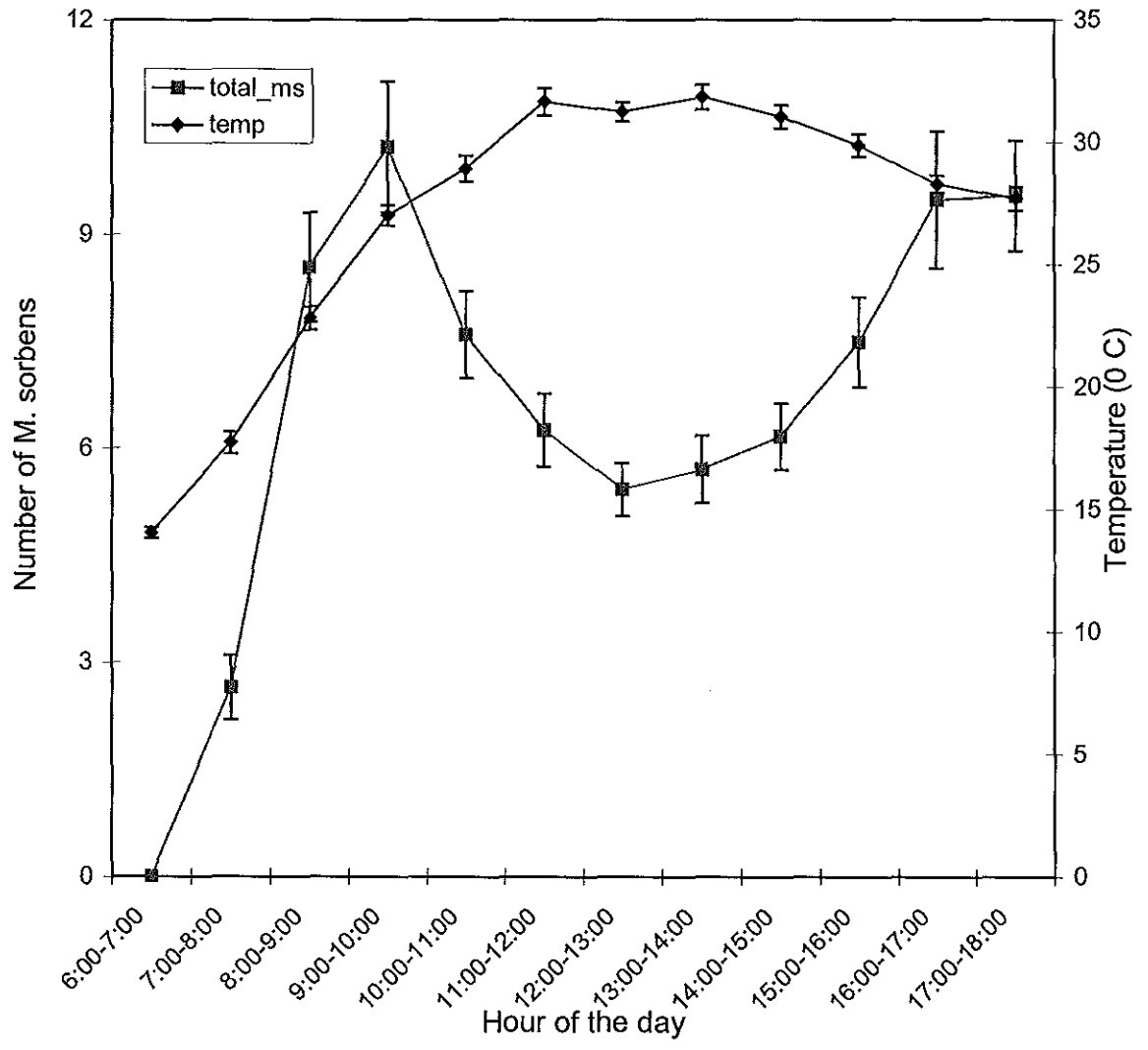


Fig. 9. Diurnal activity of *M. sorbens* compared with temperature (°C).

