Ecology and control of the trachoma vector Musca sorbens

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The work described in this thesis was conducted in rural Gambia and builds a body of evidence incriminating the fly *Musca sorbens* as a vector of the blinding disease, trachoma, which is caused by ocular infection with *Chlamydia trachomatis*. Literature on hygiene promotion, environmental change and flies and trachoma is reviewed in the context of the SAFE strategy for trachoma control advocated by the World Health Organization. *M. sorbens* was present throughout the year in trachoma endemic communities; was responsible for the majority of fly-eye contacts; *C. trachomatis* DNA was found on it; and trachoma transmission dropped when they were removed from the environment. In a large cluster-randomised trial communities receiving fly control with insecticide for six months had a mean reduction in trachoma prevalence of 56% (95% CI 19-93%; P=0.01) compared to controls and 37% (4-70%; P=0.068) fewer new prevalent cases of trachoma. Breeding media choice experiments showed that isolated human faeces were the preferred larval medium for *M. sorbens* and were capable of supporting the production of large numbers of adults. However, other animal faeces were also able to support *M. sorbens* development. This suggested that a community-based strategy to reduce the quantity of human faeces on the soil surface by providing latrines would have the effect of reducing the population of *M. sorbens*, and hence reduce fly-eye contact and trachoma transmission. The provision of latrines gave encouraging results, which were not statistically significant; 30% less active trachoma than controls after six months (-22-81%; P=0.210) and 28% (-5-60%; P=0.146) fewer new prevalent cases. Provision of latrines warrants further investigation as a method to control trachoma, particularly when used in conjunction with other control methods. The potential role of fly control in the SAFE strategy for trachoma control is discussed.

Acknowledgements

Having the opportunity to conduct the work outlined in this thesis has often felt like a personal indulgence, and I am immensely grateful to have been able to devote the last four years to it. Many people have contributed to it's successful completion, and I wish to extend my thanks to them all. In particular to Steve Lindsay, my supervisor, mentor and friend, for giving me the freedom to aim for the goals that I wanted to, and the structure to help me attain them. To my field supervisors Robin Bailey and Gijs Walraven who have provided immeasurable help on the ground and always shown great belief in me, and commitment to the project. Robin Bailey additionally introduced the field of trachoma research to me, and me to those in the field of trachoma research, for which I am extremely grateful.

The work was based at the Farafenni Field Station of the Medical Research Council Laboratories in The Gambia, and I wish to thank the following for their tireless effort and contribution to this work: Director, Keith McAdam; Station Administrator, Batch Cham; Field Supervisor, Mafuji Dibba; Field Staff, Pateh Bah, Mamadi Jallow, Tumani Kuyateh, Fama Manneh, Pateh Makalo, Yamundow Samba-Jallow, Ngansu Touray and Matarr Tunkara; Drivers, Tijan Cham, Babucar Njie, Karang Njie and Yaya Keita; Data manager, Maimuna Sowe; Data entry clerks, Mamaram Drammeh and Bakary Sonko and all the other support staff who kept the project going.

The project was conducted in partnership with the Gambian National Eye Care Programme (NECP) and with the Gambian Government Department of Community Development (DCD). It would not have been possible to do the project without these partners and I wish to thank the following people for their commitment and unstinting support: NECP; Modou Bah, Bakary Ceesay, Hannah Faal, Sarjo Kanye, Kebba Lowe, Jerreh Sanyang, Omar Sey and Ansumana Sillah; DCD; Foma Ceesay, Seiko Suwareh and Alieu Tunkara. I wish to extend particular thanks to Kebba Lowe and Alieu Tunkara who were attached to the project. Kebba Lowe was not only responsible for approximately 20,000 eye examinations and 82 surgical operations conducted during the work but also for preliminary screening and the provision of medical care to study participants in the villages. I thank him for being 'ever ready' to go to work, for his punctuality, good humour and for sharing my enthusiasm for the project. Alieu Tunkara is another enthusiast for whom I have the greatest praise and admiration; he proved himself to be absolutely reliable, single-handedly conducting the masonry work and overseeing the construction of 596 of our 677 latrines serving 7,450 people – all without deviating from our exacting schedule.

The research was supported by generous grants from the Department for International Development of the British Government and the Edna McConnell Clark Foundation. I am particularly grateful to Pene Key and Neil Squires at DfID and to Joe Cook and Jeff Mecaskey at EMCF for their personal interest, without which it would not have been possible to complete this work. The insecticide used was donated by Agrevo Environmental and I thank John Invest and John Goose for co-ordinating its safe delivery.

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This has been a community-based research project which has operated in 37 separate villages in the North Bank and Central River Divisions of The Gambia. I am indebted to the 8,600 residents of these villages who co-operated with interviews, attended for eye exams and allowed us free access to their homes. The enthusiastic participation of these people allowed this project to take place and often inspired us to keep going.

I dedicate this thesis to my parents Ron and Beryl Emerson; wife, Amy Ratcliffe; and daughter, Charlotte Emerson, in recognition of their bountiful support and encouragement. In particular I thank my parents for indulging me as a 'perpetual student' and hope that they can feel proud to see their investment reach this point.

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List of abbreviations

Standard SI units are used throughout the text, in addition the following abbreviations are used.

CI	Confidence Interval
CON	Community Ophthalmic Nurse
DCD	Department of Community Development of the Gambian Government
DFID	Department for International Development of the UK Government
ELISA	Enzyme-linked Immuno-sorbent Assay
EMCF	Edna McConnell Clark Foundation
GET	Global Elimination (of blinding) Trachoma
ITI	International Trachoma Initiative
IQR	Inter Quartile Range
MRC	Medical Research Council
N	Number of subjects, participants, or repetitions of a trial
NECP	National Eye Care Programme of the Gambian Government
OR	Odds Ratio
P	Probability Value (in statistical analysis)
PCR	Polymerase Chain Reaction
RACO	Royal Australian College of Ophthalmologists
SAFE	Surgery, Antibiotic, Facial cleanliness, Environmental improvement
ULV	Ultra Low Volume
WHO	World Health Organization

Declarations

None of the material contained in this thesis has been previously submitted for a degree in this or any other university. Five of the chapters have appeared as multiple-author papers in peer-reviewed journals in a different format. The authors on the papers are given at the start of each chapter concerned and their contributions listed below.

Contribution of other authors to papers

Chapter 2: 'A review of the evidence base for the "F" and "E" components of the SAFE strategy for trachoma control'

Working in collaboration with the other authors, Paul Emerson was responsible for the identification and collation of papers for inclusion in the review; wrote the first draft; and had primary responsibility for editing. All authors contributed to the form of the paper and had editorial input. David Mabey oversaw the project.

Chapter 3: 'Pilot study on the effect of fly control on trachoma transmission and childhood diarrhoea'

All the investigators contributed to the design and implementation of the study. Paul Emerson was responsible for the field work and Kebba Lowe conducted all eye examinations. Analyses were performed by Paul Emerson, Steve Lindsay and Robin Bailey. Paul Emerson wrote the paper which was edited by all investigators.

Chapter 4: 'Transmission ecology of the fly *Musca sorbens*, a putative vector of trachoma'

Paul Emerson and Steve Lindsay were responsible for the design and statistical analysis of the paper. Field work was co-ordinated and conducted by Paul Emerson, Kebba Lowe conducted eye exams. Paul Emerson wrote the paper and all authors contributed to editing it.

Chapter 5: 'Human and other faeces as breeding media of the trachoma vector *Musca sorbens*'

Paul Emerson and Steve Lindsay were responsible for the design of the study and statistical analysis. Paul Emerson co-ordinated and conducted the field and laboratory activities and wrote the paper. Additional statistical advice was given by Amy Ratcliffe. All co-authors helped edit the text.

Chapter 6: 'The Flies and Eyes Project: Design and methods of a cluster-randomised intervention study to confirm the importance of flies as trachoma vectors in The Gambia and to test a sustainable method of fly control using pit latrines'
Paul Emerson, in collaboration with; Steve Lindsay, Gijs Walraven, and Robin Bailey wrote the original project description. Paul Emerson wrote the paper and all authors had editorial input.

Contribution of field team and data manager
It would not have been possible for a single person to have conducted all the field work described in this thesis. Field work was carried out by a team of up to 10 village assistants and five field assistants, supervised by a senior field assistant and field supervisor. Paul Emerson had primary responsibility for the selection, training and overall supervision of all field staff. Data forms were checked by the field supervisor and Paul Emerson, and entered by dedicated data entry staff. Double entry and validation of data sets was supervised by a data manager.

Chapter 1

Background and introduction

Figure 1.1 *Musca sorbens* visiting the eye of a young boy (*reproduced with permission from the parents*)

Background and introduction

Background

Any visitor to a Gambian village will be well aware of the annoyance and irritation caused by domestic flies as they buzz around food and crawl over skin. They are a considerable nuisance, disturbing every activity from laundry to brewing green tea. Flies are tolerated by the villagers, even though they are labelled in people's minds as unhygienic and associated with poor health. And they are not included in disease control programmes. The work described in this thesis is the result of four and half years of intensive field-based investigations conducted with the goal of putting domestic flies back onto the public health agenda. The specific aims were to demonstrate whether flies are vectors of the blinding disease trachoma and to identify a practical method to control them.

Non-biting, domestic flies act as mechanical vectors of disease, picking up pathogens from a contaminated material that people would normally avoid, and transferring them to people, or onto food and eating utensils, that are not infected. Domestic flies are not usually the sole vector of any disease or condition and provide one transmission route among many. The relative contribution they make to disease transmission will vary between locations and years and depend, among other factors, on: the fly species present; the size of the fly population; the availability and attractiveness of infectious materials; and climatic conditions. Since flies only contribute to transmission, demonstrating the link between them and disease relies on removing them from the system whilst leaving other variables the same. Most attempts to incriminate them have been uncompelling and beset with methodological flaws; other evidence presented has been incomplete or anecdotal (full reviews in Esrey, 1991; Levine & Levine, 1991 and Graczyk *et al.*, 2001)). As there is a lack of substantive evidence linking flies to disease transmission, and there is a perception that it is difficult to control them, fly control has not been included among disease control programmes (e.g. Feachem, 1986), and it is time that this situation was redressed. This thesis examines the role of the fly *Musca sorbens* Wiedemann in trachoma transmission and, with reference to its ecology, proposes a sustainable method of fly control using pit latrines.

A general introduction to *Musca sorbens* and trachoma

Musca sorbens

Musca sorbens, variously known as the Bazaar fly, Filth fly, Sweat fly, Face fly and Bush fly is a close relative of the ubiquitous Housefly *M. domestica* Linnaeus, and the species are frequently sympatric. They are both medium sized, predominantly grey, flies, usually about 6-8mm in length with a wing span approximately twice the body size. *M. sorbens* can be distinguished morphologically from *M. domestica* by *M. sorbens'* overall more metallic silvery-grey appearance and the pattern of stripes on the dorsal thorax: *M. sorbens* has two broad thoracic stripes below the transverse suture and *M. domestica* has four. The sexes of *M. sorbens* are dimorphic with the male being holoptic (the eyes practically meeting on the top of the head, Figure 4.4) and the female dichoptic (the eyes separated on the top of the head, Figure 4.5) and the thoracic stripes on the female bifurcating anterior to the transverse suture, but remaining continuous on the male (Figure 1.2 and 1.3). *Musca sorbens* is characterised by its close association with people, its attraction to human faeces and its appetite for bodily exudate such as tissue fluid, mucus and pus, on which it feeds avidly.

Figure 1.2 Female *Musca sorbens* showing bifurcating thoracic stripes

Figure 1.3 Male *Musca sorbens* showing continuous thoracic stripes

The Musca sorbens *complex*

Musca sorbens is frequently referred to as though it is a single species. Through cross-breeding experiments with two African populations and an Australian population, Paterson and Norris (1970) showed that *M. sorbens* is probably a species complex consisting of at least three species; *M. sorbens*, *M. biseta* Hough and *M. vetustissima* Walker. *M. sorbens* can be differentiated from *M. biseta* and *M. vetustissima* by the possession of a relatively broad frons (the gap between the eyes, on the top of the head) in the male and more numerous proclinate setulae (fine downward pointing hairs) on the face of the female (Figures 4.4 - 4.7). There is general agreement among taxonomists (Paterson & Norris, 1970; Pont, 1991; Crosskey & Lane, 1993) that the narrow frons form from Australia (the Bush fly) is *M. vetustissima* and those from Africa and the Middle East are *M. biseta*. However the species complex is recorded across the southern Palaeartic and Oriental regions, with both broad and narrow frons forms represented, and it is not clear where the species boundary – if indeed there is one – between *M. biseta* and *M. vetustissima* exists. Throughout this thesis I will use the term *M. sorbens* generically to refer to the African members of the species complex; *M. sorbens* and *M. biseta* as the existing evidence suggests that the ecology of the two species is very similar (Skidmore, 1985; Pont, 1991).

Relationship with man: Synanthropy and euanthropy

The term 'synanthropic' - from the Greek *syn* (with) and *anthropos* (man) - is applied to certain species of flies and rodents which are associated with people and seldom found far away from them or their dwellings. Euanthropic organisms are those that have co-evolved with man, and that are not only adapted to an environment which is shaped by human activity, but are reliant on humans to provide a suitable habit. *M. sorbens* is a euanthropic fly and is closely associated with man. It probably originated in Africa and followed the human migration from that continent, colonising any area that the people colonised and only restricted in its expansion by its trophic requirements (Povolný, 1971). The fly is now distributed throughout the Afrotropical, southern Palaeartic, Oriental and Australasian regions (Pont, 1991), but failed to follow the human migration over the transient land bridges to the New World, and has not been reported from either North or South America. The absence of *M. sorbens* from Mexico and Brazil could well be the reason that flies have not been linked with trachoma transmission in these countries (Taylor *et al.*, 1985; Luna *et al.*, 1992). Adult *M. sorbens* feed directly from people or on food gathered by people, they lay their eggs on the faeces of people, or their domestic livestock, (Hafez & Attia, 1958a; Chapter 5) and they even rest at night on the outside walls of peoples' houses (Greenberg, 1971). It is the combination of this affinity with people and its aggressive feeding on substances that may contain pathogenic organisms that make *M. sorbens* a potential mechanical vector of human disease including trachoma.

Life cycle of Musca sorbens

Musca sorbens is a typical holometabolous insect with complete metamorphosis and four distinct life cycle stages: egg, larva (3 stages), pupa and adult. Eggs are laid in cracks on the surface of a suitable larval medium and they hatch to release the first larval instar (L1) which immediately burrows into the food source. Larvae feed continuously and moult twice to the third instar (L3); when the L3 are mature they descend into the underlying soil for pupation. The pupa forms within the tanned skin of the L3, which is then correctly termed a puparium (Crosskey, 1993). The emerging adult forces its way out of the puparium and through the soil with the aid of the ptilinum, a bladder-like organ temporarily extruded from the head, and never used again. Hafez and Attia (1958a) report that a female lays an average of 3-4 batches of up to 32 eggs in a life-time with a pre-oviposition period of 4-9 days. Larger egg masses of hundreds of eggs observed on particularly attractive media are probably the result of several females ovipositing together, which may indicate the existence of an oviposition pheromone, although this has not been investigated. The duration of development is temperature dependent with lower temperatures resulting in longer incubation periods; the minimum average incubation time observed in Egypt was 5.3 hours at 40ºC, with higher temperatures resulting in the death of the egg (Hafez & Attia, 1958b), incubation took an average of 8.0 hours at 28ºC. Larval development is similarly temperature dependent. At Egyptian room temperature (usually above 32ºC) Hafez and Attia (1958b) report average larval periods as 7 hours for L1; 14 hours for L2; and 40 hours for L3. The total mean larval period at Egyptian room temperature was 61 hours. At a constant 32ºC this rose to 74 hours and rose to 90 hours at 28^{0}C. The puparial period lasted an average of 3.9 days at 32^{0}C and 4.3 days at 28^{0}C. Thus, at a constant 28^{0}C total development time from egg to adult is around 8.4 days.

The theoretical capacity for population growth is therefore tremendous. Assuming limitless larval media, no predation at any stage, a mean life span of just 13 days and a constant temperature of 28^{0}C, one female emerging on the 1st of August could give rise to 17.8 million adult flies by the 14th October. Thankfully the theoretical growth potential is far in excess of that possible under valid ecological conditions and the population of flies is limited by predators, parasitoids, disease and, probably most importantly; the availability of suitable larval media (Hughes *et al.*, 1972). Given the rapid reproductive potential of *M. sorbens* control strategies aimed at adults are unlikely to be successful for very long unless they are also accompanied by efforts to reduce the availability of suitable breeding media.

