Transmission ecology of the fly *Musca sorbens*, a putative vector of trachoma

Paul M. Emerson[1,2,3], Robin L. Bailey[1,2], Olaimatu S. Mahdi[1], Gijs E.L. Walraven[1] and Steve W. Lindsay[1,3]

[1]*Medical Research Council Laboratories, PO Box 273, Banjul, The Gambia;* [2]*Clinical Research Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 5HT, UK;* [3]*Department of Biological Sciences, Durham University, South Road, Durham, DH1 3LE, UK*

Address for correspondence: P. Emerson, MRC, PO Box 273, Banjul, The Gambia; fax (+220) 735 512; email pemerson@mrc.gm

Abstract

Recent evidence suggests that eye-seeking flies are important trachoma vectors. We conducted a series of investigations to identify which species of synanthropic flies are potential vector(s) of this blinding disease in The Gambia. Several species of fly were caught in fish-baited attractant traps placed in villages throughout the year, but only two species; *Musca sorbens* and *Musca domestica* were caught from the eyes of children. *M. sorbens* comprised <10% of the total number of flies caught with attractant traps but was responsible for >90% of fly-eye contacts, the remainder were made by *M. domestica*. All fly species were more numerous in the wet season than the dry season. Eyes of young children are considered to be the main reservoir of *Chlamydia trachomatis*, the causative agent of trachoma. Collections of eye-seeking flies from children showed frequent fly-eye contacts (median [interquartile range], 3 [1.5-7] every 15 minutes). Children with potentially infective ocular or nasal discharge had twice as many fly-eye contacts than children with no discharge ($P<0.001$). There was no difference in exposure to fly-eye contacts if a child sat inside or outside a house ($P=0.273$). Female flies were more commonly caught from eyes than males ($P<0.001$). The presence of

Chlamydia DNA was demonstrated by PCR on 2 of 395 flies caught from the eyes of children with a current active trachoma infection. Both positive flies were *M. sorbens*, one male and the other female. Further elucidation of *M. sorbens* behavioural ecology and the development of sustainable strategies to control them should be a priority. It is likely that *M. sorbens* is the principal insect vector of trachoma in The Gambia.

Introduction

Trachoma is an infectious blinding disease caused by the bacterium *Chlamydia trachomatis.* Worldwide, between 300 and 500 million people are believed to be affected, of whom an estimated 5.8 million are blind, making it second only to cataract as a cause of blindness and the most common form of infectious blindness (THYLEFORS *et al.*, 1995).

The challenge of controlling trachoma has led to the Global Elimination of Trachoma by the Year 2020 Initiative (GET 2020), a WHO alliance whose aim is to eliminate trachoma as a blinding disease by 2020. It is understood that blindness from trachoma is a result of frequent infections over many years (MABEY *et al.*, 1992), but the routes of infection are incompletely understood. Several routes, with varying epidemiological significance, may exist. The most likely being fingers, fomites (infective discharges on clothes, bed sheets, towels etc.) and flies (MACCALLAN, 1931; MUÑOZ & WEST, 1997). It is believed that in endemic areas the eyes of children with active trachoma are the principal reservoir of disease (MABEY *et al.*, 1992). Studies in The Gambia (BAILEY *et al.*, 1989) and Tanzania (WEST *et al.*, 1991) have found that cases of active trachoma cluster by household, supporting the idea that transmission occurs between subjects in close proximity.

Until recently the evidence implicating synanthropic flies as vectors of trachoma was largely anecdotal or circumstantial. Flies had been shown capable of transferring fluorescein between

children's eyes (JONES, 1975), *C. trachomatis* had been cultured from flies after they were fed on heavily infected laboratory cultures (FORSEY & DAROUGAR, 1981) and high fly densities had been associated with outbreaks of trachoma in Morocco (REINHARDS *et al.*, 1968) and Egypt (MAXWELL LYONS & ABDINE, 1952; HAFEZ & ATTIA, 1958a).

A recent study from The Gambia (EMERSON *et al.*, 1999) provides the best evidence to date that flies are important in trachoma transmission. The study was conducted in two pairs of villages; one village from each pair received fly control using insecticide for three months, the other acted as a control. Fly control decreased the numbers of Muscid flies by around 75% and reduced fly-eye contact by >95% compared to controls. Cross-sectional trachoma surveys conducted at baseline and after three months showed that in the absence of flies there were 75% (95% CI 36-91) fewer new prevalent cases of trachoma in intervention villages compared to controls. On the basis of this result, fly control is likely to be the focus of interventions to interrupt trachoma transmission. This study was designed to identify the most likely vector(s) and to characterise exposure to the flies.