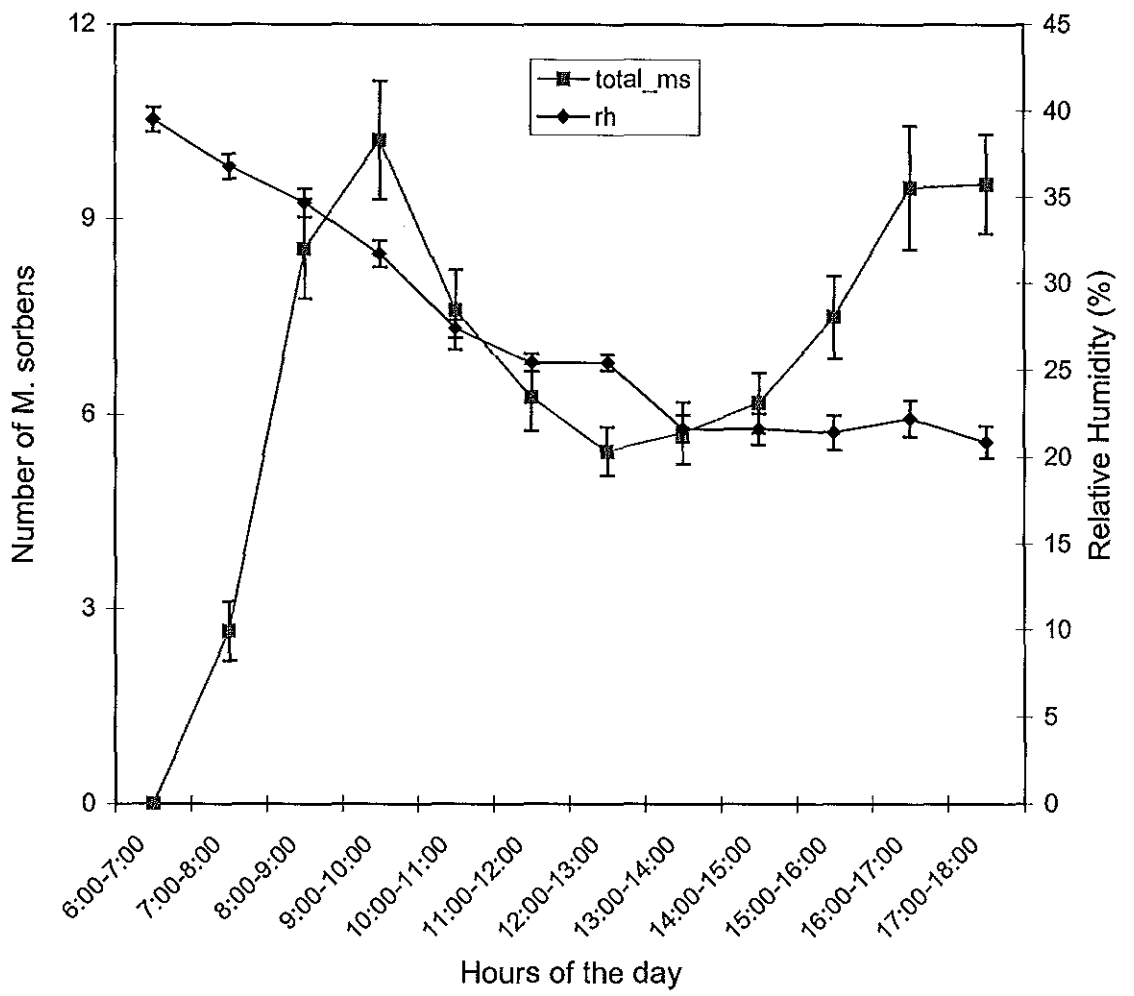


Fig. 10. Diurnal activity of *M. sorbens* compared with relative humidity (rh).

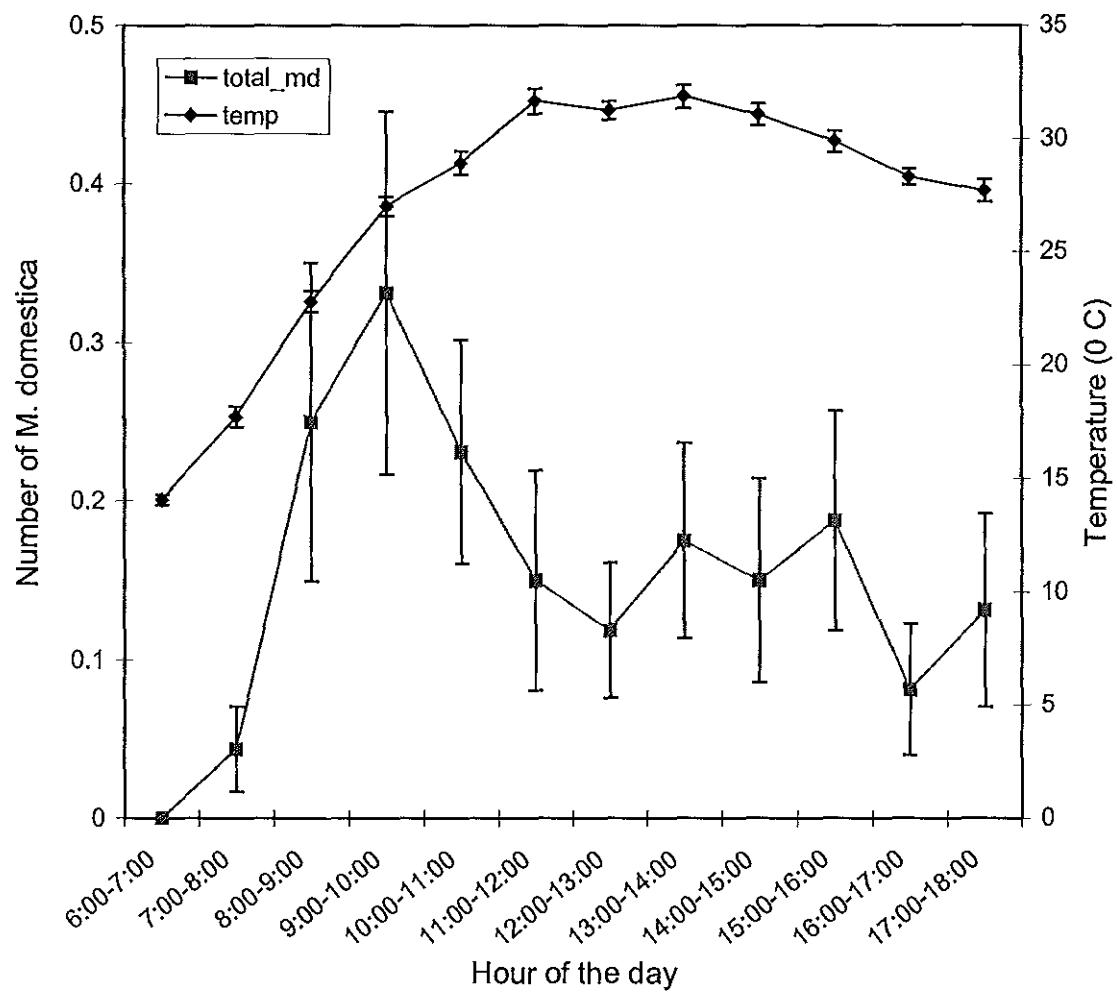


Fig. 11. Diurnal activity of *M. domestica* compared with temperature (°C).