Trachoma

Trachoma is an infection of the conjunctiva – the lining covering the eye and inside of the eyelid. It is the second leading cause of blindness world-wide and the greatest cause of infectious blindness, with some 300-500 million people affected of whom an estimated 5.8 million are blind (Thylefors *et al.*, 1995). The specific etiological agent, the bacterium *Chlamydia trachomatis*, is an obligate intracellular organism with no free-living state and no known animal reservoir (Muñoz & West, 1997). The absence of an environmental reservoir of the pathogen is of great significance to control efforts, as it implies that a reduction of human infection would lead to fewer opportunities for transmission, and the possibility for elimination of the bacteria from treated regions. *Chlamydia trachomatis* can be separated into several serovars, the primary serovars responsible for trachoma are A, B, Ba and C whilst serovars D-K are associated with genital infection (Dawson *et al.*, 1981). Whilst it is possible for ocular infection with the genital serovars to occur (particularly at birth), transmission of them does not appear to occur between eyes (Brunham *et al.*, 1990).

Life cycle of Chlamydia trachomatis

Chlamydia have a unique developmental cycle in which the organism has two forms; the elementary body and the reticulate body. The elementary body can be considered the transmission form as it is infectious but metabolically inert, encased in an environmentally resistant peptide shell. When in contact with a susceptible host cell the elementary body enters by endocytosis and transforms into the metabolically active reticulate body and multiplies rapidly to form an inclusion body that can occupy up to 90% of the cell cytoplasm. After about 20 hours the reticulate bodies transform into elementary bodies and, with rupture of the host cell, are released leading to infection of neighbouring epithelial cells (West & Muñoz, 1998).

Disease cycle of trachoma

The onset of trachoma is often unnoticed and gradual. In mild cases there is slight ocular discomfort and minimal sensitivity to light accompanied by some watering of the eye, possible foreign-body sensation and a little discharge. Severe cases, which may also have corneal involvement, are characterised by marked photophobia and considerable watering and pain. Unless there is an additional associated bacterial infection trachoma does not cause copious purulent discharge (Dawson *et al.*, 1981). The consequence of repeated ocular infection is scarring of the tarsal conjunctiva and thickening of the eyelid. The inflexible scar tissue pulls the inner lining of the eye lid tighter and causes entropion trichiasis, in which the eye lashes turn in and touch the surface of the eye. Patients with trichiasis experience dry eyes and constant pain as the lashes rub against the cornea and frequently seek temporary relief by epilating. Bacterial and fungal infections leading to ulceration, and physical damage to the cornea by the lashes, lead to eventual corneal opacity and blindness that can only be reversed by corneal transplant.

Although clinical signs of trachoma often correlate with current active infection with *C. trachomatis* detectable levels of bacteria can be present in the absence of clinical signs, and clinical signs can be apparent in the absence of bacteria (Taylor *et al.*, 1989a; Schachter *et al.*, 1999). This is interpreted to indicate that there are non-patent infections in some people and also that there is a lag between infection and the presentation of clinical signs. Active infection is required for a period of time before clinical signs appear, and those signs remain as relics after the infection has been resolved. Despite this inconsistency in the correlation of actual infection with clinical signs it is still reasonable and useful to classify clinical trachoma signs as either 'active' or 'non-active'. Active cases indicate current or recent infection and on each occasion are believed to be relatively benign. Non-active cases are an indication of the historic clinical picture, and in the presence of a high prevalence of active cases suggest that blinding trachoma will continue to be a problem (Mabey & Bailey 1999).

How can Musca sorbens *be a vector of trachoma?*

There are several recognised routes by which non-biting flies can act as disease vectors: carriage of pathogens on the surface of the fly; carriage of pathogens by ingestion and regurgitation; carriage of pathogens by ingestion and defaecation; or acquisition of pathogens by ingestion, multiplication of the pathogen in the fly gut and deposition of the pathogen in vomitus or faeces (Hewitt, 1910; West, 1951; Greenberg, 1973; Crosskey & Lane, 1993).

The elementary body form is the transmission stage of *C. trachomatis* and can be detected from eye and nose swabs from current active cases (West *et al.*, 1991a; Bailey *et al.*, 1994). The surface of the conjunctiva and ocular or nasal discharge from currently infected people (including that on clothes or handkerchiefs) can be considered as the source of infection for flies. Elementary bodies will be encountered by flies when they feed directly from the eye or on ocular and nasal discharge from active cases. The fly can either suck and filter food into its mouth via the labella or rasp the surface from which it is feeding with its prestomal teeth and then suck up the substrate. Hookworm eggs with dimensions 60μm by 40μm have been recovered from the gut of *M. sorbens* feeding on human excrement (Sulaiman *et al.*, 1988) so the *C. trachomatis* elementary bodies (6-10μm) should be swallowed with ease. In common with other Muscids *M. sorbens* has chemoreceptors in the tarsi of the front feet with which it 'tastes' potential food before feeding (West, 1951). The proboscis and front feet, with their uneven integument and myriad tiny hairs, could therefore become contaminated with *C. trachomatis* elementary bodies for surface carriage and elementary bodies could also be ingested for internal carriage and later deposition in the faeces and vomit. For transmission to take place the elementary body must come into contact with the conjunctival epithelium of a different human host. This is not likely to occur with elementary bodies in the faeces, but is possible for those regurgitated and likely for those that remain on the feet and proboscis. Eye-seeking flies in Iran (most likely *M. sorbens*, but not identified) have been shown capable of transferring flourescein from one eye to another in this way (Jones, 1975; Jones, 1980). The likelihood of transmission by the surface route is increased by the relative resistance to desiccation and ultra violet light of the elementary body, and the aggressive nature of *M. sorbens* when feeding, which moves directly from eye to eye. Other species, such as *M. domestica*, that feed on a wide range of substrates, only occasionally visiting eyes, are less likely to transmit *C. trachomatis* as they may deposit any attached elementary bodies before returning to an eye, so a transmission event is less likely to take place.

Flies are at risk from infection acquired when feeding and groom themselves frequently. In the Malaysian study of carriage of human helminths by flies (Sulaiman *et al.*, 1988), helminth eggs were found in the gut contents a hundred times more frequently than on the surface of the flies. This may have been because the flies were held in cages for a number of hours before being killed by freezing, and whilst trapped they will have been grooming, which may have removed any eggs on the integument. The likelihood of surface carriage taking place will be greatest when the fly behaviour is to move directly from infectious source to potential new host, without diversion, and without grooming. By apparently exhibiting a preference for feeding from eyes and on ocular and nasal discharge, and readily moving directly from eye to eye there is considerable potential for *M. sorbens* to be a trachoma vector.

Chapter 2

A review of the evidence base for the "F" and "E" components of the SAFE strategy for trachoma control[1]

Figure 2.1 Flies on the face of a child
(*reproduced with permission from the parents*)

[1] This Chapter appeared as a paper with the same title by P.M. Emerson, S. Cairncross, R.L. Bailey and D.C.W. Mabey in *Tropical Medicine and International Health* (2000): **5**; 515-527.

A review of the evidence base for the "F" and "E" components of the SAFE strategy for trachoma control

Abstract

Community control of trachoma as a blinding disease is based on the SAFE strategy of Surgery, Antibiotic therapy, Facial cleanliness and Environmental improvement. Surgery and antibiotic therapy presently dominate most programmes. Blindness from trachoma results from frequent infections repeated over many years, so ultimate success requires the reduction of transmission. This is only likely to be sustainable through the F and E components of SAFE.

Environmental improvement with access to water, enhanced hygiene and better sanitation reduces trachoma transmission and the blinding sequelae eventually disappear. Transmission routes and factors that cause this are not known and consequently no single specific tool for F and E is in place.

Evidence from intervention studies shows that the promotion of face-washing gave modest gains for intense effort and a pilot study showed that trachoma transmission was reduced in the absence of eye-seeking flies. Other studies have shown that the presence of latrines and improved access to water are associated with a lower prevalence of active trachoma. There is likely to be a long-term beneficial effect of a combination of improved water supplies, provision of latrines, facial hygiene promotion through established infra structure and control of eye-seeking flies. Each of these interventions offers additional public health and other benefits in its own right.

Further research on the routes of transmission, the role of hygiene and the means of sustainable fly control should be a priority.

Introduction

Trachoma is a contagious eye disease caused by ocular infections with the bacterium *Chlamydia trachomatis*. It is the commonest cause of infectious blindness world-wide with hundreds of millions at risk and an estimated 5.8 million blind (Thylefors *et al.*, 1995). Blindness from trachoma is linked to poverty and is two to three times more likely to occur in women than men (Tabbara & Ross-Degnan, 1986; West *et al.*, 1991b). Trachoma afflicts the most deprived people in the world, people marginalised and without political voice (Mecaskey, 1998; Mabey & Bailey, 1999), it causes disability, dependency and poverty and is a barrier to development.

The challenge posed by the control of this disease has resulted in the formation of the WHO alliance for the Global Elimination of Trachoma as a blinding disease by the year 2020 (GET 2020). The alliance aims to control blindness from trachoma through the SAFE strategy, that is the provision of surgery, antibiotic therapy, facial cleanliness and environmental improvement. Corrective lid surgery is effective for delaying the onset of blindness in people with trichiasis (Bog *et al.*, 1993) and is a priority where there is a backlog of people at immediate risk. Regrettably surgical compliance is usually poor: only 18% of 205 women offered free treatment and transport had actually undergone an operation after two years in one study from Tanzania (West *et al.*, 1994). Treating trichiasis by mechanically sticking the lashes to the outside of the lid has been proposed as an alternative to surgery (Graz *et al.*, 1999), but the technique is yet to be adopted by the GET 2020 alliance. The rational use of antibiotics relies either on frequent mass treatment or targeted treatment to 'at risk' groups such as all children under ten in hyperendemic areas or, because ocular chlamydial infection is transmitted within the family, cases and their families. Treatment is with either a single oral dose of azithromycin or topical application of tetracycline for six weeks. Treatment is effective on an individual basis (Bailey *et al.*, 1993) and mass treatment can reduce infection and transmission in the short term, but has not given lasting control (West *et al.*, 1993; Schachter *et al.*, 1999). The frequency of mass azithromycin administration required to eliminate active trachoma from an area has been modelled (Lietman *et al.*, 1999). In hyperendemic areas (where >50% of children are active cases) the model suggests that with 100% coverage biannual treatment of all people would be required for the elimination of active cases. In areas where trachoma is moderately prevalent (<35% active cases in children) annual treatment of all people would be sufficient.

Blindness from trachoma is a result of frequent infections repeated over many years and the ultimate success of GET 2020 relies on blocking transmission and reducing the community prevalence of infection. It is logistically impractical to achieve this by surgery and antibiotic therapy alone and control programmes need to be enhanced by the inclusion of strategies that reduce transmission – this is the F and E part of SAFE. It is known that overall development of an area causes the prevalence of trachoma to decline and the blinding sequelae to eventually disappear (Nichols *et al.*, 1967; Bobb & Nichols, 1969; Dolin *et al.*, 1997) and that community based programmes can reduce the prevalence of active trachoma (Sutter & Ballard, 1978; Sutter & Ballard, 1983), but the specific mechanisms by which this occurs are not known.

This chapter is a response to a call from affected countries at the third meeting of the WHO Alliance for the Global Elimination of Trachoma for a review of the evidence base linking facial cleanliness and environmental improvement with trachoma control. There is a need to incorporate these aspects of the SAFE strategy into national trachoma control programmes and the potential public health contribution of the strategies available are reviewed. Papers were identified by referring to the earlier works of Prost and Négrel (1989) and Marx (1989), from a multilingual MEDLINE and BIDS search of publications from 1975 to 2000 and from citations noted in these studies.

Trachoma as a disease

For simplicity of diagnosis and consistency of reporting between areas, clinical trachoma and its complications have been divided into grades (Figure 2.2) (Thylefors *et al.*, 1987). Throughout this review the term 'active trachoma' refers to grades TF and TI and is used as an indicator of current trachoma prevalence. 'All trachoma' also includes TS, TT and CO which demonstrate past infection and are a measure of the historical impact of trachoma. The WHO grading system describes the condition of the upper conjunctiva at one point in time and the grades should only be considered sequential in that TS, TT and CO occur only after a history of the active grades. Each active infection is self-limiting and does not necessarily lead to an inexorable decline to blindness. A history of past infection manifests itself as scars on the upper conjunctiva which start to become prevalent among older children in hyperendemic areas. If the scarring is severe it can cause the lid margin to become distorted, turning the lashes inwards so they rest against the globe. In addition to the intolerable discomfort this must cause, the constant abrasion and secondary infections can lead to opacity of the cornea, loss of vision and eventually to blindness. The prevalence of scarring and the blinding sequelae increases with age and is most commonly seen in older adults (Muñoz & West, 1997).

Grade	Description
N	Normal eye, no evidence of current or past infection.
TF	Follicular trachoma: Presence of 5 or more follicles >0.5mm in diameter in central zone.
TI	Trachomatous inflammation- intense: pronounced inflammatory thickening that obscures more than half of the normal deep tarsal vessels.
TS	Trachomatous scarring: presence of easily visible scarring.
TT	Trachomatous trichiasis: at least one eyelash rubs on the eye ball. Evidence of recent removal of inturned lashes should also be graded as trichiasis.
CO	Corneal opacity: presence of easily visible corneal opacity over the pupil.

Figure 2.2 WHO simplified system for grading trachoma (upper eyelid is everted and the tarsal conjunctiva visually examined with a x2.5 binocular loupe and adequate light)

Many active infections are asymptomatic and appear to cause little or no discomfort (Dawson *et al.*, 1981; Mabey *et al.*, 1991a). Each individual active infection probably presents insignificant long term risk, but the contribution is not known. Community prevalence rates of active disease of up to 20% can be associated with levels of trichiasis below 1% (Luna *et al.*, 1992; Sukwa *et al.*, 1992) but because of demographic changes the population in many affected areas is ageing, implying that more people will survive to old age when blindness from trachoma develops (Schachter & Dawson, 1990). To diminish the risk of blindness from trachoma it may not be necessary either to find and treat all cases, nor to eradicate the infection from the population. The transmission intensity need only be reduced such that the community prevalence falls below an as yet undefined threshold for the development of blinding complications.

Geography vs poverty

The current geographical distribution of trachoma is largely, but not entirely, associated with hot and arid parts of the world such as the Sahel, the interior of Australia and the highlands of Ethiopia and Nepal. This has lead to a general perception that trachoma is linked with dry environments and a scarcity of water. Trachoma has not always followed this distribution and has in the past been of major public health concern in more temperate climates. Indeed, the disease was so widespread in London at the start of the 19th century that Moorfields Eye Hospital was founded in 1805 largely to deal with it (Jones, 1980). In 1931 MacCallan included Latvia, Poland, Estonia, Finland and Czechoslovakia in a list of countries where trachoma was considered 'very common', and special trachoma clinics continued to be held until 1949 in Finland (Prost & Négrel, 1989). Within a geographical area the prevalence of trachoma is not necessarily greater where there is poor access to fresh water. Mann (1967) reported the prevalence of signs of trachoma as 8.6% in people from the Marshall Bennett islands, where there was 'no access to drinking water' - the people instead 'relying on the water from green coconuts', and 45.9% in the wet and humid capital of Papua New Guinea, Port Moresby.

Studies from India (Taylor *et al.*, 1958), Brazil (Luna *et al.*, 1992), Australia (Royal Australian College of Ophthalmologists, 1980; Tedesco, 1980), Japan (Marshall, 1968), Malawi (Tielsch *et al.*, 1988) and Saudi Arabia (Barenfanger, 1975) have all shown a higher prevalence of trachoma in households with lower income than their neighbours and in which the head of the household is poorly educated (reviewed in Marx 1989). Trachoma is a problem among the disadvantaged and dispossessed, whether they be nomads eking out an existence in the Australian desert, subsistence farmers on the edge of the Sahara or impoverished workers in pre-industrial cities. Hot and arid may simply be a proxy for poverty in the modern world.

The transmission of trachoma

Reservoir

Cases of active trachoma that shed the elementary body stage of *C. trachomatis* are believed to be the main source of infection. Many studies have shown that the prevalence of active trachoma is greatest among pre school-age children and then decreases with age (Kupka *et al.*, 1968; Dawson *et al.*, 1976; Taylor *et al.*, 1985; Tielsch *et al.*, 1988; West *et al.*, 1991b). Infections in children persist longer than in adults (Bailey *et al.*, 1999) suggesting that infected children form a reservoir of infection. *C. trachomatis* can also be found in the genital tract and in the naso-pharynx.