Materials and Methods

Study site

The study was conducted in the Sanjal region of The Gambia between May 1997 and May 1998. Flies were collected from Wollof hamlets (300-700 inhabitants) which consisted of 12 to 20 family compounds. Houses in the hamlets were typically (>90%) single roomed and constructed from mud blocks. Most houses were roofed with grass thatch, the remainder with corrugated iron. Goats, sheep, dogs and poultry roamed freely between the houses during the day but were shut in or penned at night. Horses, donkeys and cattle were enclosed in pens or tethered adjacent to the owner's house. Houses and compounds were swept daily, with the refuse being piled outside each compound and occasionally burnt. There were few latrines and most adults defecated away from the settlement in the bush. Young children defecated in the compounds and carers cleaned up after them, throwing the faeces onto the refuse piles.

Background trachoma prevalence

Prior to the collection of entomological data all people over three months old resident in the hamlets were screened for trachoma by a community ophthalmic nurse from the Gambian National Eye Care Programme. Screening was conducted by everting the upper eyelid and visually examining the tarsal plate with a torch and a x2.5 binocular loup. Eyes were graded according to the WHO simplified scale (THYLEFORS *et al.* 1987), which classifies active trachoma as the presence of follicular trachoma (five or more follicles >0.5mm visible) or intense trachoma (50% of tarsal plate obscured by inflammation). People with symptomatic trachoma were offered tetracycline eye ointment, and those with trichiasis were referred to the district eye clinic where surgery was available.

Fly collections

Flies were collected using fish-baited attractant traps, directly from children's faces with hand-nets, from sticky targets and in exit traps placed over latrines.

Attractant traps. Each trap was made locally from a blue 10 L plastic bucket strapped beneath a WHO mosquito exit trap (W.H.O., 1975). Two semicircular holes of 12 cm diameter, were cut from the sides of the bucket near the base to allow flies to enter. Half a fish of a standard type was used as bait. The fish was tied to the base of the bucket to prevent cats and other animals removing it. Flies have a tendency to fly upwards after taking off; those attracted into the bucket by the fish flew up into the exit trap and were unable to escape. On collection, the traps were labelled, detached from the bucket and the funnel blocked. Flies were killed by placing the entire cage in a freezer at -20°C for 10 minutes, and then turned out onto a sheet of white paper for sorting. To test whether the height of bait presentation was important, traps were initially set together in pairs; one on the ground, the other suspended at 1-1.5 m. There was no significant difference between the number of Muscid flies caught when the traps were set on the floor or hanging. Routine collections were made using hanging traps set for 24-hour periods, as these were less likely to be disturbed by animals. Traps were set in similar positions in each village chosen to reflect the distribution of flies: at an animal tethering area; by a latrine; in the centre of a compound and by the banta ba (a shaded platform where members of the community gather and relax).

Hand-net collections. These collections were carried out between 07:30 and 13:00. Child volunteers aged between 2 and 6 years, sat on a low stool and flies that touched the eyes were caught by a trained field-worker in a hand-net. The presence of ocular or nasal discharge was recorded for each child. Children's faces were considered clean if neither type of discharge was present. Flies were caught from each child for 15 minutes, except when comparing catches inside and outside a house; in which case the child sat for 10 minutes outside, rested briefly then sat for a further 10 minutes inside. The live flies were transferred to holding tubes and killed by freezing in the laboratory. Flies caught from the eyes of children with a known current trachoma infection were tested for the presence of *Chlamydia* DNA by PCR. To reduce the likelihood that the bacteria would be removed by the flies' grooming behaviour these flies were killed with diethyl ether and put on ice immediately after capture.

Sticky traps. Transparent acetate sheet coated on one side with adhesive (AgriSense, Pontypridd, MidGlamorgan, UK) was wrapped, sticky side out, round yellow polythene targets (150x2x450 mm) and suspended inside houses. Traps were hung at a height of around 1.5m in the corner of the room closest to the door. Sticky traps were set for 24-hour periods.

Exit traps over latrines. W.H.O. mosquito exit traps (W.H.O., 1975) were placed over the drop hole of randomly selected pit latrines in the hamlets for 24-hour periods. Latrine users were asked to replace it after visiting the latrine.

Identification of flies

Flies were identified in the laboratory using a dissecting microscope and relevant taxonomic keys (PONT, 1991; CROSSKEY & LANE, 1993).

Identification of Chlamydia trachomatis *on flies*

Flies were stored at -20°C for up to 10 weeks prior to testing for the presence of *C. trachomatis* DNA with a modified version of the cryptic plasmid PCR test developed for use on eye swabs (BAILEY *et al.*, 1994).