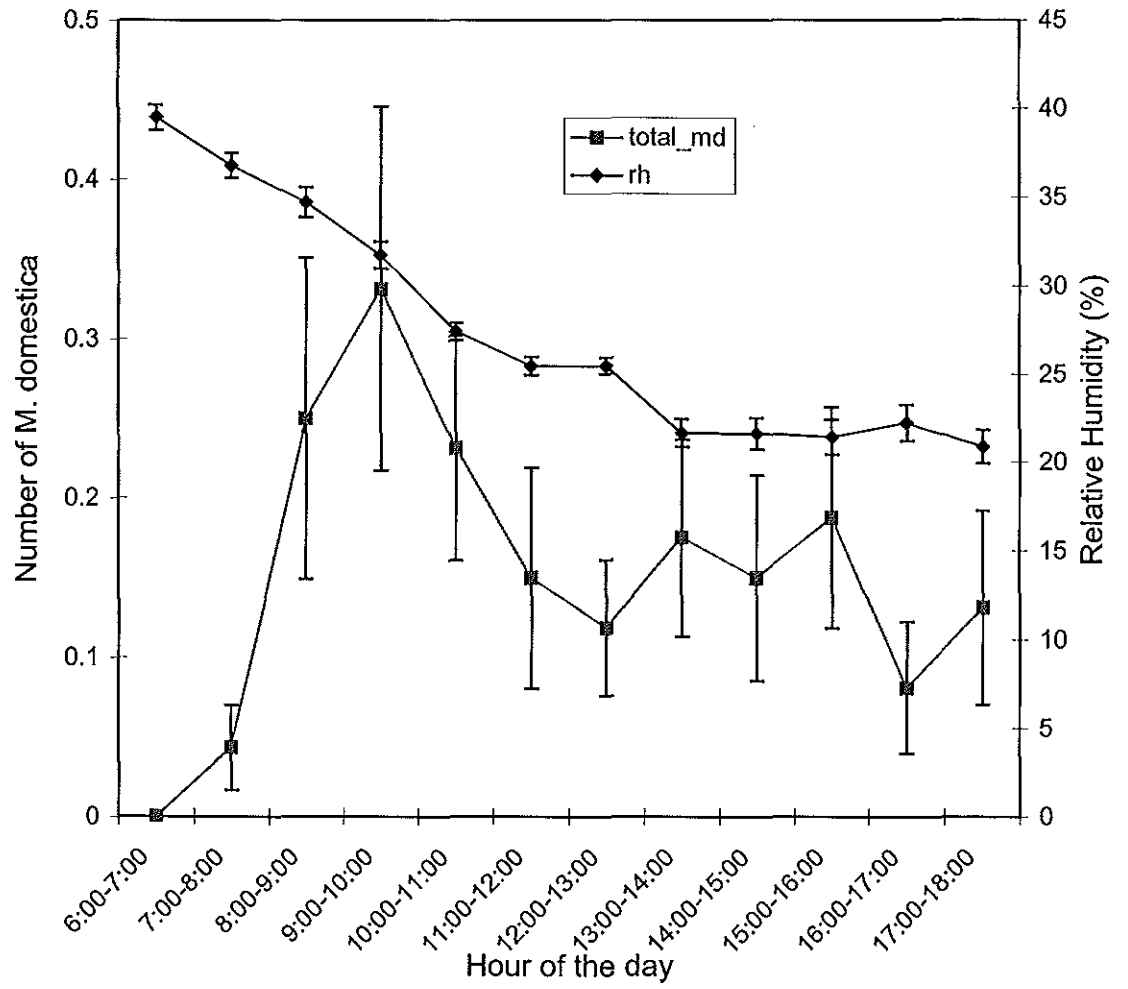


Fig. 12. Diurnal activity of *M. domestica* compared with relative humidity (rh).