Cervical infections, caused by *C. trachomatis* serotypes D-K, can be transmitted vertically during birth and this was previously believed to be an important route of transmission (Jones, 1975). It is now known that these genital serotypes are only rarely transmitted between eyes (Brunham *et al.*, 1990). Naso-pharyngeal *C. trachomatis* may be important in trachoma transmission but could be a result of ocular infection and probably does not constitute a separate reservoir. West *et al.* (1993) found that children with active trachoma were also likely to have nasal specimens positive for *C. trachomatis*. However, the rate of reinfection after topical eye treatment was similar in children with a positive nasal swab at baseline compared to those who were negative, when faster reinfection in those with positive swabs may have been expected if there were a naso-pharyngeal reservoir. Systemic antibiotic treatment would remove a non-ocular reservoir, so we might expect that it would offer longer protection from reinfection than topical treatment. Bailey *et al.* (1993) showed that the rate of reinfection in children treated with a systemic antibiotic was the same as in those treated topically. No animal reservoir has been found, and extra-ocular sites of infection do not appear important in trachoma epidemiology. The eyes of infected people, particularly children, should be considered as the main reservoir of infection.

Clustering of cases

The prevalence of signs and symptoms of trachoma is not uniform within a geographical area and can vary greatly between villages. Within a village trachoma is strongly clustered by household (Katz *et al.*, 1988; Tielsch *et al.*, 1988; Mabey *et al.*, 1992a), suggesting that the type of prolonged close contact found within families is required for high levels of transmission. Strong evidence implying that intimate contact is important has come from multivariate studies which identify sharing a sleeping room with an active case as a major risk factor for trachoma (Bailey *et al.*, 1989; Courtright *et al.*, 1991; West *et al.*, 1991b). Crowded living conditions within a family have been shown to increase the risk of trachoma in some studies (Assad *et al.*, 1969; Barenfanger, 1975), but not others (Bailey *et al.*, 1989). It is unclear whether this is the result of crowding *per se*, or because crowded families are likely to be larger with more pre school-age children and less well off than their neighbours.

Transmission routes

Knowledge of the mechanisms of transmission has not changed substantially since the seminal work of MacCallan (1931) who proposed four of the five routes listed below over 50 years ago. Possible routes of transmission include:

i) Direct spread during play or when sharing a bed.

ii) Conveyance on fingers.

iii) Indirect spread on fomites (shared handkerchiefs, towels etc.).

iv) Eye-seeking flies.

v) Coughing/sneezing.

The relative importance of all of these routes may be different in different places and at different times and it is unlikely that any single route is responsible for all the transmission in a particular area. It will be difficult to establish the relative importance of these routes of transmission even using multivariate analyses of risk factors, which control for the effects of age and clustering, or intervention trials that seek to examine the effect on transmission of blocking a suspected route. In the absence of a single route of infection the findings of such studies may only be of local relevance and it is doubtful that a single globally applicable 'magic bullet' to stop transmission can be found. Nevertheless, attempts to elucidate transmission may be important for the formulation of locally appropriate 'F' and 'E' strategies.

Trachoma and water

Although trachoma is currently associated with arid parts of the world where access to water is limited and consequently there is a low standard of personal hygiene, the published evidence linking trachoma with water is not conclusive. The mechanisms by which improved access to water is supposed to influence trachoma transmission are seldom explained and are not entirely clear. It has been postulated that increased frequency of laundry - specifically bed sheets and handkerchiefs - may reduce transmission on fomites (Taylor *et al.*, 1958). If transmission on fingers is important the use of water for personal hygiene purposes may reduce the frequency of infection (Wilson *et al.*, 1991). Face washing may decrease the accessibility of infectious discharge to flies (Taylor *et al.*, 1989b) and make infected eyes less attractive to eye-seeking flies, since it is known that the presence of ocular or nasal discharge increases the number of flies attracted to a face (Chapter 4). Whilst stating that face washing ...'obviously has no effect on the course of disease'... Muñoz and West (1997) conjecture that it ...'may reduce the likelihood of autoreinfection'..., presumably by flushing the elementary body form of the pathogen from the eye when rinsing.

The distance to a water source has been used as a predictor of disease; some studies have shown that an increased distance to water increases the risk of trachoma (Mathur & Sharma, 1970; Tielsch *et al.*, 1988; West *et al.*, 1989), whereas others have shown no effect of distance (Kupka *et al.*, 1968; West *et al.*, 1991a). One study from Ethiopia reports that people living greater than 15 minutes walk from water actually had less active trachoma than those living less than 15 minutes away (Zerihun, 1997). These apparently contradictory results may be explained by the observation that water consumption *per capita* is remarkably constant between households where the round trip to a water source is 30 minutes or less (Figure 2.3). The 'water use plateau' (Cairncross & Feachem, 1993) has been documented by studies from East, West and Southern Africa, Asia and Central America and suggests that water consumption will differ from the cultural norm only where the supply is in-house or further than 15 minutes walk away (i.e a round trip of greater than 30 minutes).

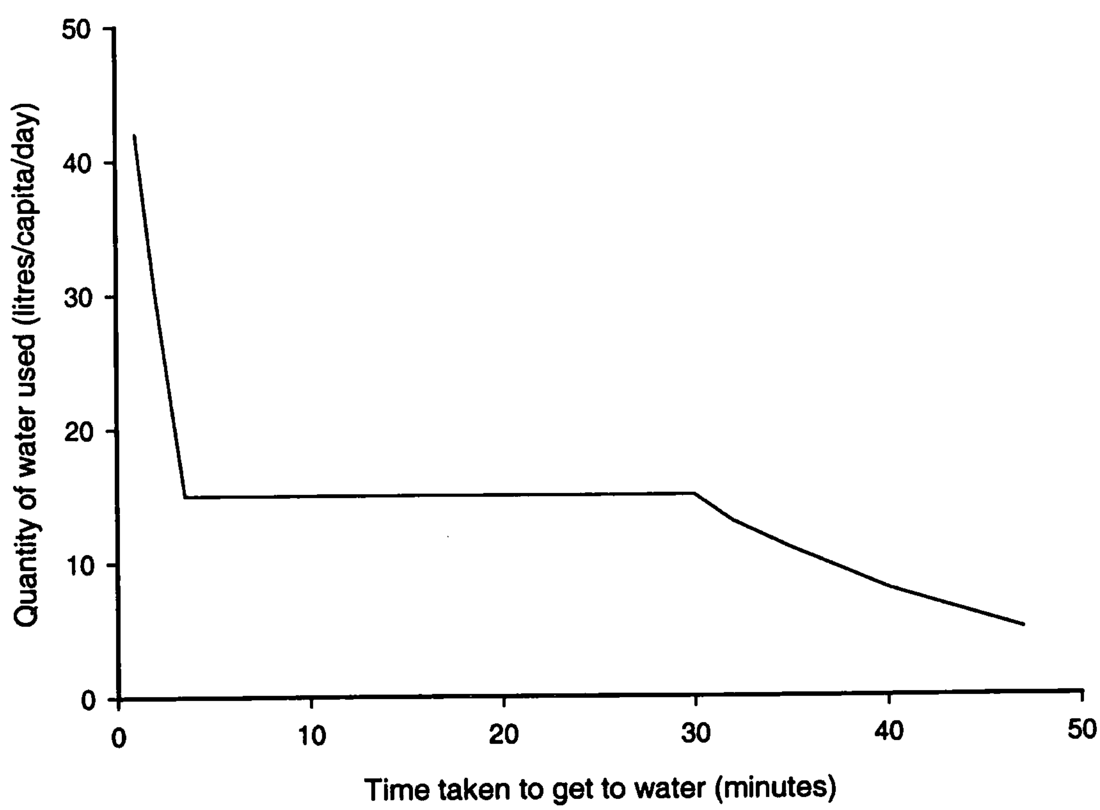

Figure 2.3 Domestic water consumption and time required for water collection

The quantity of water brought into a household may be of more importance than the distance it has come. In Morocco the quantity of water brought into a house was found to be independent of the distance, and children from households with a greater quantity of water had less active trachoma (Kupka *et al.*, 1968). Conversely two other studies controlling for distance have found that the total quantity of water used has no effect on the prevalence of active trachoma (West *et al.*, 1989; Bailey *et al.*, 1991). One of these studies (Bailey *et al.*, 1991) observed how the water was used in the household and found that trachoma free households used more water for washing children than households with trachoma cases.

The evidence suggests that the availability of water may well have an impact on the epidemiology of trachoma, but it is difficult to disentangle the effect of water availability from other indicators of socio-economic development and the living standard of the study subjects. In areas with poor water availability the provision of water not only provides the means for improved hygiene and cleanliness, it also releases the time needed for these activities to take place – the two greatest inhibitory factors to facial cleanliness identified in a survey conducted in Tanzania (McCauley *et al.*, 1990).

Literature on hygiene and environmental risk factors for trachoma- problems of methodology

Data from the literature (Table 2.1) come from two types of study; observational and intervention. The majority of the information available comes from observational studies that compare the trachoma situation in a number of different locations in an attempt to identify risk factors. There are only two well-conducted intervention studies (West *et al.*, 1995; Emerson *et al.* 1999) that have sought to investigate the effect of a specific change on trachoma transmission whilst keeping potentially confounding variables the same. In common with the literature on the impact of water supply on diarrhoeal diseases (Blum & Feachem, 1983) the trachoma literature is beset with a number of problems of methodology that make interpretation difficult:

1. Lack of adequate controls. An environmental change or intervention can have a positive, neutral or deleterious effect on the prevalence of trachoma in a community. In order for meaningful conclusions to be made it is vital that adequate controls are in place. Problems of control arise in several forms; the comparability of groups not established at baseline (Portney & Hoshiwara, 1970; Cairncross & Cliff, 1987), obvious differences between groups making them incomparable (De Sole, 1987), surveys conducted in different seasons (Marshall, 1968; RACO, 1980), or even years apart (Kupka *et al.*, 1968).
2. It is known that trachoma clusters by household which results in a lack of independence between cases which should be taken into account during analyses at the individual level (Taylor, 1958; Marshall, 1968; Assad, 1969; RACO, 1980; Tedesco, 1980; Zerihun, 1997)

3. Confounding variables such as sex and age not taken into account. Active trachoma is more prevalent in children and blinding trachoma more prevalent in women. Comparisons of active trachoma prevalence should be standardised by age and blinding trachoma by age and sex (Kupka, 1968; Marshall, 1968; Mathur, 1970; RACO, 1980; Taylor, 1985).

4. Repeatability and validity of survey methods. It is reasonable to assume that if a single observer screened all subjects then systematic errors would occur evenly between groups. Where two or more observers screen different groups then the inter-observer variation should be investigated to determine the reliability of the results (Marshall, 1968; Assad *et al.*, 1969; Mathur & Sharma, 1970). Observations of categoric details such as the presence/absence of a latrine and distance to water should also be validated (Majcuk, 1966). Measuring behaviour related variables such as face washing, the frequency of fly-eye contact or latrine use present difficulties of validation and need to be carefully defined to ensure repeatability.

5. Behaviour reporting. Relying on questionnaires to measure behaviour is problematic. There may be a tendency to over-report what is considered positive behaviour (Traore *et al.*, 1994; Cousens *et al.*, 1996; Manun'Ebo *et al.*, 1997) and to exaggerate for effect if the respondent feels that he may benefit from his suffering. Hence frequency of washing may be over-reported and long distances to water sources exaggerated (Tielsch *et al.*, 1988; Taylor *et al.*, 1989b; West *et al.*, 1989).

Table 2.1 Literature providing evidence for the F and E components of the SAFE strategy for trachoma control (for notes see pages 30-31).

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Australia; national survey (RACO 1980)	29,424 aborigines <21 years old.	Access to water: tap outside, compared to inside. Presence of human faeces in environment. Housing quality (includes water access, sanitation and waste disposal).	Prevalence of active trachoma 34.8% when tap outside and 7.4% when inside; faeces present 29.7%, when faeces absent 7.8%; European style housing 6.6%, tents/iron huts 30.8%	1,2,3	Can not disentangle variables such as water availability, housing type, waste management or geographical location from each other.
Australia; Northern Territory (Tedesco, 1980)	2,038 aborigines <21 years old. (sub-sample of above)	Population put into 4 zones. Zones classified with reference to water availability, housing, sanitation and nutrition. Zone 1 worst; 4 best.	Point prevalence of active trachoma decreased with zone: 1, 57.4%; 2, 46.7%; 3, 19.9%. Sample in zone 4 too small.	1,2,3	Overall improved environmental conditions associated with a lower prevalence of active trachoma.
Brazil; São Paulo (Luna, 1992)	Stratified sample of 2908 people of all ages.	Per capita water use, frequency of trash collection, source of water, educational level of head of household, face washing, frequency of laundry.	High water use, frequent trash collection, piped water into house and educated household head all protective. No effect of face washing or frequency of laundry .	5	Low prevalence of active trachoma in population. Active trachoma linked to deprived households. Questionnaire used for hygiene variables.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Egypt; Nile Delta hamlet (Courtright, 1991)	225 children aged 1-5.	Presence of socio-economic markers, presence of domestic animals, crowding, educational level, latrine ownership.	Predictors of active disease were presence of an infected school-age sibling, absence of latrine and higher numbers of children		Well controlled multivariate logistic regression analysis with no subjective variables.
Ethiopia: Jimma (Zerihun, 1997)	Random sample of 7,423 people of all ages.	Presence or absence of latrine. Distance to water, <15 minutes or >15 minutes.	Presence of a latrine protective for active trachoma. Increased distance to water linked to less active trachoma.	2,3,5	Distance to water based on recall. Majority of people between 15 and 30 minutes.
India; Jaipur (Mathur, 1970)	Cross-sectional survey. 4,209 people from 10 villages, stratified by size.	Distance to water (<200 yards, or >200 yards), personal hygiene, quality of housing.	Trachoma (all signs) higher in people >200 m from water source. Active trachoma decreased with higher standards of hygiene. Signs of all trachoma greater in poor housing	2,3,4, 5	Subjective grading of personal hygiene based on respondent's appearance. Analyses not restricted to active trachoma. No age standardisation.
Japan; Okinawa (Marshall, 1968)	60, 307 school children in four groups.	Presence or absence of a piped water supply in urban or rural settings.	Prevalence of active trachoma in urban with piped water, 4.1%; urban without, 24.1%; rural with piped water 3.3%; rural without 41.5%	1,2,3, 4	Surveys conducted by different teams, in different years. Largest group with 58,480 children, smallest with 323. Confounding not considered.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Malawi; Lower Shire Valley (Tielsch, 1988)	Cross-sectional survey of 5,436 children <6 from 71 randomly selected villages.	Distance to water, crowding, latrine ownership, face washing, nose blowing.	Active trachoma higher among children >60 min walk from water. Use of rag or handkerchief when nose blowing and latrine ownership protective. No effect of frequency of face washing.	5	Multivariate analyses to control for confounders. No estimation of quantity of water used. Only 71 in group >60 min walk from water source.
Mexico; Chiapas (Taylor, 1985)	469 children aged<10 years from 2 rural villages.	Frequency of face washing, and other housing and hygiene variables.	Active trachoma more prevalent in those who washed face <7 times per week (when 2 villages pooled). No effect of distance to water or housing type.	3,5	Prevalence of active trachoma and frequency of face washing different between the 2 villages. No significant effect of face washing when villages taken alone.
Morocco; Skoura, (Reinhards, 1968)	2,127 children <8.	Fly control.	Fly control had limited effect on its own, but was effective when combined with chemotherapy.	1,3	No record of the level of fly control achieved. Only paper to use speed of resolution as the outcome measure.
Morocco; Erfoud and Zagora (Kupka, 1968)	1900 people of all ages from villages in 2 geographical zones.	Distance to water, quantity of water used.	Active trachoma decreased in children aged 1-14 with increased water use. No effect of distance to water.	1,2,3, 5	No effect of distance on the quantity of water used. Surveys conducted in different seasons and years. Ages estimated.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Mozambique; Mueda (Cairncross, 1987)	798 people of all ages from 2 villages with different water supply.	Village 1 with easy access to water 300m from homes, estimated per capita consumption of 14 l; village 2 a 90 minute round trip to collect water, 8 l per capita consumption.	Prevalence of any signs of trachoma (including scarring) in village 1 was 19.2% and 38.1% in village2.	1,2,3, 4,5	"Daily bath" reported for 100% children in village 1 and 39% in village 2. Prevalence rate includes scarring which would not be affected by a water supply installed for only 18 months.
Saudi Arabia (Bobb, 1969)	436 children <10.	Oases villages or town compared on level of sanitation, fly density, access to water and incomes. Oases villages poorer in all respects.	Prevalence of active trachoma 3.4% in townsite village and 29.6% in oases village.	2,3,4	Townsite village also had access to health care. Impossible to disentangle effect of any single risk factor.
Somalia and Ethiopia (De Sole, 1987)	2,555 people of all ages for camel *vs* cattle herding. 407 children<10 for presence or absence of cattle	Herding camels or cattle. Presence of cattle as a proxy for fly numbers – estimated that 75% of children near cattle had flies on face compared with 20% in absence of cattle.	Prevalence of active trachoma 0.9% in camel herders, 44.8% in cattle herders. Active trachoma 52.3% in presence of cattle, 53.3% in absence. Intense trachoma higher with cattle.	1,2,4	Camel herders and cattle herders of different ethnicity and not comparable. Population with or without cattle similar ethnicity, but absence of cattle caused by drought.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Sudan (Atbara) (Majcuk, 1966)	478 people.	Daily or occasional bathing.	Prevalence of active trachoma 18.0% in daily bathers, 42.1% in occasional bathers.	2,3,4, 5	Only 50 people in daily group, 428 in occasional
Taiwan, (Assad, 1969)	33,014 people of all ages.	Distance to water, type of water supply.	Prevalence of active trachoma increases with distance to water supply. No effect of type of supply.	2,4	Effect of water confounded by other indicators of socio-economic development.
Tanzania (Kongwa) (West, 1989; West, 1988)	Random sample of 2,116 households from 20 villages. 3,830 children aged 1-7 screened.	Presence of village pumps, estimated walking time to water source, estimated quantity of water brought to household, facial cleanliness.	Active trachoma reduced in house holds <30 mins walk to water source but unaffected by presence of pump or quantity of water used. Higher prevalence in households where all children had dirty faces.	4,5	Effect of facial cleanliness restricted to households where all children dirty.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
Tanzania (Kongwa) (Taylor, 1989b)	Same sample as above	Frequency of face washing, presence of flies, presence of latrines, towel or handkerchief use, cattle herding.	Prevalence of active trachoma reduced by handkerchief and towel use but unaffected by frequency of face washing or presence of latrine. Higher prevalence associated with high fly counts and cattle herding.	4,5	No effect of face washing. Method for collecting fly data later invalidated (Brechner *et al.*, 1992) and hygiene data collected by questionnaire.
Tanzania (Kongwa) (West, 1991a)	Subsample of 1,574 children from above.	Estimated walking time to water source, presence of flies, cattle herding, facial cleanliness.	Prevalence of active trachoma reduced by handkerchief use but unaffected by distance to water or facial cleanliness. Higher prevalence associated with presence of flies and cattle herding.	5	Effect of distance to water source and facial cleanliness, shown in West 1988 and West 1989, lost in this subsample.
Tanzania, (Kongwa) (West, 1995)	6 villages (3 intervention, 3 control), 1,417 study children aged 1-7.	Promotion of face washing in intervention villages after mass treatment. Facial cleanliness defined by presence of nasal discharge, ocular discharge or flies on face.	Hygiene promotion increased prevalence of clean face, but this narrowly missed significance. Effect of hygiene promotion limited to a reduction in TI, no effect on all active trachoma.		Only study to measure impact of a hygiene intervention. When all data pooled prevalence of active trachoma lower in children with a sustained clean face.