DNA Extraction. Pools of five flies in 300 µL of physiological saline were vigorously washed by vortexing for five minutes to dislodge bacteria. The washing solution was removed and centrifuged at 10,000 *g* for 30 minutes to pellet cellular material. After removal of the

supernatant the pellet was resuspended in 20 µL distilled water. 0.5 volume phenol (BDH) and 0.5 volume chloroform (BDH) were added and vortexed briefly to dissolve proteins and fats. Aqueous and organic layers were separated by centrifugation at 10,000 g for 5 minutes. The aqueous layer containing DNA was removed and added to a tube containing 0.1 volume 4M NaCl solution. 2.5 volumes of cold absolute ethanol was added and the tubes chilled at -20°C for 30 minutes to precipitate DNA. DNA was pelleted by centrifuging at 10,000 g for 10 minutes; the supernatant removed and tubes left to bench dry. The crude DNA pellet was resuspended in 20 µL distilled water and stored at -20°C.

PCR Amplification. PCR amplification was performed using primers derived from the sequence of the cryptic plasmid (BAILEY *et al.*, 1994) (Oswell Diagnostics, Edinburgh, UK) and reagents from Promega (Southampton, UK). Reaction volumes were 50 µL containing 1 U Taq DNA polymerase, 50 mM KCl, 10mM Tris-HCl (pH8.0), 1.5 mM $MgCl_2$ 0.2 mM of each dNTP, 1µM of each primer and 5.0 µL of crude DNA preparation. 34 amplification cycles were carried out using a Techne PHC-3 Cycler (Cambridge, UK) consisting of denaturation at 94°C for 1 minute, annealing at 53°C for 2 minutes and extension at 72°C for 2 minutes. Standard precautions to avoid contamination were made. Positive controls (ocular sample from an active trachoma case previously proved to be positive by ELISA and PCR), and negative controls (template-free) were included for every 20 samples. 5 µL of PCR product was added to 2 µL loading buffer (15% Ficoll 400, 0.05% Bromophenol blue in Tris-Acetate-EDTA) and run on a 2% agarose (Sigma) gel for 30 minutes at 85 V, 400 mA and stained with ethidium bromide (Sigma). A positive result corresponded to a band of 370 bp.

Statistical Analysis

Tests of normality showed that the fly catch data from all collection methods was not normally distributed. It was described by the median and interquartile range and analysed with non-parametric tests. Wilcoxon matched-pair signed-rank tests were used when comparing paired data and Wilcoxon rank-sum tests for comparing two independent samples. Confidence intervals for proportions were calculated from the exact binomial distribution. Analyses were performed using STATA® and Epi Info 6.

Ethical clearance

The study was approved by the joint Gambian Government and MRC Ethical Committee. Prior consent was obtained from the guardians of volunteer children involved in fly catches.

Results

Background trachoma prevalence

Of 924 people aged three months and over screened in the study villages 120 (13.0%) showed signs of an active trachoma infection. The age distribution of the active cases is shown in Table 1.

Fly traps

The attractant fly traps baited with fish reflected the presence of large numbers of flies living in close proximity to people in the hamlets. The number of flies caught per trap in 24 hours ranged from 0 to 3543, the median number of flies per trap per day was 54.5 with an interquartile range of 16 - 195. Of 71 362 flies caught throughout the year in 383 fish-baited traps, 3 species dominated the catch (96.4% of the total): *Chrysomya albiceps* (68.5%); *Musca domestica* (19.3%) and *M. sorbens* (8.6%) The remainder of the flies were *Lucilia sericata*, *Wohlfahrtia*, *Sarcophaga*, *Chrysomya*, and *Cordylobia* species. *C. albiceps* was never caught in hand-net from eyes and was therefore not considered a potential trachoma vector, consequently further analyses were restricted to the two Muscid species *M. sorbens* and *M. domestica*.

The number of *M. sorbens* caught in the wet season was twice that in the dry season. In the wet season the number of flies caught per trap each day averaged 20 (Interquartile range; 13 - 46) and in the dry season there were 9 flies per trap each day (Interquartile range; 4 - 13) (Z= 2.72, *P*<0.01). There were 5 times more *M. domestica* caught in the wet season compared to the dry season; 37 flies per trap per day (Interquartile range;16 - 229) in the wet season and 7 flies per trap per day (Interquartile range; 5 - 10) in the dry season, Z=3.313, *P*<0.001. Of 678 flies caught from 110 sticky traps set inside houses during the dry season 66.5% were *M. domestica* and 16.4% *M. sorbens*. The sex ratios for both species were virtually 1. For *M. domestica* 48.4% were males and 51.6% were females, for *M. sorbens* 49.5% were males and

50.5% were females. Of 1876 flies caught in exit traps placed over latrines 100% were *C. albiceps*. No Muscid flies were caught exiting latrines.