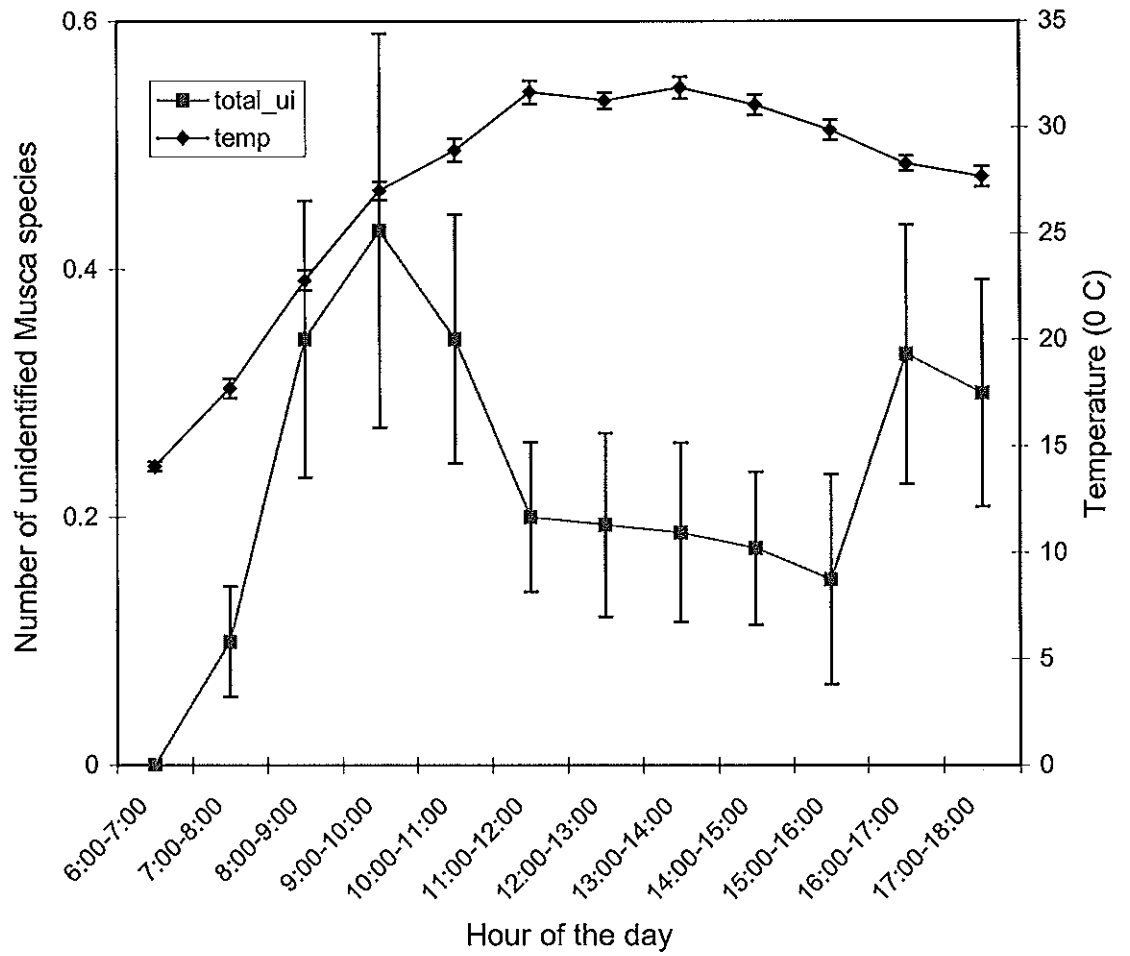


Fig. 13. Diurnal activity of unidentified *Musca* species compared with temperature (°C).

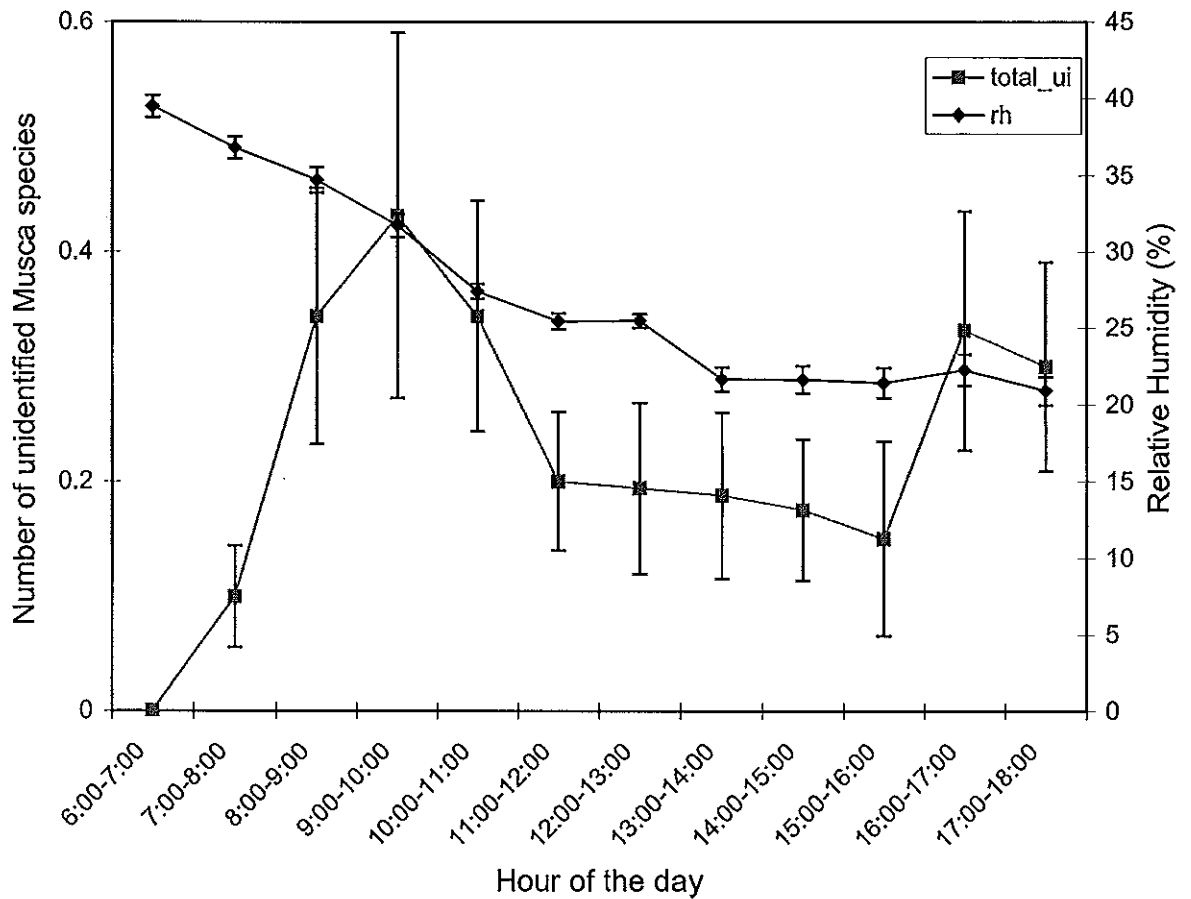


Fig. 14. Diurnal activity of unidentified *Musca* species compared with relative humidity (rh).