Country	Sample	'F' and 'E' Risk Factors	Observations	Notes	Comments
The Gambia (Kiang), (Bailey, 1991)	Case control. 68 case children, 50 control.	Quantity of water brought into household. Quantity of water used for washing children from direct observation.	Similar quantity of water brought into household between groups. Case households used less water for washing children.	5	Small sample size. Case and control children matched for age. Objective measure of water use and distance.
The Gambia (Sanjal), (Emerson, 1999)	4 villages (2 intervention, 2 control) 924 people of all ages.	Fly control in intervention villages	Fly control reduced fly-eye contact by 96%. Prevalence of active trachoma reduced by fly control, new prevalent cases reduced by 75%.	4	Only properly controlled intervention study against flies. Reduction in flies obvious, so possible bias during screening.
USA; Arizona, (Portney, 1970)	441 people on an Indian reservation	33 environment and sanitation variables including washing, refuse collection and flies	No relationship found between trachoma and any sanitation variable. Limited floor space protective.	1,4	Absence of link between factors and trachoma probably due to small sample sizes, low rates of infection and homogeneity of sample.

Facial Cleanliness

A questionnaire-based study conducted in two rural villages in Mexico (Taylor *et al.*, 1985) found that those children for whom face washing more than seven times per week was reported had a significantly lower risk of having active trachoma than those for whom face washing was reported less often (relative risk = 3.1). This finding suggested that the promotion of face washing would be a suitable tool for intervention deliverable through primary health care and education systems. However, the two villages in the study were significantly different at baseline and the relative risk was calculated from pooled data. Pooling data from different populations can lead to spurious results, an effect known as Simpson's Paradox (Rothman, 1986); in this instance the data should have been stratified and not pooled. Mabey and colleagues (1992b) reanalysed after stratifying for village and showed a more modest relative risk of 1.85 (95% CI 1.15-2.8).

In a large (N=3,832) multivariate study of Tanzanian preschool children (Taylor *et al.*, 1989b) active trachoma was associated with unclean face (defined ...'by general facial appearances, especially the presence or absence of dirt, dust, or crusting on the cheeks and forehead'...) OR 1.30 (1.11-1.54). In the same multivariate model two other hygiene associated factors also appeared protective of active trachoma; handkerchief use (OR 0.67) and towel use (OR 0.76). The same sample were additionally questioned about the frequency of face washing and no effect was found - doubts were later raised about the validity of self-reporting of what was considered a desirable practice (Muñoz & West, 1997). 1,085 children from the same population went on to have laboratory tests for trachoma (West *et al.*, 1991a); 589 were clinically positive (i.e. were grade TF, TI or both) and 354 had a positive laboratory test for *Chlamydia*. Under regression analyses there was a positive association between clinical signs and unclean face for all children (N=1,085) which did not achieve statistical significance OR 1.13 (0.83, 1.54). The same pattern persisted for those either clinically or laboratory positive; clinical trachoma (N=589) OR for unclean face 1.21 (0.77, 1.90), positive laboratory test (N=354) OR for unclean face 1.01 (0.72, 1.23). Additional 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 again these were based on self-reporting and may have been affected by reporting bias.

To test if face washing was a suitable intervention for reducing the prevalence of active trachoma among children, an intensive participatory strategy to change hygiene behaviour was undertaken in central Tanzania (McCauley *et al.*, 1990; Lynch *et al.*, 1994). This succeeded in increasing the prevalence of clean faces seven fold (from 4% to 27%) over 12 months. Facial cleanliness was assessed on two days for each survey; clean faces were defined as having only one or none of: 'sleep' in the eye, nasal discharge or the presence of flies observed on the two days. This definition correlated with objective evidence of face-washing based on the use of invisible fluorescent cream applied to the subject's forehead (Lynch *et al.*, 1994).

Based on this result an intervention trial on the effect of face-washing on active trachoma in children was conducted in three pairs of Tanzanian villages (West *et al.*, 1995). One year after baseline children in the intervention villages were more likely to have a sustained clean face than those in the control villages (defined as the presence of a clean face on at least two of three post intervention surveys); OR 1.61 (0.94, 2.74). There was no difference in the prevalence of all active trachoma cases (TF and TI) between intervention and control villages, but face-washing was associated with a lower prevalence of severe trachoma (TI) OR 0.62 (0.40, 0.97). When all participants from intervention and control villages were pooled, children who had a sustained clean face were less likely to have active trachoma than those who ever had a dirty face OR 0.58 (0.47, 0.72). This is the only published hygiene-based intervention trial against trachoma and it shows that even under intensive conditions, and after painstaking preparation, sustainable changes in hygiene-based behaviour were difficult to achieve. For this reason, face washing may be better incorporated into the general promotion of hygiene rather than advocated as a control measure in its own right. The trial indicated, after controlling for confounders, that children who had a sustained clean face were less at risk of trachoma than those who had dirty faces. The factors that predict sustained clean face need to be elucidated.

Environmental Improvement - Flies, cattle and latrines

The presence of flies has been associated with trachoma for at least four hundred years. Duke-Elder (1965) cites the 1598 memoirs of the Bohemian Baron Harant of Poljitz and the papers of Lucien Howe 1888, as the first anecdotal and scientific evidence (respectively) of the involvement of flies in trachoma transmission. High fly densities have been associated with outbreaks of trachoma in Morocco (Reinhards *et al.*, 1968) and Egypt (Maxwell Lyons & Abdine, 1952; Hafez & Attia, 1958c) but they have been absent from Mexico (Taylor *et al.*, 1985). When combined with antibiotic therapy fly control reduced reinfection in Morocco, but was of little effect on its own (Reinhards *et al.*, 1968). Jones (1975) has shown that flies were capable of transferring fluorescein between children's eyes in Iran and later asserted that they were ...'most exquisite passive vectors of ocular discharge from one person to another'... (Jones, 1980). *C. trachomatis* has been cultured from flies after they were fed on heavily infected laboratory cultures (Forsey & Darougar, 1981) and identified by PCR on 2/395 *Musca sorbens* caught from the eyes of swab positive children (Chapter 4). The PCR technique used was a modification of that developed for use with ocular swabs (Bailey *et al.*, 1994) and this result is interpreted as evidence that it is possible for wild-caught flies to be carrying the bacteria and not an indication of vectorial capacity.

The presence of flies around the house was associated with a greater risk of trachoma in Tanzania (Taylor *et al.*, 1989b; West *et al.*, 1991a), but the sugar-board method used to estimate the size of the fly populations (Taylor, 1988) was later shown to be unreliable (Brechner *et al.*, 1992). Working in Ethiopia and Somalia, De Sole (1987) has suggested that there is an association between cattle herding and trachoma on the basis that cattle encourage flies and flies transmit trachoma. There were obvious differences of ethnicity, behaviour and geographical location in the study groups rendering them incomparable, and the findings should be interpreted with care. Cattle herding as an occupation, as compared to the physical presence of cattle in a village, appeared to be a risk factor for active trachoma in the Tanzanian study (Taylor *et al.*, 1989b; West *et al.*, 1991a). This could be a marker for other socio-economic factors since children that herd cattle probably live a more traditional way of life than those who are involved in other activities such as schooling and may represent the poorer segments of society or marginalised ethnic groups.

There are many species of fly in the domestic environment, but only the Bazaar fly, *M. sorbens*, has been proposed as a vector of trachoma (Hafez & Attia, 1958a; Saccà, 1964; Jones1980). *M. sorbens* is a species complex, and although no true distribution maps exist it has been recorded from Australia and all the African and Middle Eastern countries affected by trachoma (Paterson & Norris, 1970; Pont, 1991). It is strongly attracted to pus, mucus, open sores and eye secretions on which it feeds aggressively. Catches of flies from children's eyes (Hafez & Attia, 1958a) have shown that a disproportionate number of females are caught. In Chapter four I hypothesise that pre-gravid or part-gravid females which require protein-rich food for egg development are attracted to the discharge from infected eyes. This subset of flies may move appetitively between eyes, which would greatly enhance transmission potential.

A pilot intervention study was conducted in The Gambia to assess the contribution of eye-seeking flies to the transmission of trachoma (Chapter 3). The study was conducted for three months in two pairs of villages; one in the wet season (N=566), the other in the dry season (N=358). Baseline characteristics of the village pairs were similar. One village from each pair was randomly assigned the intervention and fly control was effected by the application of insecticide. Fly populations were monitored with multiple attractant traps and by catches of eye-seeking flies directly from children aged under five years in the dry season. Spraying reduced the population of *M. sorbens* by 75% and, in the dry season, reduced the child fly-eye contact by 96%. Where fly control was carried out the number of new prevalent cases of trachoma at three months was 75% lower than in the comparison group RR 0.25 (0.09,0.64). Fly control in this intervention was based on the use of insecticide, which was expensive and labour intensive, so unsuitable as a long term community based control measure in most countries. The larval medium for *M. sorbens* is faeces, and it shows a marked preference for human faeces over any other type. It only utilises human faeces available on the soil surface: larval stages have not been found in latrines and adults have not been caught emerging from them (Curtis & Hawkins, 1982). Since latrines effectively remove the larval habitat from the environment it is possible that they may be a suitable method to control *M. sorbens* and hence reduce trachoma transmission. The presence of pit latrines has been shown to be protective against trachoma in risk factor analyses in Egypt (Courtright *et al.*, 1991), Malawi (Tielsch *et al.*, 1988), Australia (Tedesco, 1980) and Ethiopia (Zerihun, 1997). The observed protection in each of these studies was at the household level. If the observation is explained in terms of diminished larval habitat for *M. sorbens* it would imply that the flies do not move between households, which does not seem reasonable. A community-wide protection would seem more likely in the presence of latrines as the local population of *M. sorbens* would decline. The studies in which latrines were protective have observed existing latrines built to fulfil a perceived need by the user. Providing latrines to communities where they do not exist without generating the perceived need may not have the desired effect of reducing faecal contamination of the environment.

The role of F and E in the SAFE strategy

A review of the evidence presented in the literature shows that there is currently no single specific tool for trachoma control that can be recommended for inclusion in the F and E part of SAFE. There is good evidence that children with sustained clean faces are less likely to be at risk of trachoma but the results of an intervention based on the promotion of face-washing show that on its own this had limited success (West *et al.*, 1995). Other studies have shown that the presence of an adequate water supply is associated with a reduced community prevalence of active trachoma, but although necessary, this is only a part of what is required and not the whole answer (Prost & Négrel, 1989). The presence of latrines appears to be associated with a lower prevalence of active trachoma and the evidence that flies are vectors of trachoma has been strengthened by a pilot intervention study.

The fact remains that for GET to be a success there has to be an impact on transmission to reduce the community prevalence of active disease. This calls for a community approach to both the perception of the disease and the management of it. Trachoma is a community disease that is clustered by individual families and is not a series of isolated cases (Bailey *et al.*, 1989). The need for the provision of surgery and the use of antibiotics is not in doubt, but on their own they are unlikely to be successful. When used rationally antibiotics have a positive effect, but it is unrealistic to think that this can be maintained (Mabey *et al.*, 1991b). The majority of cases of active trachoma are asymptomatic or mild, and affected people rarely seek treatment; this means that antibiotics need to be delivered to cases identified by health personnel, which has huge implications for health budgets, or via mass treatment programmes. When used as the only method to combat trachoma, antibiotics have not achieved lasting control (Reinhards *et al.*, 1968; Dawson *et al.*, 1976; Bailey *et al.*, 1993) and the projected frequencies of mass administration of azithromycin required (Lietman *et al.*, 1999) are daunting when applied to trachoma endemic regions.

Success of GET will require a combined approach that couples surgery and the use of antibiotics with hygiene promotion and environmental improvement. The long-term solution will not be arrived at quickly and it will not be cheap. The need for trachoma control has to be incorporated into government policy in the affected areas and should impact on the ministries responsible for water and education in addition to health. The evidence suggests that it is likely that the provision of latrines and adequate water to the affected areas would be beneficial. Trachoma should be included with other diseases such as malaria and taught about in schools. Simple messages about the importance of good hygiene, the role of flies and how to identify trachoma could be incorporated in the science, home economics and civics curricula of both primary and secondary schools.

Simple, cost-effective and sustainable measures to control trachoma are urgently needed to support the GET programme. Research into the modes of trachoma transmission and meaningful risk factor analyses may suggest further areas for investigation. The reasons why the presence of latrines and clean faces are protective need to be clarified and the recent evidence that *M. sorbens* is a likely vector needs to be verified and sustainable methods to control it developed.