Fly-eye contacts

Observations of hand-net collections of eye-seeking flies suggested that this technique was practically 100% efficient when there were few flies, but up to 5% of flies escaped when the numbers were greater. Table 2 gives the sex and species of flies caught from the faces of children. Almost 3 times more female *M. sorbens* were caught from the eyes than males (73.1% *vs* 26.9%) and 16 times more female *M. domestica* than males (94.1% *vs* 5.9%). Flies contacted children's eyes frequently (3 contacts every 15 minutes [95% CI 1.5 - 7]). Children with ocular or nasal discharge had double the number of fly-eye contacts of children with clean faces. The median number of flies caught from children with ocular or nasal discharge was 8 (95%CI 6-12) and the median number caught from children with neither ocular nor nasal discharge was 4 (95%CI 2-6), $Z=-3.83$, $P<0.001$. There was no difference in the exposure to fly-eye contact as measured by the number of flies caught in the hand-net collections if a particular child was inside or outside a house. The median number of flies caught in ten minutes from a child seated inside was 3 (95%CI 0.25 - 5); when the same child moved outside the median was 3.5 (95%CI 2 - 5.75), $Z=-1.10$, n=36 children, $P=0.273$.

Identification of C. trachomatis *on flies*

The species and sex of the 395 flies tested for the presence of *C. trachomatis* DNA by PCR is shown in Table 2. None of the *M. domestica* were found to be positive, 2 (0.5% of the total) *M. sorbens* were PCR positive. Of these one was male and the other female.

Discussion

Flies as trachoma vectors

We have shown elsewhere (EMERSON *et al.*, 1999) that the control of all fly species in this environment with insecticide resulted in a reduction in the transmission of trachoma. This provides strong evidence that flies are acting as vectors of trachoma, but because of differences

in behavioural ecology between species is it not reasonable to assume that all species of fly are involved in transmission. For a fly species to be a considered as a potential trachoma vector it must satisfy a number of criteria: It must be present when there is disease transmission; it must come into contact with infectious material; it must pick up pathogens and it must transfer sufficient pathogens to a susceptible host to cause a new infection.

The most numerous species caught was *C. albiceps*, a large green fly commonly seen in huge numbers around latrines and market stalls that sell fish, meat or mangoes. Despite being present in large numbers in the hamlets *C. albiceps* was never caught from the eyes of children and therefore should be discounted as a trachoma vector. *M. domestica* was the second most abundant species caught in attractant traps and the species most commonly caught on sticky traps inside houses. *M. domestica* is of public health importance as a proven vector of diarrhoea (WATT & LINDSAY, 1948; COHEN *et al.*, 1991; CHAVASSE *et al.*, 1999) and comprised 8% of flies caught from eyes in this study. *M. 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 for it to be of major significance as a vector. *M. sorbens* accounted for only 8.6% of the total number of flies caught in attractant traps, but was responsible for 92.2% of the fly-eye contacts. It fed aggressively on exudate and could be seen clustered shoulder-to-shoulder on sores or open wounds. We identified *C. trachomatis* on two *M. sorbens* caught from the eyes of children with a known current trachoma infection. This shows that it is possible for flies to pick up the bacteria in a natural setting and provides essential evidence that it is possible for them to be vectors, yet it does not indicate that they are able to transfer the bacteria to a susceptible host, or if sufficient bacteria are transferred for a new infection to develop. The PCR test used in this study was developed for use on eye swabs and modified for use on flies. It is possible that the sensitivity of the test was reduced by the presence of inhibitors washed off the flies during processing and that a greater proportion of flies visiting infected eyes actually pick up the pathogen. Female *M. sorbens* accounted for 73% of those caught in fly-eye catches despite there being an overall sex ratio of 1:1 as measured by the sticky traps placed indoors around the hamlets. A similarly skewed sex ratio was reported from Egypt where 79% of *M. sorbens* caught from "children with diseased eyes"

were female and there was an observed sex ratio of 1:1 in flies caught in traps placed around the village (HAFEZ & ATTIA, 1958b). It is possible that the observed skewed sex ratios in flies caught from eyes is because part-gravid females are attracted to ocular discharge since it represents a protein-rich food source needed for successful egg production. If this hypothesis were correct this subset of the *M. sorbens* population may fly appetitively from eye to eye enhancing transmission potential.