4.3. Investigation of breeding habitat for eye-seeking flies

The results of the experiment on the potential breeding habitat (animal and human excreta) for the eye-seeking flies under natural conditions are depicted in Table 3. Flies emerged from all types of media except for the faeces of donkey, garbage and the control experiment. An average sum of 14.7 *Musca* species emerged from the faeces over the six trials, of which 88 (97 %) were *M. sorbens*. The majority of were females (65%); with 35 % being males (see Appendix III).

Musca sorbens emerged from all six replicates of human and dog faeces, but only two *M. sorbens* emerged from one replicate each of the calf dung and goat droppings, and only

one from one replicate of the cow dung. The highest number of flies emerged from dog faeces (on average 7.35 flies) followed by humans (on average 6.5 flies).

Table 3. Mean number of adult *Musca sorbens* emerged from the eight potential breeding habitats*

Trial	Dog faeces	Human faeces	Calf dung	Cow dung	Goat droppings	Donkey dung	Garbage	Control	Total
I	6	3.3	0	0	0.7	0	0	0	10
II	8.7	9.7	0.7	0.3	0	0	0	0	19.4
Total average	7.35	6.5	0.35	0.15	0.35	0	0	0	14.7

Note:-* All the flies that emerged were *M. sorbens* except three unidentified *Musca* species (see Appendix for details III).

Flies emerging from dog faeces appeared smaller in size compared to those emerging from other media and also smaller than those flies collected from the faces of children as observed by simply eye inspection. Except dog faeces, the size of flies emerging from other faeces seem to have similar size to those collected from children's faces.

To compare statistically whether the numbers of flies emerging from the eight different media were the same or different (Table 3), a non-parametric statistical test, the Kruskal-Wallis Several Independent Sample Test was applied in the data. The Kruskal-Wallis Several Independent Test have showed that at least one of the media tends to yield higher number of flies than the other media ($\chi^2=38.875$, d.f=7, P= 0.00).

Significant variation was observed in the number of adult *M. sorbens* emerging from human faeces compared from that produced from each of the cow, calf, goat and donkey (Wilcoxon's rank sum test, P=0.002). In addition, production of flies from dog faeces was significantly different from that produced from, calf and goat (Wilcoxon's test, P= 0.004); cow and donkey (Wilcoxon's test, P=0.002) respectively. However, production of flies from human faeces was not significantly different from dog faeces (Wilcoxon's test, P= 0.589). Moreover, production of flies was not significantly higher between calf and donkey, cow and donkey, goat and donkey (Wilcoxon's test, P=0.699); cow and calf, cow and goat (Wilcoxon's test, P=0.937); calf and goat (Wilcoxon's test, P=1.00) respectively.

4.4. Prevalence of trachoma in the study subjects and risk factors.

The prevalence of trachoma among 40 children examined in the study area is shown in Table 4. Clinically, active trachoma cases were detected in 75 % of the study subjects whereas 25 % of them were free from all forms of trachoma. It was also seen that the prevalence was much higher in females (95%) than in males (57%). Trachomatous scarring was also detected in 12.5 % of the total children observed.

Table 4. Prevalence of trachoma in the study subjects in Melefenya village
(December 2002- March 2003).

Sex	No. of children examined	(%) with signs of active trachoma	(%) with trachomatous scarring
Male	21	12 (57)	1 (5)
Female	19	18 (95)	4 (21)
Total	40	30 (75)	5 (12.5)

Logistic regression analysis on the risk factors of trachoma have shown that trachoma is directly related with sex, age, education, household size, number of children below the age of 10 years, cohabitation with cattle, fly density, and face drying (Table 5).

Table 5. Logistic regression out put for risk factors of trachoma.

Factors	B	S.E.	Wald	d.f.	Sig.	Exp (B)
Sex	3.611	0.209	298.9	1	0	37.009
Eye secretion	0.171	0.167	1.050	1	0.305	1.187
Household size	0.197	0.040	24.765	1	0	1.218
Face wash	0.132	0.421	.098	1	0.754	1.141
Face dry	1.177	0.392	9.040	1	0.003	3.246
Share cloth	-0.310	0.419	0.550	1	0.458	0.733
Housing	0.211	0.164	1.658	1	0.198	1.235
Cohabitation with cattle	3.013	0.288	109.3	1	0	20.347
No. of children	0.348	0.087	15.973	1	0	1.416
Age	0.738	0.191	14.933	1	0	2.091
Fly density	0.706	0.405	3.044	1	0.081	2.027
<i>Musca sorbens</i>	1.005	0.413	5.912	1	0.015	2.732
<i>Musca domestica</i>	0.268	0.220	1.485	1	0.223	1.305
Unidentified spp.	0.463	0.203	5.211	1	0.022	1.589
Education	2.336	0.199	138.0	1	0	10.341

The result showed that trachoma was 37 times more in females than males. A unit increase in the number of households has increased the risk of trachoma by 1.218 times (this

tendency was also observed from an increase in the number of children). Cohabitation with cattle was the second highest risk by increasing the risk of trachoma 20.347 times compared to those who do not pass the night with cattle. Children below the age of six were 2.091 times at risk than children greater than or equal to six years. The presence of one or more fly on the face of a child will increase the risk of having trachoma by 2.027 times, than having no fly. Education has also shown that being illiterate result in increasing trachoma risk by 10.341 times as compared to literate. Drying faces after washing have also shown a protective effect.