Chapter 3

Pilot study on the effect of fly control on trachoma transmission and childhood diarrhoea[2]

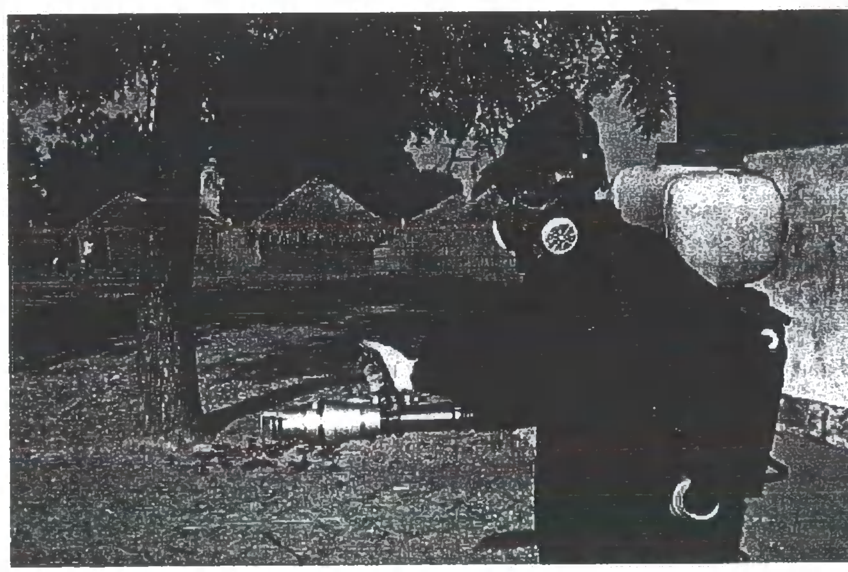

Figure 3.1 A field assistant demonstrating the MicronAir AU8000 spraying machine

[2] This chapter is a modified version of the paper '*Reduction of trachoma and diarrhoea by fly control*' by Paul M Emerson, Steve W Lindsay, Gijs EL Walraven, Hannah B Faal, Claus Bøgh, Kebba O. Lowe and Robin L Bailey which appeared in *Lancet* 1999; **353**: 1401-03.

Pilot study on the effect of fly control on trachoma transmission and childhood diarrhoea

Abstract

Background The extent of the public health problem posed by domestic flies is incompletely understood. They are accepted vectors of diarrhoea, but their role in trachoma transmission has never been quantified and no study has demonstrated that fly control reduces trachoma. We evaluated the public health impact of fly control in a pilot study in two pairs of Gambian villages.

Methods One pair of villages was studied in the 1997 wet season, the other in the 1998 dry season. Deltamethrin space spraying to control flies was conducted for three months in one village of each pair whilst the other acted as a control. Fly populations were monitored with fish-baited traps. Trachoma surveys were conducted at baseline and at three months, and daily diarrhoea surveillance was conducted for children aged between three months and five years.

Findings Fly control reduced Muscid flies by 75% in the intervention villages compared to controls. Trachoma prevalence was similar at baseline, but after three months of fly control there were 75% fewer new prevalent cases of trachoma in the intervention villages, rate ratio and relative risk 0.25 (adjusted 95%CI 0.09,0.64), P=0.003; 22% less childhood diarrhoea in the wet season, period prevalence ratio 0.78 (0.64,0.95), P=0.01 and 26% less diarrhoea in the dry season, period prevalence ratio 0.74 (0.34,1.59), P=0.6 compared to the controls.

Interpretation Muscid flies are important vectors of trachoma and childhood diarrhoea in this environment. Deltamethrin is effective for controlling flies and may be useful in some situations. Research on sustainable methods of fly control should be a priority.

Introduction

The association of flies with filth, faeces, faces and food affords ample opportunity for them to act as mechanical vectors of many pathogens, but they are often over-looked in public health interventions. Childhood diarrhoea causes 3.3 million deaths worldwide per year, with an estimated 1 billion cases of diarrhoea annually in children under 5 years of age (Benn *et al.*, 1992). The body of evidence implicating flies as vectors of diarrhoea causing pathogens, particularly those requiring a minute inoculum such as *Shigella,* has been strengthened by an intervention trial in Israel (Cohen *et al.*, 1991) and the demonstration of many pathogens on the flies (Wright, 1983; Monzon *et al.*, 1991; Fotedar *et al.*, 1992; Grübel *et al.*, 1998). *Chlamydia trachomatis*, which causes trachoma, has been cultured from flies after they were fed on heavily infected laboratory cultures (Forsey & Darougar, 1981) and an association between flies and trachoma has often been noted in the literature (for example MacCallan, 1931; Reinhards *et al.*, 1968; Taylor, 1988), but as yet there are no field-based or epidemiological studies that directly incriminate them as vectors of trachoma.

Trachoma is the main cause of preventable blindness world-wide (Thylefors *et al.*, 1995). Such blindness is more common in women than men (West *et al.*, 1991b) and results from repeated ocular *C. trachomatis* infections over many years. In less-developed countries young children are the reservoir of infection, and transmission is clustered within villages (Bailey *et al.*, 1989), but the mechanisms by which infection is spread are poorly understood. An intervention that lowers the frequency of transmission is likely to have a beneficial effect on the prevalence of trachoma-related blindness in the future.

The challenge posed by the prevention of blindness from trachoma has resulted in the WHO Global Elimination of Trachoma by the year 2020 initiative (GET 2020) in which control is to be based on a strategy of lid surgery, antibiotic treatment, facial cleanliness and environmental improvement with the acronym 'SAFE'. However, the implementation and sustainability of antibiotic therapy is beset by problems caused by low compliance with the topical application of ophthalmic tetracycline eye ointment (Mabey *et al.*, 1991b) and the prohibitive cost of oral azithromycin. Trachoma is associated with poverty and largely disappears where there are improved environmental conditions and access to better sanitation (Dolin *et al.*, 1997). Nonetheless the prospects of a rapid global improvement in living standards remains bleak. The SAFE strategy would be strengthened by the inclusion of realistic and sustainable methods to reduce trachoma transmission. We undertook a pilot study designed to investigate the role of domestic flies in the transmission of trachoma and childhood diarrhoea.

Methods

Study population

The study was conducted in four villages for three months in the previously described Sanjal area of The Gambia (Greenwood & Pickering, 1993) one pair was studied from September to December 1997 (the wet season) and the other pair from January to April 1998 (the dry season). The two pairs of villages were selected from 23 available small villages with a population of less than 400 in the Sanjal district on the basis of trachoma prevalence, lack of immediate access to primary health-care, homogeneous (Wollof) ethnic composition and willingness to participate. The village pairs were 3km and 12km apart respectively. One village from each pair was arbitrarily assigned the study intervention (fly control) on the toss of a coin, and the other acted as a control. The study was approved by the joint ethical committee of The Gambia Government and the Medical Research Council and we obtained the informed consent of the villagers and guardians of participating children.

Fieldwork

Flies were controlled by ultra-low-volume application of deltamethrin (Aqua K-Othrine, Agrevo, UK) at 0.175% volume to volume using Hudson Portapac® equipment (Hudson Manufacturing, Chicago, IL, USA). Spraying was conducted throughout the village and up to 20 metres outside each village in a concentric circle. Control started with an 'attack' phase of spraying every two days for two weeks to kill the current population of adult flies and those emerging from the breeding sites, followed by a 'maintenance' phase where spraying was carried out twice weekly in the wet season when fly numbers were highest, and weekly in the dry season when they were lower. No adverse effects of spraying were recorded.

Fly populations were monitored using four fish-baited traps (Figure 4.2) placed in each village at an animal tethering area, next to a latrine, in the centre of a domestic compound and the *banta ba* (area where people gather to meet and relax) for 24 hours every two weeks. The same fixed locations were used for traps for the duration of the trial. To measure fly-eye contact in the dry season hand-net collections of eye-seeking flies were conducted fortnightly from ten seated children for fifteen minutes each. Flies that touched the children's eyes were collected and taken to the laboratory for identification.

The whole of each village community was screened for clinical signs of trachoma at baseline and at three months by the same Community Ophthalmic Nurse who was nominally unaware of the status of each village. Screening was carried out by everting the upper eyelid and visually examining the tarsal plate using a x2.5 binocular loupe. A torch was used to provide extra light where necessary. Eyes were graded according to the WHO simplified scale (Thylefors *et al.* 1987), which classifies active trachoma as the presence of follicular trachoma 'TF' (5 or more follicles >0.5 mm visible) or intense trachoma 'TI' (50% of tarsal plate obscured by inflammation). Subjects with symptomatic trachoma were offered tetracycline eye ointment and those with trichiasis referred to the district eye clinic where free surgery was made available.

Diarrhoea surveillance for all children aged between three months and five years was carried out using 'diarrhoea diaries' completed by the mother or guardian of each child. Mothers were asked to record daily whether the child had experienced diarrhoea (Wollof: *biir bu dow*) and diaries were checked and renewed on a weekly basis. Analyses were performed on the period prevalence (proportion of days on which diarrhoea was reported) as a reflection of morbidity to avoid the difficulty of defining diarrhoeal episodes (Black *et al.*, 1982; Morris *et al.*, 1994). Oral rehydration salts were given to the mother of any child with diarrhoea and any child identified to have had diarrhoea for five consecutive days was referred to the local health centre for management.

Statistical analysis

Statistical analyses were performed using Epilnfo (version 6) and SPSS (version 6.1). Categorical data such as trachoma or diarrhoea prevalence were described by proportions or percentages. Fly count data, which were non-normally distributed were described by the median or adjusted geometric mean. For each village, counts from the four traps set on the same day were obtained, and the adjusted geometric mean for the catch from these traps was calculated by adding one to each count, calculating the geometric mean of the resulting four counts and then subtracting one, this was done to avoid the problem posed by zero fly catches. Non-normally distributed variables were compared using non-parametric methods, the Kruskal Wallis procedure and the Wilcoxon matched-pairs signed-ranks test. Categorical outcomes such as prevalence rates were initially compared using the χ^2 test with Yates' correction and Mantel-Haenszel estimates of relative risk and rate ratios used for stratified data. Because neither trachoma cases, nor episodes of diarrhoea can be assumed to occur independently within households, confidence intervals and P values for these outcomes were adjusted to allow for intra-household correlation using Miettinen's test-based approach, as advocated by Rothman (1986). The Kruskal Wallis H statistic derived from ranking of household or family disease rates was used in the adjustment.

Results

Entomology

Muscid flies (*Musca sorbens* - the Bazaar fly and *Musca domestica* – the Housefly) were more abundant in the wet season control village than the dry season control village. The median (range) of adjusted geometric mean numbers of *M. sorbens* per trap per day in the wet season was 8.8 (2.3, 37.3) versus 6.0 (2.4, 38.3) in the dry season and, for *M. domestica* 10.2 (2.5, 102.9) in the wet season versus 4.2 (1.1, 12.1) in the dry season. In the wet season intervention village spraying resulted in 76% fewer *M. sorbens*, median (range) 2.2 (0.2, 5.6) Wilcoxon test on paired observations P=0.02, and 57% fewer *M. domestica* 4.4 (1.2, 36.3) P=0.04 compared to the control village. In the dry season intervention village spraying resulted in 75% fewer *M. sorbens* (1.5 (0, 5.2) P=0.002), and 71% fewer *M. domestica* (1.2 (0, 5.3) P=0.006) compared to the control village.

Observations of hand-net collections of eye-seeking flies suggested that the process was virtually 100% efficient when there were few flies, but up to 5% of flies escaped when there were more flies present. Collections in the dry season during the spraying period showed that 96% fewer flies were caught from the eyes of children in the intervention village compared with those in the control village; adjusted geometric mean number of flies caught per child in 15 minutes 0.06 versus 1.54; 4/54 fly positive catches in the spray village compared to 38/49 in the control village; χ^2 =49.8, P<0.001). 92% of eye-seeking flies were *M. sorbens* and the remaining 8% were *M. domestica*. Other fly species, predominantly *Chrysomya albiceps* Wiedemann, were caught in the traps, but were never caught from eyes or recorded on faces.

Trachoma

Village communities were of similar size, age composition (Table 3.1) and ethnicity (all Wollof). Their total population in the 1993 census was 1,020. We recruited and enumerated 1,124 people of all ages who were screened for clinical signs of trachoma at baseline, of whom 924 (82%) were also screened at three months. Loss to follow up, mainly due to the inclusion of temporary migrants in the baseline data, was similar for the intervention and control groups, rate ratio 1.13 (0.83, 1.54).

Table 3.1 Selected characteristics of the four pilot study villages

	Wet season		Dry season	
	Control	Intervention	Control	Intervention
Census population(1993)	285	334	185	217
Number <10†	112	136	79	87
Number aged 3-60 months	82	77	51	53
Number aged 3-60 months per family‡				
Mean	3.9	3.1	3.6	3.1
Median	4	3	3	3
Range	1-11	1-7	1-7	1-9
Ocular exam:				
at baseline	319	381	227	207
at follow up (%)	271 (85)	295 (77)	189 (91)	169 (74)
% days diarrhoea data	90.9	91.6	93.7	90.3

† refers to those seen in both trachoma surveys.

‡ 'family' indicates those normally eating from the same food basin.

Data on trachoma prevalence (Figure 3.2) shows that there was no difference in the community prevalence of active trachoma at baseline in either village pair: wet season; spray village 26/295 (8.8%) versus control village 33/271 (12.2%); dry season spray village 34/189 (18.0%) versus control village 27/169 (16.0%). The prevalence of trachoma after three months was lower in the spray village than the control village in both seasons. In the wet season the prevalence of active trachoma in the spray village was 11/295 (3.7%) versus 37/271 (13.7%) in the control village, prevalence ratio 0.27 (adjusted 95%CI 0.08-0.93 P=0.04). In the dry season the prevalence in the spray village was 19/189 (10.0%) compared to 32/169 (18.9%) in the control village, prevalence ratio 0.53 (0.25, 1.12 P=0.09). Overall there was a 61% lower community prevalence of active trachoma in the spray villages associated with fly control; prevalence ratio = 0.39 (0.20, 0.77 P=0.007).

The relative risk of becoming a new active trachoma case – being graded as an active case (TF or TI) at three months if the baseline grade was non-active (N or TS) – was lower in the intervention than the control villages. Wet season relative risk = 0.20 (95% CI 0.05-0.82) P=0.026; dry season relative risk = 0.33 (0.1-1.06) P=0.064. Overall the relative risk of being a new case of active trachoma was 75% lower in villages where fly control was practised than the controls; relative risk = 0.25 (0.09-0.64) P<0.003.

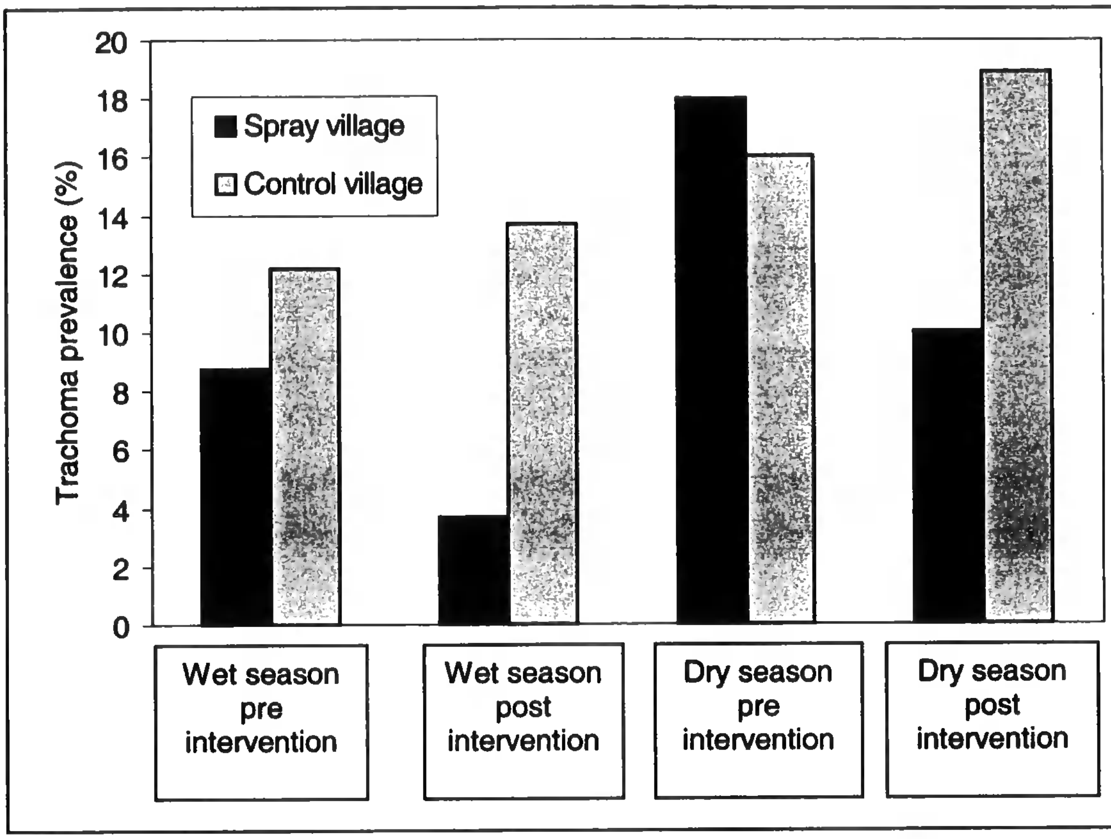

Figure 3.2 Prevalence of active trachoma before and after fly control in wet and dry season pairs of study villages

Diarrhoea

All resident children aged from 3 to 60 months were recruited for diarrhoea surveillance, comprising 263 children in total. Data were obtained for over 90% of possible child-days of observation, with no significant differences between villages or age groups. The distribution of numbers of children under 60 months per family was not significantly different in the four villages (Table 3.1).