Active trachoma infection is associated with ocular and nasal discharges that contain *C. trachomatis*. The infection is active in the conjunctiva and bacteria probably enter the nasal discharge via the naso-pharygeal sinus. We found that children with these potentially infectious discharges had twice as many fly-eye contacts as those without discharge.

We conducted hand-net collections in the mornings because there was a general perception that flies were less active in the heat of the afternoon. Thus the median frequencies of fly-eye contacts obtained should not be multiplied to predict the total number of contacts in a day. Establishing the pattern of fly-eye contact during the day was not operationally possible as it necessitated using the same child repeatedly at regular intervals during the day, and we did not consider this justifiable.

The evidence implicating *M. sorbens* as a vector of trachoma in The Gambia is strong. It is present throughout the year in trachoma endemic communities, it frequently contacts the eyes of children – particularly those with ocular or nasal discharge, it can harbour *C. trachomatis* and trachoma transmission drops when they are removed from the environment. However, we observed that the number of *M. sorbens* caught in traps increases during the wet season but no seasonality of trachoma transmission has been detected. Further studies on the seasonality of fly-eye contact are required.

Implications for fly control in the prevention of blindness from trachoma
The WHO GET 2020 initiative aims to eliminate trachoma as a blinding disease. Control is based on the "SAFE" strategy of lid Surgery, Antibiotic treatment, Facial cleanliness and

Environmental improvement. For valid operational reasons, national control programmes may put initial emphasis on the S and A part of the strategy; surgery and the use of antibiotics. However, the ultimate sustainability of trachoma control relies on reducing the frequency of infection in addition to the provision of surgery and medication.

Corrective lid surgery is effective for delaying the onset of blindness in people with trichiasis (BOG et al., 1993), but has a poor compliance and requires skilled personnel. The need for lid surgery arises from a history of past infection and will increase as the populations of affected countries age, unless transmission among children is interrupted (SCHACHTER & DAWSON, 1990).

Effective use of antibiotics relies on case detection followed by mass treatment with either a single oral dose of azithromycin or topical application of tetracycline for six weeks. There are problems associated with distribution, the cost of azithromycin and of compliance with tetracycline (MABEY et al., 1991). These may be reduced by drug donation with azithromycin. Mass treatment is effective for reducing infection and transmission in the short term, but has not given lasting control (BAILEY et al., 1993; WEST et al., 1993). If the use of antibiotics is coupled with health education or other methods to control transmission the long-term control of trachoma required to prevent future blindness may be achieved. Supervised face washing of children after a mass treatment campaign has been shown partially effective in an intervention trial in Tanzania (WEST et al., 1995), reducing the chance of having a severe case by 42%, but having no significant effect on all active trachoma. The campaign was very labour intensive and not sustainable in the long term. Facial hygiene may be important in preventing transmission by attracting fewer flies to the face, but hygiene promotion is probably best implemented via existing health education programmes and in the schools rather than as a campaign in its own right.

Although M. sorbens is known to breed in human faeces they were not observed emerging from pit latrines, suggesting that they do not breed in them. Consistent use of pit latrines may therefore effectively remove the M. sorbens larval habitat from the environment. The presence of basic pit latrines has been associated with a lower prevalence of trachoma in risk factor

analyses conducted in Egypt (COURTRIGHT *et al.*, 1991) and Malawi (TIELSCH *et al.*, 1988) although no mechanism for the observed effect was proposed. The provision of basic latrines is likely to be met with community support and may lower trachoma transmission by reducing the *M. sorbens* population.

There is a clear need for feasible and cost-effective methods that reduce trachoma transmission, but because there are several routes it is most unlikely that a "magic bullet" can be found. Assuming that *M. sorbens* is a vector, methods to control it effectively would have a long-term beneficial impact on the prevention of blindness from trachoma – particularly when combined with the use of antibiotics and the provision of surgery.

Acknowledgements

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Figure Legends

Table 1: Background trachoma prevalence by age in the study area

Table 2: Species and sex distribution of eye-seeking flies caught from Gambian children

Table 1

Age	n	Number with signs of active trachoma	%
3 – 59 months	214	42	19.6
5 – 9 years	200	41	20.5
≥ 10 years	510	37	7.3
Total	924	120	13.0

Table 2

	Number caught	% of total catch	Number tested with PCR	% of those tested
♂ *Musca sorbens*	162	24.8	105	26.6
♀ *Musca sorbens*	441	67.4	260	65.8
All *M. sorbens*	603	92.2	365	92.4
♂ *Musca domestica*	3	0.5	3	0.8
♀ *Musca domestica*	48	7.3	27	6.8
All *M. domestica*	51	7.8	30	7.6
Total flies	654		395	

No other species caught