5. DISCUSSION

Flies swarming to the eyes of children is a common occurrence in Melefena village (Derra Woreda). Attempts to quantify these fly-eye contacts were done in two manners: simply counting the numbers of flies flying the eyes and collecting the flies. The average number of flies counted were around 4.63/min. and for collections, 1.4/min. The counts may be an overestimate and the collections an under estimate. It is probable that some of the same flies came back to the eyes when waved away if they had not completed their feeding. Estimates of how long a fly remains at an eye were not made. Perhaps if the counts had been made over a three- or five-minute period without disturbing them, a better estimate could be made, but because we wanted to reduce the possibility of transmission resulting from our experiments, longer counts cannot be made. On the other hand, the collections may be an underestimate because every fly probably was not captured. Nevertheless, the number counted and the number collected follows similar trends in the weekly averages and diurnal activity. These data suggest that the proportions of the different species collected most probably reflect the true proportions and that the discrepancy between counts and collections is not due to unidentified flies.

This study clearly showed that most of the fly-eye contacts were made by *M. sorbens*, accounting for 94.5% of flies coming to the eyes. Only 2.2 % of the flies were female *M. domestica*. Similar proportions were found in The Gambia (Emerson *et al.*, 1999, 2000). If *M. sorbens* and *M. domestica* are able to carry *C. trachomatis* equally well, the sheer number *M. sorbens* makes it the more important potential vector. A small percentage of the flies were unidentified, but their contribution of trachoma transmission is unknown.

In this study and those of Emerson *et al.* (1999; 2000), the majority *M. sorbens* making eye contacts were females (approximately 70 %) and only female *M. domestica* were

collected from the eyes. Female flies need proteinacious food at the age of about 3-5 days and during each gonotrophic cycle (Tyndale-Biscoe and Hughes, 1968 cited in Emerson *et al.*, 2001). This is a common phenomenon in blood sucking insects such as *Anopheles* mosquito species, which requires a blood meal for the development of eggs in females. Assuming that nasal and ocular discharges represent a protein rich, source of food needed for the production and development of eggs as suggested by different studies, this might be the possible reason that female flies seek eyes more often as compared to males. If this assumption was true, no question that eye-seeking flies will move repeatedly from eye to eye for feeding this proteinacious food and on the way transmit trachoma pathogen.

Likewise, Emerson *et al.* (1999; 2000), in The Gambia have found that children with nasal and ocular discharges showed twice the number of fly- eye contacts than children with no discharges. Besides, the eyes of young children were considered the main reservoir of *C. trachomatis* the causative agent of trachoma.

From the above observations, it can be suggested that development of trapping devices using attractant baits having similar characteristics to the discharges of children should be considered in the future. These could be advantageous since trapping females will reduce the reproductive capacity of the fly population.

Assessments of the fly activity throughout the day demonstrated that the highest fly densities were observed in the morning hours from 09:00 to 11:00 hours and then another peak between 16:00 to 18:00 hours. This same pattern of diurnal activity was observed throughout the study period, from December 2002 to March 2003. Although peaks of activity were observed in the morning and later in the afternoon, the density of flies throughout most of the day was high enough to maintain transmission. Other authors have not measured the eye contacts throughout the day, yet perceptions in Emerson *et al.* (2000) were that flies were

less active in the heat of the afternoon in The Gambia suggesting that at least a peak of activity occurs in the mornings. In Tanzania, as in the present study, fly numbers were low in the early morning. However, in the same study, the fly activity between 17:00 and 18:00 hours was lowest (Brechner *et al.*, 1992) unlike in the present study where a second peak was detected.

Because eye-seeking flies were heavily swarming the faces of children during the morning hours of the day starting from 09:00 to 11:00 hours and in late afternoon from 16:00 to 18:00 hours, these are the two important periods where children's should be encouraged to keep their face clean so as to prevent transmission of trachoma. However, washing of faces by children in these hours is not a common practice as most of them wash their faces after completing their daily work associated with cattle keeping. They usually wash their faces after the peak hours when they come back home for breakfast after doing the farm and cattle keeping.

Two environmental factors (temperature and relative humidity) that may affect fly activity were measured during the course of this study. Of the two, only temperature appeared to be linked with fly activity. Peak fly activity was recorded between the temperatures of 23°C and 27°C. Fly activity decreased as temperatures increased above 27°C and began to increase again as temperatures descended below 30°C in the afternoon. Extreme temperatures below 14°C and above 41°C appeared to be non conducive for the activity of flies. The trend and range of temperature obtained in the different *Musca* species collected from the faces of children was similar to the general fly score trend in all the study periods.