Children in the dry season study villages experienced 58% fewer days with diarrhoea than those studied in the wet season: period prevalence ratio = 0.42 (0.23-0.76) P=0.003. The proportion of days on which diarrhoea was reported was significantly lower with increased age (χ^2 for trend P<0.001) in all the villages, but there was no association with family size. The period prevalence of diarrhoea was lower in the intervention than the control village in both seasons: in the wet season the period prevalence of diarrhoea in the spray village was 14%, and 19% in the control village relative risk =0.78 (0.64-0.95) P=0.01. In the dry season spray village the period prevalence of diarrhoea was 6% compared to 8% in the control village; relative risk =0.74 (0.34-1.59) P=0.6. The period prevalence of diarrhoea in the children under five years of age was 22% lower in the wet season fly control village (P=0.01) and 26% lower in the dry season (P=0.6) compared to the control villages.

Discussion

In this pilot study the control of Muscid flies lowered the transmission of trachoma and the period prevalence of diarrhoea in children. However, the study was conducted in only four small settlements and may have been affected by bias – for example, it is possible that a realisation that there were fewer flies might have induced differential reporting of diarrhoea and differential observations of trachoma by the ophthalmic nurse. We cannot exclude this possibility but it does not seem likely to have affected our findings since the diarrhoea data show similar age trends in all villages, and the ophthalmic nurse performed consistently at validation sessions. This is the first published study, that quantifies a reduction in trachoma transmission using fly control.

Collections of flies directly from the children's eyes confirm that despite comprising less than 10% of the total fly population as measured in fish-baited traps *M. sorbens* was responsible for over 90% of the fly-eye contacts and is therefore the most likely insect vector of trachoma. *M. domestica* was responsible for the remainder of the eye contacts and should not be discounted as a potential vector. We have tested 395 flies caught from the eyes of swab positive children with PCR for the *C. trachomatis* common cryptic plasmid PCR (Chapter 4) and successfully identified two *M. sorbens* which were positive for Chlamydia DNA. This is supporting evidence of their role as vectors. Our field observations on fly behaviour show that *M. domestica* and *M. sorbens* are strongly attracted to both human faeces and prepared food and are probably mechanical vectors of diarrhoea in The Gambia.

Ultra-low-volume spraying with deltamethrin was effective, reducing fly-eye contacts by 96%. Since catching eye-seeking flies in the hand-nets may have been less efficient when more flies were present, even this may be an underestimate. Fly control was associated with 75% (95% CI 36-91%) fewer new prevalent cases of trachoma and a 61% (95% CI 23-80%) decrease in the community point prevalence of trachoma, which compares favourably with the results of a trial of supervised face washing (West *et al.*, 1995). However, it is hard to envisage fly control with insecticides as a sustainable, routine public-health measure in most countries in which trachoma prevalence is highest, although it may be practical in oil-rich areas such as the Middle East. Spraying would be a useful adjunct to trachoma elimination strategies or during outbreaks and would have the additional merit of reducing morbidity from diarrhoea. A definitive study on the role of flies in the transmission of trachoma would require external validation of trachoma grading, perhaps with photographs, and a larger number of clusters with a correspondingly larger study population. It would need to be run over all seasons to take account of seasonal differences in fly populations and the possibility of seasonality in the prevalence of trachoma. Such a study was carried out and is described in Chapters 6 and 7. The development and evaluation of sustainable and cost effective methods for the control of Muscid flies, such as the provision of latrines, identification and clearance of breeding sites and assessing the feasibility of locally made traps, should be a priority.

Chapter 4

Transmission ecology of the fly *Musca sorbens*, a putative vector of trachoma[3]

Figure 4.1 Kebba Lowe and Mamadi Jallow screening for trachoma

[3] This chapter is an expanded version of a paper with the same title by Paul M. Emerson, Robin L. Bailey, Olaimatu S. Mahdi, Gijs E.L. Walraven and Steve W. Lindsay which appeared in *Transactions of the Royal Society of Tropical Medicine and Hygiene* (2000): **94**; 28-32.

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

Abstract

Recent evidence suggests that eye-seeking flies are important trachoma vectors. A series of investigations were conducted 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 traps placed in villages throughout the year (1997-1998) 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 the fish-baited traps but was responsible for >90% of the fly-eye contacts. The remainder were made by *M. domestica*. All fly species were more numerous in the wet season than in 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 [IQR], 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 ($P=0.27$). Female flies were more commonly caught from eyes than males ($P<0.001$). The presence of *Chlamydia* DNA was demonstrated by PCR on two 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. Two of the three recognised species of the *M. sorbens* complex, *M. sorbens* and *M. biseta*, are found in The Gambia. Both species were caught from eyes. Morphological separation of males by the frons/head-width ratio gave unambiguous results. Separating females on facial characteristics was harder and less reliable. 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* (*sensu lato*) is the principal vector of trachoma in The Gambia.

Introduction

Trachoma is an infectious blinding disease caused by the bacterium *Chlamydia trachomatis*. World-wide, 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.*, 1992b), 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.*, 1992b). Studies in The Gambia (Bailey *et al.*, 1989), Tanzania (West *et al.*, 1991b) and elsewhere 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, 1958c).

A recent study from The Gambia provides the best evidence to date that flies are important in trachoma transmission (Chapter 3). 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 75% and reduced fly-eye contact in the dry season by >95% compared to controls. Cross-sectional community-based 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 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 free corrective surgery was made 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 (WHO, 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 -20ºC 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 1-1.75 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 prone to disturbance 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).

Figure 4.2 Fish-baited fly trap set in Samba Sotor village

Hand-net collections. These collections were carried out between 07:30 and 13:00. Child volunteers aged between two and six 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.

Figure 4.3 Sticky fly trap set inside a participant's house

Exit traps over latrines. W.H.O. mosquito exit traps (WHO, 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 Chlamydia trachomatis on flies

Flies were stored at -20ºC 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 -20ºC 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 -20ºC.

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 Unit Taq DNA polymerase, 50 mM KCl, 10mM Tris-HCl (pH8.0), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM 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 94ºC for 1 minute, annealing at 53ºC for 2 minutes and extension at 72ºC 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.

Identification of flies

Flies were identified in the laboratory using a dissecting microscope and relevant taxonomic keys (Pont, 1991; Crosskey & Lane, 1993). Members of the *M. sorbens* complex were identified by the methods described by Pont (1991). For males the frons and maximum head widths were measured with an eyepiece micrometer and the frons/head-width ratio calculated (a/b in Figure 4.4). The narrow fronsed form with a frons/head-width ratio between 0.031 and 0.065 were classified as *M. biseta*, the wide fronsed form with a range between 0.071 and 0.120 were classified as *M. sorbens.* For females the number of proclinate setulae (fine, downward pointing hairs) below the lowest strong seta (thick hair) on the fronto-orbital plate (Figure 4.5) were counted. Specimens with 12 or fewer setulae were classified as *M. sorbens* (Figure 4.6) and those with 14 or more, *M. biseta* (Figure 4.7).

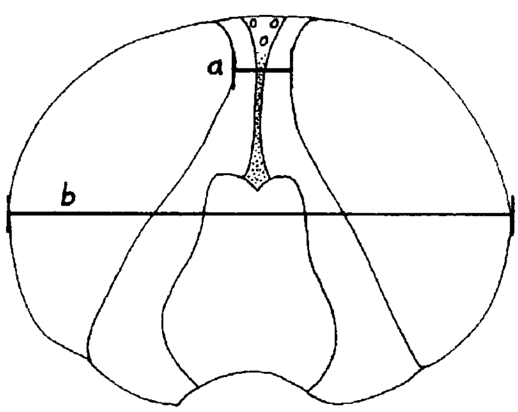

Figure 4.4 Head of male *M. sorbens* showing measurements taken to calculate frons/head-width ratio

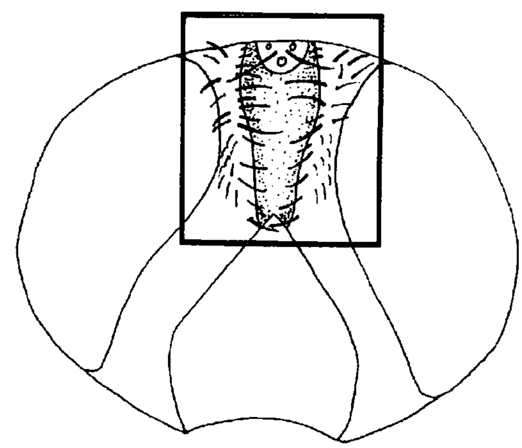

Figure 4.5 Head of female *M. sorbens* showing area enlarged in Figures 4.6 and 4.7

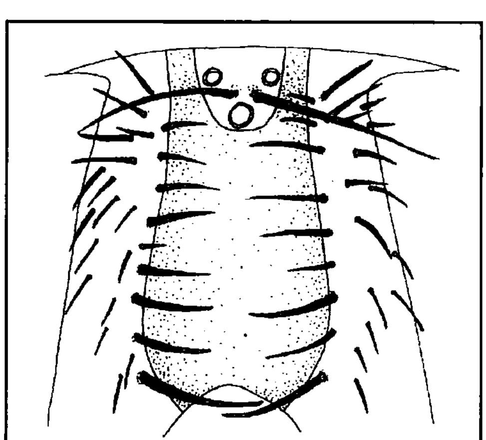

Figure 4.6 Fronto-orbital plate of female *M. sorbens*

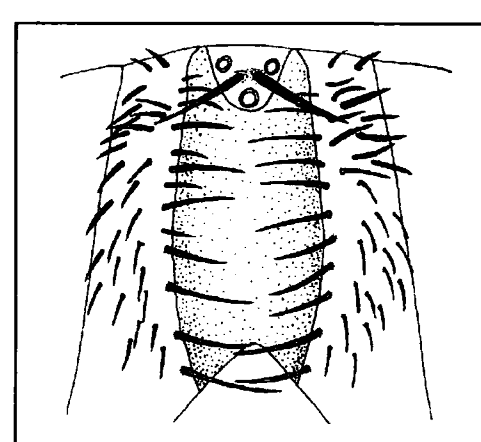

Figure 4.7 Fronto-orbital plate of female *M. biseta*

Statistical Analysis

Tests of normality showed that the fly catch data from all collections were non-normally distributed. The data were described by the median and inter-quartile range (IQR) and analysed with non-parametric tests. Wilcoxon matched-pair signed-rank tests were used when comparing paired data and Wilcoxon rank-sum tests to compare two independent samples. Confidence intervals for proportions were calculated from the exact binomial distribution. Analyses were performed using STATA® (version 6) and Epi Info (version 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 4.1.

Table 4.1 Background trachoma prevalence by age in the study area of The Gambia (1997)

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

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 (IQR) number of flies per trap per day was 54.5 (16-195). Of 71 362 flies caught throughout the year in 383 fish-baited traps, three species dominated the catch (96.4% of the total): *Chrysomya albiceps* (68.5%); *M. 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 catches 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 median number of flies caught per trap per day was 20 (13-46) and in the dry season the median catch was 9 flies per trap per day (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 (16-229) in the wet season and 7 flies per trap per day (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 caught on sticky traps was virtually 1:1. 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 4.2 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 (6-12) and the median number caught from children with neither ocular nor nasal discharge was 4 (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 10 minutes from a child seated inside was 3 (0.25-5); when the same child moved outside the median was 3.5 (2-5.75, Z=-1.10, N=36 children, P=0.273).

Table 4.2 Species and sex distribution of eye-seeking flies caught from Gambian children (1997-1998)

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

Identification of flies

The frequency distribution of 92 male *M. sorbens sensu lato* is shown in Figure 4.8. The distribution is bimodal with the narrow fronsed form, *M. biseta*, comprising the first peak and the wide fronsed form, *M. sorbens* the second. *M biseta* constituted 39% of the sample, *M. sorbens* 61% and there were no intermediates. The frequency distribution of proclinate setulae on the right and left sides of the fronto-orbital plate of 117 female *M. sorbens s.l.* is shown in Figure 4.9. Two specimens had 13 setulae on both their left and right side and one had 13 on the right only, hence three specimens were classified as indeterminate. Species distribution was essentially the same if the left or right side of the fronto-orbital plate was used, 21% being the more setulous *M. biseta*, 77% the less setulous *M. sorbens* and 2% being indeterminate.

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 4.2. None of the *M. domestica* were found to be positive, two (0.5% of the total) *M. sorbens* were PCR positive. Of these one was male and the other female.

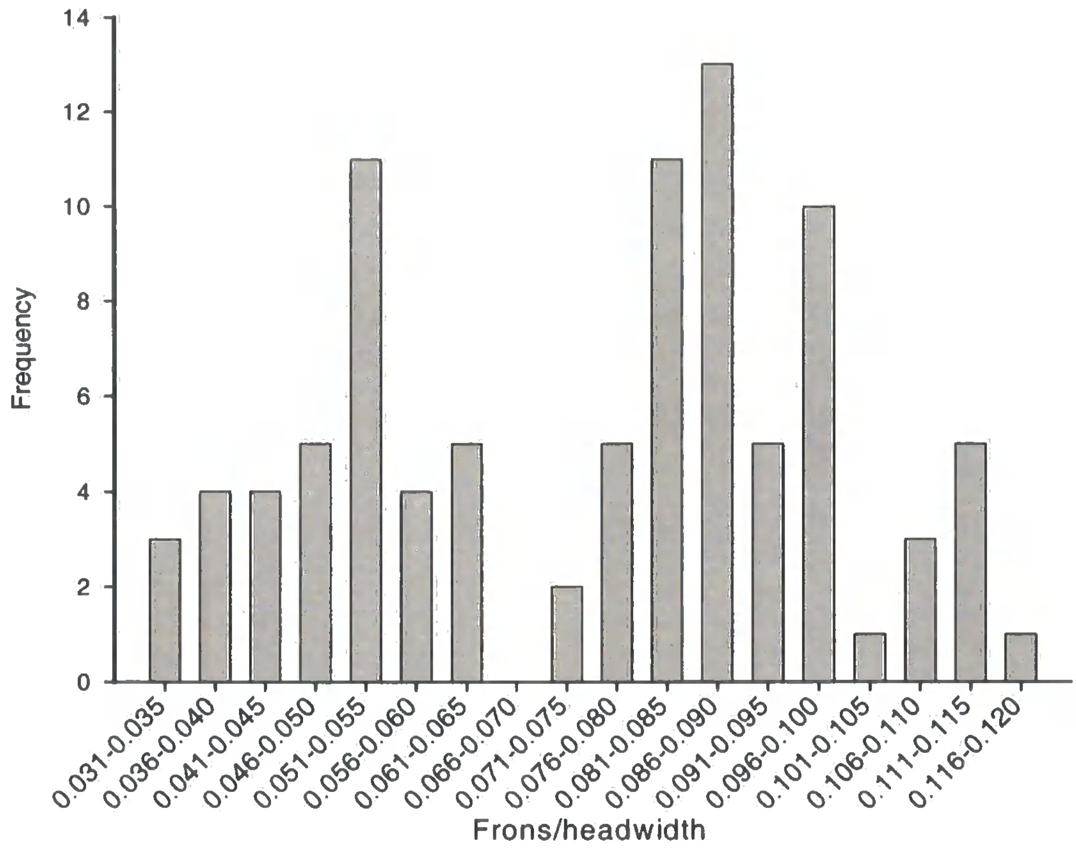

Figure 4.8 Frequency distribution of frons/head-width ratios of 92 male *M. sorbens*. The left peak is *M. biseta*, the right peak is *M. sorbens*