The second environmental factor measure, relative humidity, could not be correlated with daily fly activity, although peak activities were measured between 32 and 35%. Higher relative humidity was measured in the early morning hours which decreased continually

throughout the day. It is probable that flies are active at higher relative humidity since this study was done during a season of no rain where the relative humidity is low. However, other studies suggest that higher number of flies were observed during wet seasons (Emerson *et al.*, 2000; Brechner *et al.*, 1992).

Other environmental factors such as light intensity and wind speed were not measured, but probably could play important roles in fly behaviour. For example, sunrise and sunset may affect fly activity, but the study began at 6:00 in the morning before sunrise (around 6:30) and was terminated at 18:00 before sunset (around 18:30). It would be interesting to know if fly activity stopped by 19:00. In addition, the interaction of several environmental factors may affect fly activity.

The highest density of eye-seeking flies has been obtained in February 2003 and the lowest was in December 2002. On average, the fly score increased from December 2002 to February 2003 and during which the average monthly temperature was also increasing. In March the fly score decreased as the daily average temperature increased above the average monthly temperature. It was not possible to see the effect of relative humidity since measurement commenced on the third month of the study period.

Since this study was limited to four months, the seasonal variation of fly numbers could not be assessed.

A study in The Gambia by Emerson *et al.* (2000) showed that the number of *M. sorbens* caught in traps increased during the wet season, but no seasonality of trachoma transmission has been detected. From this it can be extrapolated that small number of flies can maintain the transmission throughout the year. However, Brechner *et al.* (1992) reported the

A study in Hawaii (Yu, 1971 cited in Legner *et al.*, 1974) has shown that dog faeces were the most important breeding habitat for *M. sorbens*. Another study in The Gambia by Emerson *et al.* (2001) had shown that the best breeding media for *M. sorbens* was found to be isolated human faeces in the environment. However, *M. sorbens* was also seen emerging from calf, cow, dog and goat faeces. The breeding habitat for *M. sorbens* may show variation depending on the local conditions in different geographical conditions. Hence, the contribution of the droppings of cattle, calves and goats might be significant if human and dog faeces are not available in the open field.

Data in the present study suggest that dog and human faeces contributed equally to the *M. sorbens* population, but the size of flies emerged from dog faeces were much smaller than those caught from the faces of children. Nevertheless, flies emerging from human faeces seem to have a similar size to those collected on the faeces of children.

Anecdotal observations indicate that human and dog faeces are swarmed with more flies compared to other animal faeces. Our data suggest *M. sorbens* have strong affinity to human and dog faeces. A substantial number of dogs were found in the village and pass almost all the time within the village. Thus fresh faeces are available during the day when other animals are away from the village. This provides flies to have a continuous source of breeding habitat for oviposition. This strongly suggest the importance of environmental sanitation to prevent transmission of blinding trachoma by interrupting the life cycle of the major potential vector (*M. sorbens*) through larval media depletion. By doing so it is also possible to reduce the chance of fly-eye contacts and thus trachoma transmission.

Trachoma was detected clinically in 75 % of the children indicating the severity of trachoma in this area. This could be the result of repeated infections made by flies on the faces of children consistently throughout the day. Higher prevalence of trachoma has been

presence of seasonal effect on the prevalence of trachoma where the rainy season appears to show a higher prevalence of trachoma than the dry season in Tanzania.

Knowledge on the diurnal variation and monthly fluctuations of eye-seeking flies on children's faces is an important issue to consider for the development of fly suppression strategies and for monitoring trachoma prevalence in the area.

From group discussions made with the dwellers in the study area high fly density is normally observed during autumn (from May to June) and then decreases from July to August when the rain becomes torrential and continuous. Starting from September the fly population tends to increase as the amount of rainfall decreases. These were the two most important peak seasons considered where fly density becomes higher. During these periods farmers find it very difficult to do farming activities because the oxen used for farming and the dwellers themselves were bitten by biting flies probably, *Stomoxys* spp. The bite is so intense that it makes their cattle restless and difficult to graze. In order to solve this problem villagers use smoke in the house to repel the biting flies. These biting flies are named by the dwellers "Ewur-Zimb" which means blind fly. In the study area flies are perceived as major evils not from the public health problem point of view, but from the problems brought on their cattle. As they said, if flies are eradicated from the area the wealth of cattle will double progressively.

Results obtained from the investigation of the breeding habitat of eye-seeking flies have shown that *M. sorbens* breeds in human and other animal faeces. The number of flies emerging from dog and human faeces was comparable, but were much higher than those emerging from other breeding media.

observed in females than male children. This might be due to the close association of female children with their mother in relation to activities linked to female jobs performed in the house and vicinity that brings them in contact with higher number of flies while males tend to follow their father away from villages. Surprisingly 12.5 % of the study subjects showed trichomatous scarring, of which 80 % were females and such condition was not expected in children below the age of 10 years. The logistic regression has shown a higher prevalence of trachoma in children of less than 6 years than those above 6 years old. This is in agreement with the work of Brechner *et al.* (1992) where about 60 % of active trachoma cases were observed in children aged 3-5 years.

From the multiple logistic regression model we have seen that the presence of flies on the faces of children was found increasing the risk of trachoma in the study population indicating the presence of a strong association. Likewise, Brechner *et al.* (1992) and Taylor (1988) in Tanzania have found that the risk of active trachoma was highly associated with the presence of flies on the faces of children.

We also observed that the study area has marked water source problem. Spring water was available during the rainy season and until the month of December. From January to June the spring water diminishes and completely stops flowing. Hence, villagers were observed fetching unhygienic pond water that collected in deep depressions made by the spring water and has been collected from the previous flow. Hence, this is the key area where governmental and non-governmental organizations have to work hard in order to improve the health of the community in particular and the standard of life at large.

6. CONCLUSION

The dominant eye-seeking fly in the study area was found to be *M. sorbens*. The second important eye-seeking fly has been *M. domestica*. These are the potential vectors maintaining the high transmission of trachoma in the area, which is manifested by the presence of high prevalence of trachoma.

Female flies have been collected 2.5 times than males. This has been postulated by the presence of protein on the discharges utilized as source of food for egg production.

The density of flies has been shown to increase between 09:00 – 11:00 hours in the morning and 16:00 – 18:00 hours in the afternoon and its maximum peak were recorded within the morning hours (09:00 – 11:00 hours).

Knowing the density of flies in a given area will indicate the effect of control interventions in the future and it helps also for monitoring fly control programmes.

The most important breeding habitat for the dominant potential vector in the area was found to be dog and human faeces excreted in the environment.

High prevalence of trachoma has been found in the study population. A strong association has been obtained between trachoma and sex, education, household size, number of children below the age of 10 years, cohabitation with cattle, fly density, and face drying.

These findings will contribute to the success of trachoma prevention and control activities and to the general health promotion at large through the implementation of the F and E components of the SAFE strategy by providing base line levels of the fly species, fly density and their breeding source

7. RECOMMENDATION

The information obtained in this study provides baseline information which serve as a basis to design appropriate control measures against the potential vectors of trachoma. The study clearly indicated that eye-seeking flies are abundant in the area and it is time to act against these vectors to prevent further spread of the disease. Therefore, the following recommendations are suggested among others: -

Removal of faecal materials in the environment through the provision of basic sanitation in order to avoid breeding grounds of eye-seeking flies should be considered seriously since the study has shown that the most dominant vectors are reproducing in the faecal materials left on exposed bare grounds.

Face washing should be encouraged early in the morning and also during early afternoon to keep flies away from the faces of children as most of the children were swarmed with flies during morning hours and in the late afternoon.

An extensive effort should be made by governmental and non-governmental organizations to improve water supply and sanitation in the area since the existing water supply is very poor. According to Neatherline (2001) only 2% of the population gets piped water and the other 13.3 % from protected well, 53.4 % from unprotected well and 31.1 % from running water.

Tethering dogs around the house and removing their droppings should be given special attention since dog faeces may contribute equally to that of human faeces for the proliferation of the most common eye-seeking fly *M. sorbens*.

Health education especially on hygiene should be considered critically so that the beneficiaries would recognize the importance of flies as the agents of diseases.

Development of attractant baits which could be made from local materials with similar characters of eye and nasal secretions should be considered as these flies are attracted more to children's with these secretions.

Mass treatment should be considered for active cases of trachoma in the area. The prevalence of active cases of trachoma is very high in the Woredas, which is 81.2 % according to Neatherlin (2001) and 75 % in our study.

Chemical control should be also considered during an epidemic situation to reduce the fly density for short period of time. For this the diurnal and nocturnal resting sites of these flies should be investigated.

As a whole integrated approach is the most important in reducing the burden of trachoma in the area by strengthening the F and E components of SAFE for sustainable control.

Special attention should be given to the females, and the possible risk factors that make them more susceptible should be assessed.

Similar studies should be considered on the species type and trends of fly density on the faces of children and on the seasonal variation in different parts of the region to develop sustainable and cost effective strategies to control eye-seeking flies in the country where trachoma is prevalent.

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APPENDIX I

Logistic Regression

Dependent Variable

Original	Internal
No	0
Trachomatu	1

Categorical Variables Codings

		Frequency	Paramete
			(1)
Education catagorized	Illiterate	960	1.000
	Literate	960	.000
Eye Secretion	No secretion	601	.000
	With secretion	1319	1.000
Washing of face	Do not Wash	1728	.000
	Wash	192	1.000
Drying of face	Do not dry	1632	1.000
	Dry	288	.000
Sharing of cloth	Do not Share	1824	.000
	Share	96	1.000
Kind of house	Tukul	1196	1.000
	Metal	724	.000
Shelter of cattle	Under the same roof	144	.000
	Separately	1776	1.000
Age catagorized	1-5	816	1.000
	6-10	1104	.000
Un identified fly catagorized (Ui)	No Ui	1627	.000
	One or more Ui	293	1.000
Musca domestica catagorized (Md)	No Md	1705	.000
	One or more Md	215	1.000
Musca sorbens catagorized (Ms)	No Ms	242	1.000
	One or more Ms	1678	.000
Fly density catagorized	No fly	235	.000
	One or more fly	1685	1.000
Sex	Female	912	1.000
	Male	1008	.000

Appendix III. Number and species of flies emerged from the eight potential breeding media*

Trial	Replicate	Dog droppings				Human faeces				Calf dung				Cow dung				Goat droppings				Donkey dung				Garbage				Control					
		MS		UI		MS		UI		MS		UI		MS		UI		MS		UI		MS		UI		MS		UI							
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F						
I	1	3	8	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	4	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	1	4	8	0	0	8	2	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	4	8	0	0	4	7	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	1	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		13	31	0	0	15	24	0	1	2	0	0	2	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0

Key: - M= Male; F = Female; MS = *Musca sorben*; UI = Unidentified *Musca* species;

*: - No *Musca domestica* was detected in all the trials.