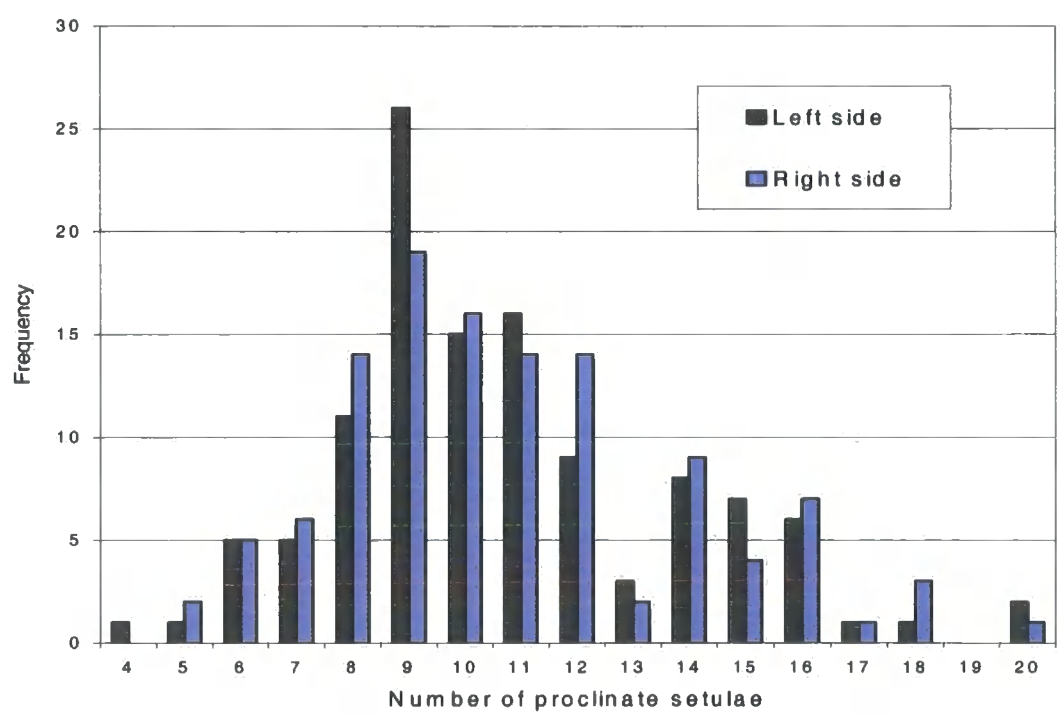

Figure 4.9 Frequency distribution of proclinate setulae on the right and left sides of the fronto-orbital plate of 117 female *M. sorbens*. Less than 13 setulae are *M. sorbens*, greater than 14 are *M. biseta*

Discussion

Flies as trachoma vectors

We have shown that the control of all fly species in this environment with insecticide resulted in a reduction in the transmission of trachoma (Chapter 3). 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 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; Levine & Levine, 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% 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, 1958a). 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 slight ocular and nasal discharges from which it is possible to isolate *C. trachomatis* elementary bodies. The infection is active in the conjunctival epithelium 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.

Morphological separation of M. sorbens sibling species

The separation of male *M. sorbens* from *M. biseta* by the frons/head-width ratio was simple to perform, objective and provided unambiguous results since the distribution of the ratios was bimodal. However, counting setulae on the fronto-orbital plate of females proved to be more time consuming and difficult, 2% of females were classified as 'indeterminate' by this method. A subjective decision of what constituted 'the lowest strong orbital seta' had to be made, followed by carefully counting. Setulae were occasionally missing after damage in the hand-nets and the resulting scar could be confused with a missing scale or other abrasion. Further work utilising genetic or molecular techniques could be undertaken to validate the morphological methods used here.

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 6 weeks. There are problems associated with distribution, the cost of azithromycin and of compliance with tetracycline (Mabey *et al.*, 1991b). 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 being a severe case (grade TI) by 42% in those with a sustained clean face, but having no significant effect on all active trachoma (grades TF and TI). The hygiene promotion 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 (West *et al.*, 1991c), 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. Because of the uncertainty surrounding the precise nature of the breeding media of *M. sorbens* in The Gambia a study was carried out to ascertain the preferred breeding media of this fly and is described in the following chapter.

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 single answer 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.

Chapter 5

Human and other faeces as breeding media of the trachoma vector *Musca sorbens*[4]

Figure 5.1 Female *Musca sorbens* about to oviposit at the base of a human stool lying on the soil

[4] This chapter appeared as a paper with the same title by P.M. Emerson, R.L. Bailey, G.E.L. Walraven and S.W. Lindsay in *Medical and veterinary Entomology* (2001): **15**; 314-320.

Human and other faeces as breeding media of the trachoma vector *Musca sorbens*

Abstract

The fly *Musca sorbens* apparently transmits *Chlamydia trachomatis*, causing trachoma. The literature indicates that *M. sorbens* breeds predominantly in isolated human faeces on the soil surface, but not in covered pit latrines. This study was carried out to identify breeding media of *M. sorbens* in a rural Gambian village endemic for trachoma. Test breeding media were presented for oviposition on soil-filled buckets and monitored for adult emergence. *M. sorbens* emerged from human (6/9 trials), calf (3/9), cow (3/9), dog (2/9) and goat faeces (1/9) but never from horse faeces, composting kitchen scraps or a soil control (0/9 of each). After adjusting for mass of medium, the greatest number of flies emerged from human faeces (1426 flies/Kg). Median time for emergence was 9 days (inter quartile range = 8-9.75) post-oviposition. Of all flies emerging from faeces 81% were *M. sorbens*. Male and female flies emerging from human faeces were significantly larger than those from other media, suggesting that they would be more fecund and longer-lived than smaller flies from other sources. Female flies caught from children's eyes were of a similar size to those from human faeces, but significantly larger than those from other media. Thus human faeces appear the best larval medium for *M. sorbens*, although some breeding also occurs in animal faeces. Removal of human faeces from the environment, through the provision of basic sanitation, is likely to greatly reduce fly density, fly-eye contact and hence trachoma transmission, but if faeces of other animals are present *M. sorbens* will persist.

Introduction

It is now becoming accepted that the fly *Musca sorbens* is a mechanical vector of trachoma and is in part responsible for the transmission of this blinding disease (Chapters 3 and 7). Studies on the behavioural ecology of eye-seeking flies (Hafez & Attia, 1958a) have shown that *M. sorbens* is responsible for the majority of fly-eye contacts, that they are highly attracted to potentially infective ocular and nasal discharge and that the causative agent of trachoma, *Chlamydia trachomatis* bacteria, can be found on them. In a pilot study in which flies were controlled by the application of insecticide there was a significant reduction in trachoma prevalence in fly-control communities compared to matched control communities (Chapter 3). It is feasible to achieve community control of adult flies with insecticides and this may be warranted in specific circumstances. However, it is unrealistic to suggest that this would be either a sustainable strategy, or a high priority, in many countries in which trachoma is endemic. In common with other Muscid flies, *M. sorbens* has a tremendous reproductive potential when conditions are favourable (West, 1951) and reductions in the adult population are probably best achieved by restricting the availability of suitable larval media. The larvae of *M. sorbens* are known to be coprophagous (Skidmore, 1985) and it has been stated that in Egypt they breed ..."almost exclusively in human excrement in the form of isolated stools"... (Hafez & Attia, 1958a). Other authors have also recovered *M. sorbens* larvae from human faeces in Egypt (Sabrosky, 1952) and Russia (Zimin, 1948). However, reports also exist of other media being utilised such as dog faeces in China (Meng & Winfield, 1944, cited in Legner *et al.*, 1974); dog and cat faeces (Mau, 1978) and cow faeces in Hawaii (Toyama & Ikeda, 1976); pig and donkey faeces in Russia (Zimin, 1948) and also horse, cow and pig dung (Hafez, 1941). All these authors found that the fly only breeds in exposed, isolated, stools lying on the soil and that larvae are not usually recovered from dung heaps or middens, in which it is replaced by other species. The failure to catch adult *M. sorbens* emerging from the drop holes of pit latrines in Botswana and Tanzania (Curtis & Hawkins, 1982) and The Gambia (Chapter 4) support this. Zimin (1948) also found that *M. sorbens* larvae could be recovered from small garbage heaps, provided they were on the soil and not in the shade, but this has not been reported elsewhere.

The aim of this study was to determine which types of faeces were the sources of *M. sorbens* in a trachoma endemic community in The Gambia in order to elucidate the likely impact of the provision of basic sanitation (pit latrines) on the fly population and trachoma transmission.

Methods

Study area

The trial was conducted at the Medical Research Council field station at Farafenni (13° 34' N, 015° 36' W) and Kunjo (Kussasa) a nearby village in the North Bank Division of The Gambia, West Africa between August and December 2000. The village was typical of those in the area. In May 2000 there were 385 inhabitants living in 38 family compounds. There were 19 pit latrines in the village serving 226 people: 41% of the population did not have a latrine in their compound. Sixteen of the latrines were of the 'local' type constructed using wooden beams over a 2-3m pit with a mud or cement covering. Three were the 'improved' type in which a ferro-reinforced cement slab was laid over the pit.

A trachoma screening exercise was conducted in June 2000 in which 85% (152/178) of children under 15 years old and 72% (71/99) of adults aged over 35 years participated. Screening was by visual examination of the tarsal plate using the WHO grading method (Thylefors *et al.*, 1987) and revealed a point prevalence of current active trachoma infection (grades TF, TI or both) of 15% (23/152) in the under 15 year olds and a prevalence of trachomatous scarring (TS) of 42% (32/71) and of trichiasis (TT) of 3% (2/71) in those over 35 years old.

Study design

A pragmatic approach was taken in which experimental conditions were kept as close as possible to those in the village. The faeces were handled minimally to retain their natural state and the experiments were sited adjacent to a compound containing active cases of trachoma. Trial media were presented on earth-filled buckets arranged in a circle approximately 1.5m apart. The same site in the village was used for each trial. To preclude the chance of earlier oviposition by flies, fresh faeces were collected from the source animals early in the morning and doubled-bagged. All trial media were weighed in the bag on an electronic scale and rounded to the nearest 10g. Media were randomly assigned to one of the buckets and turned-out of the bags at the same time. Trials were set-up between 10:00 and 11:00 a.m. In each trial human, milk-fed calf, cow, dog, goat and horse faeces and composting waste from a pile behind the compound were used as trial media. One soil-filled bucket acted as a control to verify that the soil did not already contain fly pupae. Dog, horse and goat faeces were collected in the village, milk-fed calf and cow faeces came from a small herd tethered nearby and the human faeces came from a village volunteer. Diets of the source animals and village volunteer were typical of those of the village: cows and goats foraged in the bush; horses were fed cut grass, groundnut tops, millet husk and whole millet; dogs ate human leftovers and whatever could be scavenged; the village volunteer ate a normal Gambian diet high in carbohydrate and oil (predominantly rice and millet) with a low protein content.

The buckets were left in the village for three days and then taken to the field station and kept on the shaded verandah of a building. Each bucket was covered with a numbered funnel-cage exit trap which caught all emergent flies. The traps were inspected for emergent flies every morning and evening for 16-20 days (i.e. to day 19-23 post set-up). When flies were present the entire funnel-cage was placed into a -20ºC freezer for ten minutes to kill the flies. Flies were then removed from the traps, sorted and stored frozen.

Entomological methods

Flies were identified using a dissecting microscope and relevant taxonomic keys (Pont, 1991; Crosskey & Lane, 1993). There was considerable variation in the number of emergent flies caught in each trap; if there were 15 or fewer male and 15 or fewer female *M. sorbens* all were given a unique number and pinned. If there were more than 15 of either sex the flies were laid out on a numbered grid and 15 selected for pinning using a table of random numbers. Pinned flies were desiccated at room temperature and stored in museum quality cases (Watkins and Doncaster, Hawkhurst, Kent, UK); additional flies were stored at -20ºC.

When desiccation was complete, the maximum head-width of pinned flies was measured twice using a dissecting microscope and eye-piece graticule calibrated against a stage-micrometer (Graticules Ltd., Tonbridge, Kent, UK). Flies were returned to the case between measurements and the investigator was blinded to the result of the first measurement when making the second. Flies in which the head-width measurements differed by three or more divisions (equivalent to $\geq 87\mu m$) were examined a third time and the two most concordant measurements recorded. Analysis of size was conducted using the arithmetic mean of the two measurements.

To compare flies emerging from the trial media with those visiting the eyes of children, biweekly fly collections were made from the eyes of volunteer children under five years old. Collections were made in the same compound as that in which the breeding media trials were conducted and also in a second compound in a neighbouring village. Any fly that physically touched the eye with its feet or proboscis in a 15 minute period were caught by a trained field worker using an established method (Chapter 4).

Weather data

Daily records of air temperature and precipitation were collected throughout the trial period. Temperature data was read from a maximum and minimum thermometer placed next to the trial buckets at the field station and precipitation measured in a standard rain gauge sited more than 5m away from buildings and trees.

Statistical Analysis

Normally distributed data were described by the mean and standard error, 95% confidence intervals were calculated from the exact binomial distribution and means were compared with t-tests. Confidence intervals for proportions were calculated from the exact binomial distribution. Non-normally distributed data were described by the median and inter quartile range (IQR). Comparisons between the production of flies from different media were made using Friedman two-way analysis of variance by ranks and the Wilcoxon signed-ranks test. Analyses were performed using SPSS® (version 10.0) and EpiInfo (version 6.0).

Ethical considerations

The study was approved by the joint Gambian Government - MRC Ethical Committee. Informed consent was obtained from the guardians of volunteer children involved in fly catches and from the compound heads adjacent to the bucket site.

Results

Fly production by media

A total of 991 flies were caught emerging from faeces over the nine trials, of which 806 (81.3%) were *M. sorbens*. Of the *M. sorbens*, 50.4% were male giving a practical sex ratio of 1:1. The production of *M. sorbens* from the different trial media and median (IQR) mass of the media are shown in Table 5.1. *M. sorbens* emerged from human (6/9), milk-fed calf (3/9), cow (3/9), dog (2/9) and goat (1/9) faeces but never from horse faeces, composting kitchen scraps or soil control. The proportion of human faeces that were positive for the emergence of any adult *M. sorbens* was twice that of any other media, however the observed difference did not reach statistical significance in this series of nine trials. There was significant variation in the number of adult *M. sorbens* emerging from the different media (Friedman test, χ^2=22.4, d.f.=7, P=0.002). Production of flies from human faeces did not differ significantly from that produced from milk-fed calf, cow or dog (Wilcoxon's test, z = 0.612, 0.128, 0.173 respectively, n.s.), but was significantly higher than that from other media (z = 2.201, P = 0.028 in each case).

Table 5.1 Production of *Musca sorbens* from trial breeding media: Actual number of flies/stool (flies/Kg of medium)

	Type of trial medium							
Trial number	Human faeces	Calf faeces	Cow faeces	Dog Faeces	Goat faeces	Horse faeces	Composting waste	Control
1	0	130 (241)	69 (137)	0	0	0	0	0
2	9 (63)	0	4 (14)	42 (311)	0	0	0	0
3	0	0	0	0	0	0	0	0
4	7 (20)	0	0	2 (11)	0	0	0	0
5	11 (115)	0	0	0	0	0	0	0
6	154 (1426)	58 (372)	30 (87)	0	0	0	0	0
7	40 (268)	243 (942)	0	0	2 (30)	0	0	0
8	5 (18)	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
Proportion +ve for *M. sorbens*	6/9	3/9	3/9	2/9	1/9	0/9	0/9	0/9
Mass of media g, median (IQR)	142 (108.5, 161.5)	156 (93.5, 286)	375 (309.5, 631.5)	103 (71.5, 165.5)	68 (60, 111.5)	280 (205, 533.5)	354 (256, 409)	-

Head-width size of Musca sorbens

The head widths of 468 *M. sorbens* were measured, 273 of which were female. As the sex ratio of emergent flies was 1:1, and this was constant across the different media, flies from all trial media were pooled to compare the size of males and females. Females were an average of 7% (107 μm) larger than males (t=4.30, d.f.=466, P<0.001). Further analyses were stratified by sex. Table 5.2 shows the variation in the size of head width between flies collected from the different media and from the eyes of children. Specimens collected emerging from goat faeces were not included due to the small sample size (N =2). The head-width of female flies caught from the eyes of children was significantly larger than those emerging from dog (t=9.87, d.f.=62, P<0.001), cow (t=5.20, d.f.=92, P<0.001) and calf faeces (t=5.09, d.f.=117, P<0.001), showing these were different populations. However, the head-width of female flies from human faeces were similar to those caught from children's eyes (t=1.30, d.f.=116, n.s.) indicating that these were from the same population. Male flies caught contacting children's eyes were significantly smaller than those from human faeces (t=4.87, d.f.=67, P<0.001), significantly larger than those from dog faeces (t=5.34, d.f.=49, P<0.001), and of a similar size to those from those from cow (t= 0.01, d.f.=67 n.s.) and calf faeces (t=1.55, d.f.=99, n.s.). Both male and female *M. sorbens* emerging from human faeces were significantly larger than those from other larval media.

Flies contacting children's eyes

M. sorbens accounted for 79% of 196 flies caught from the eyes of children in 159 separate 15 minute catches, the remainder being *M. domestica*. Of the *M. sorbens* 120/154 (78%) were female.

Table 5.2 Summary statistics of mean head-widths of *Musca sorbens* caught from the eyes of children compared to those emerging from the breeding media

			Summary statistics		
Source of flies	sex	N	Mean head-width (mm)	95% CI of head width	Range (mm)
Child's eyes	Male	32	1.58	1.50, 1.66	1.19 – 2.35
	Female	41	1.86	1.78, 1.94	1.25 – 2.29
Human faeces	Male	37	1.82	1.76, 1.88†	1.43 – 2.07
	Female	77	1.92	1.87, 1.97	1.25 – 2.20
Calf faeces	Male	69	1.64	1.60, 1.68	1.13 – 1.93
	Female	78	1.69	1.66, 1.72*	1.01 – 2.09
Cow faeces	Male	37	1.58	1.50, 1.66	1.22 – 1.91
	Female	53	1.59	1.53, 1.65*	1.19 – 1.97
Dog faeces	Male	19	1.25	1.19, 1.31*	1.03 – 1.54
	Female	23	1.28	1.23, 1.33*	1.07 – 1.57
All males		195	1.61	1.59, 1.63	1.03 – 2.35
All females		273	1.72	1.70, 1.74	1.01 – 2.29

* Flies significantly smaller than those caught from the eyes of children (t test, $P<0.001$).

† Flies significantly larger than those caught from the eyes of children (t test, $P<0.001$).

Physical conditions by trial

Table 5.3 shows the weather experienced during the trials and the adult development time for *M. sorbens.* Temperatures for the first eight days, when *M. sorbens* was developing, and precipitation for the first three days, when the buckets were in the village and not under cover, are shown. The mean daily maximum and minimum air temperatures during the first eight days of each trial were in the range 32.7-43.3^{0}C and 20.8-25.9^{0}C respectively. Despite the trials being conducted during the wet season no measurable quantities of precipitation were recorded during the first three days of any trial. The median day of emergence for all flies was 9 (IQR = 8-9.75). In trials where any *M. sorbens* emerged the minimum time from egg to adult was always eight days, although production continued for as many as nine days from first emergence.

Table 5.3 Air temperatures and development time of *Musca sorbens* by trial

	Trial number								
	1	2	3	4	5	6	7	8	9
Absolute max. T (°C) from day 1-8†	40.0	43.0	43.0	45.5	45.5	48.0	47.0	47.5	34.0
Mean daily max. T (°C) day 1-8†	37.7	35.2	39.0	38.1	36.9	43.3	36.9	39.6	32.7
Absolute min. T (°C) from day 1-8†	21.0	22.0	22.0	23.0	22.5	23.0	23.0	22.0	19.5
Mean daily min. T (°C) day 1-8†	23.7	25.1	24.1	24.9	25.9	26.0	24.1	23.8	20.8
Median (IQR) days for *M. sorbens* to emerge	10 (9,11)	9 (8,9)	‡	8.5 (8,9)	8 (8,8)	9 (8,9)	8 (8,8)	12.5 (10.3, 14.8)	‡
Precipitation on days 1-3 (mm)	0	0	0	0	0	0	0	0	0

† Refers to air temperature, not to soil or media temperature.

‡ No *Musca sorbens* emerged from any trial media.

Discussion

In contrast to the findings of Hafez and Attia (1958a) it was not found that *Musca sorbens* breeds 'almost exclusively' in human faeces, but instead adults emerged from a variety of animal faeces; indicating that this fly is capable of utilising a range of larval media. The trial media were kept as representative as possible of the natural system in a Gambian village and faeces were used that were commonly available. Horses, goats and sheep were kept in the village and their faeces were ubiquitous. Cows were kept tethered away from the village with their calves and their faeces were brought into the village for trial purposes. Human faeces not disposed of in the latrines were dumped onto waste heaps outside the compound and could always be found. Dogs are taken away from the village during the day and consequently their faeces were scarce in the village. Laboratory investigations into the suitability of chicken and cat faeces (Mau, 1978) concluded that chicken faeces did not support *M. sorbens* larval development, but cat faeces did. Both of these media were excluded since few cats were present, and they bury their faeces, and chicken faeces were unsuitable. Pig faeces have been identified as a source of larvae (Hafez, 1941; Zimin, 1948), but these animals were not present in this largely Muslim community, so they were excluded. In this study adult flies emerged from human faeces twice as frequently as from any other type and human faeces produced the greatest quantity of flies when adjusted for mass. Thus, in this environment human faeces are probably the primary source of *M. sorbens* with other animal faeces being of lesser importance. In other environments where cattle are kept within the village or dogs are more numerous the relative importance of different breeding media may change.

There was wide variation in the number of flies emerging from individual faeces from each species, from 0-1426 flies/kg in the case of human faeces. This may indicate that: there is a narrow range of physical conditions, such as texture or water content, or chemical conditions, such as the volatiles produced that elicit oviposition; that there is wide variation in the nutritional quality of individual faeces as a larval medium; that ovipositing females lay variable numbers of eggs on individual faeces or a combination of these factors. Despite our efforts to keep the faeces in their natural state, some smoothing of the surface took place in the bags and it also cooled down, thus our experimental procedure may not completely replicate what happens in nature. Observations of behaviour showed that flies were strongly attracted to the human and dog faeces immediately they were turned out of the bag with *M. sorbens*, *M. domestica* L., *Chrysomya albiceps*, *C. regalis* Robineau-Desvoidy and *Sarcophaga* spp usually present within 15-60 seconds of the characteristic unpleasant odour being released. Not all female *M. sorbens* attracted to a stool laid eggs, but when oviposition was observed it occurred almost immediately after the fly arrived, often less than one minute after the stool was exposed. Females typically reversed into cracks in the stool to lay the eggs or oviposited at the base of the stool where it touched the soil. Oviposition was not observed on human or dog faeces that lacked cracks, were particularly hard or that had dried and developed crusting. It is possible that the cooling of the faeces and the minor changes in shape caused by handling in the bag may have averted some oviposition responses by *M. sorbens*. Seasonal changes in nutrient and water content of the faeces of cattle have been shown to have profound effects on the breeding success of the Australian Bush fly *Musca vetustissima* (Hughes, 1970; Greenham, 1972). Similar seasonal variations in the quality of animal faeces as a larval medium for *M. sorbens* are likely to occur in Africa, and in all other regions, making generalisations difficult.

The size of insects is an important factor governing individual fecundity and survival. Head-width is normally distributed in flies and can be used as a measure of size. Dissection of laboratory reared female *M. vetustissima* has shown that flies with a larger head-width are more fecund, producing greater numbers of eggs than smaller flies (Tyndale-Biscoe & Hughes, 1968). Large flies are also better able to withstand starvation and lack of water than small ones in the laboratory (Hafez & Attia, 1958a; Hughes *et al.*, 1972) and so presumably have a greater longevity in the wild. The size of the adults is probably largely phenotypic rather than genotypic, being caused by the nutrient quality of the larval medium. As large flies have a fitness advantage over smaller ones, and we found that both males and females emerging from human faeces were significantly larger than those emerging from any other medium, we conclude that human faeces produced the 'best quality' flies. The observed difference in size may indicate that although non-human faeces were able to support the development of *M. sorbens* they are sub-optimal and the emerging flies are less fit than those developing in human faeces. However, we did not test the viability or fecundity of emergent flies.

Female *M. sorbens* are more commonly caught feeding from the eyes of children than males, and are typically caught in the ratio of 4-5:1 (Hafez & Attia, 1958c; Chapter 4). As females seek eyes more frequently, and appear to move from eye to eye appetitively they are more likely to be trachoma vectors than males. The mean head-width of females caught from the eyes of children in this study was similar to those emerging from human faeces, but significantly larger than flies emerging from other media. Although there is overlap in the head-width range, this finding indicates that the potential-vector female flies form the same population as those emerging from human faeces, but not from other faeces. This may be because the smaller flies do not survive as well as the larger ones or because smaller flies exhibit a different behavioural ecology. It is known that female flies require a protein meal when about 3-5 days old, and subsequently during each gonotrophic cycle, in order to develop eggs (Tyndale-Biscoe & Hughes, 1968). This may be the reason that they seek eyes, and why twice as many female *M. sorbens* can be caught from children with ocular or nasal discharge than from children without discharge (Chapter 4). We found that male *M. sorbens* caught from the eyes of children were significantly smaller than those emerging from human faeces, the same as those emerging from cow and calf faeces and larger than those from dog faeces. As males do not have the physiological requirement for a protein meal to develop eggs they probably visit eyes for a different reason and the observed variation in size may reflect a younger population of flies.

Development of *M. sorbens* is temperature-dependent, ranging between a theoretical minimum of 6.5 and maximum of 21 days (Hafez & Attia, 1958b). In this study the time for development from egg to adult was non-normally distributed with a median time of 9 (IQR=8-9.75) days. First emergence of adult flies was always on day 8 and *M. sorbens* was always the first species of fly to emerge. Hafez and Attia (1958b) gave the lethal temperature of eggs as 40^0C and larvae as 43^0C; the maximum air temperatures recorded in this series exceed these critical levels, but these represent peaks in air temperature that did not penetrate the larval medium.

This series of experiments has shown that *M. sorbens* is not behaviourally restricted to breeding in human faeces but that it is also capable of utilising the faeces of other animals. Further work is required to test the viability of flies emerging from the different media; to determine whether the size of adults emerging from different media is truly a phenotypic effect or if different sized flies have different preferences for oviposition, and to investigate the ecology of the small flies.

This study shows that isolated human faeces are capable of supporting the production of large numbers of adult *M. sorbens*, but flies also emerged from faeces of other animals; notably milk-fed calf, cow and dog. Human faeces were twice as likely to produce adult flies than any other breeding media in the trial; they produced the greatest quantity of flies per kilogram and flies from human faeces were the largest, suggesting they were also of the best quality. Female *M. sorbens* were most commonly caught from the eyes of children and these flies appeared to be from the same population as those emerging from human faeces, but not from those from other breeding media. These findings imply that the reduction of human faeces in the environment by the provision of basic sanitation will reduce the population of *M. sorbens*, reduce the observed frequency of eye-fly contact, and thus reduce trachoma transmission. However, it is unlikely that the provision of basic sanitation will result in a complete absence of human faeces, and latrines will not affect the amount of animal faeces in the village. Thus, the population of *M. sorbens* will persist, albeit at a lower level, and the reduction in trachoma transmission will not be as great as the level achieved by controlling flies with insecticide.

Chapter 6

The Flies and Eyes Project. Design and methods of a cluster-randomised intervention study to confirm the importance of flies as trachoma vectors in The Gambia and to test a sustainable method of fly control using pit latrines.[5]

Figure 6.1 The author catching flies from a study participant

[5] This chapter will appear as a paper with the same title by Paul M Emerson, Steve W Lindsay, Gijs EL Walraven, Sheikh-Mafuji Dibba, Kebba O Lowe and Robin L Bailey in *Ophthalmic Epidemiology* (in press).

The Flies and Eyes Project. Design and methods of a cluster-randomised intervention study to confirm the importance of flies as trachoma vectors in The Gambia and to test a sustainable method of fly control using pit latrines.

Abstract

The Flies and Eyes project was a community-based, cluster-randomised, intervention trial based in a rural area of The Gambia. It was designed to prove whether flies are mechanical vectors of trachoma; to quantify the relative importance of flies as vectors of trachoma and to test the effectiveness of insecticide spraying and the provision of latrines in trachoma control.

A total of 21 clusters each composed of 300-550 people, were recruited in groups of three. One cluster from each group was randomly allocated to receive insecticide spraying, one to receive pit latrines and the remaining to act as a control. The seven groups of clusters were recruited on a step-wise basis separated by two months to aid logistics and allow all seasons to be covered.

Standardised, validated trachoma surveys were conducted for people of all ages and both sexes at baseline and six months post intervention. The Muscid fly population was monitored using standard traps and fly-eye contact measured with catches of flies direct from children's faces.

The Flies and Eyes project has been designed to strengthen the evidence base for the 'E' component of the SAFE strategy for trachoma control. The results will assist programme planners and country co-ordinators to make informed decisions on the environmental aspects of trachoma control.

Introduction

In 1997, with the endorsement of the World Health Assembly, the World Health Organization instituted a new initiative aimed at the Global Elimination of Blinding Trachoma by the year 2020 (WHO, 1997). WHO joined with interested governments, non-governmental organisations and industry to form an alliance for the control of blinding trachoma known as the 'GET 2020 Alliance'. The cornerstone of the initiative has been the development of an integrated approach to trachoma control known as the SAFE strategy. This strategy has four elements: lid Surgery for entropion (in-turned eyelashes) and/or trichiasis (eyelashes abrading the cornea); Antibiotic treatment for active cases; Facial cleanliness and Environmental improvement.

Since the surgical correction of lid deformities has an immediate impact on preventing blindness in those at greatest risk, surgery is usually the first aspect of the strategy to be implemented. Lid surgery cannot reverse corneal opacity and there is no evidence that it improves visual acuity (Mabey & Fraser-Hurt, 2001), but it does relieve the considerable suffering caused by in-turned lashes and a high satisfaction rate has been reported by patients (Bowman *et al.*, 2000a). The community satisfaction following the provision of surgery can provide a useful entry point for implementing other aspects of the SAFE strategy. The use of antibiotics such as 1% topical tetracycline or oral azithromycin have been shown to be effective in the treatment of active trachoma cases (Schachter *et al.*, 1999). The prescription of medication is a recognised and established method of controlling disease and although reaching all cases presents logistical difficulties, it is foreseeable that existing health structures can be utilised for successful distribution. The use of antibiotics is typically the second implemented aspect of the strategy and is of particular importance where drug is available free as a result of the International Trachoma Initiative (Taylor & Taylor, 1999).

Blindness from trachoma is the result of frequent infections repeated over many years and the ultimate sustainability of trachoma control relies on reducing the frequency of infection in addition to the provision of surgery and medication. This requires that transmission be interrupted. Although it has been shown that the removal of the infectious reservoir with antibiotic therapy can theoretically halt transmission (Lietman *et al.*, 1999), the model used assumed that 100% of the population at risk could be treated and that in-migration of active cases was essentially non-existent. Since endemic trachoma is currently found in under-developed parts of Africa, Asia, South America and Australia (Muñoz & West, 1997), most of which suffer from poor infrastructure and difficult communication, this scenario is not realistic.

Achieving a reduction in the transmission of trachoma is the role of the F and E components of the SAFE strategy. Facial cleanliness and environmental improvement aim to prevent new cases, rather than cure current infections or reverse the effect of lid deformation. Because facial cleanliness through hygiene promotion requires intense effort to implement (West *et al.*, 1995), and the mechanisms to execute meaningful environmental improvements are not clear, there is a danger that the F and E components of the SAFE strategy may be sidelined in country programmes. In an effort to clarify the evidence base for F and E two recent reviews of the literature have been published (Chapter 2; Prüss & Mariotti, 2000). Both of these reviews point to a need for clinical trials to estimate the relative contribution of personal hygiene and fly control through environmental improvement to the risk of transmission of trachoma and the effectiveness of these elements on their own, or in combination with other aspects of the SAFE strategy.

This chapter describes the design and methods of an intervention study designed to test whether eye-seeking flies are vectors of trachoma and the relative contribution of this route of transmission in The Gambia. The study is also designed to show the relative effect on trachoma transmission of environmental improvement through the provision of locally designed latrines. This will provide essential evidence to allow the members of the GET 2020 alliance to make informed decisions about the promotion of environmental sanitation and fly control in their trachoma control programmes.

Background

It is believed that eye-seeking flies act as passive mechanical vectors of trachoma by picking up *Chlamydia trachomatis* on their feet and proboscis when feeding and transferring it to a susceptible host (Chapter 3). The 'Bazaar Fly', *Musca sorbens*, has been identified as the species most likely to act as a trachoma vector (Hafez & Attia, 1958c; Saccà, 1964; Chapter 4) because it is highly attracted to the eyes and is the species most frequently caught from the faces of children (Hafez & Attia, 1958a), who are considered to be the main reservoir of infection.

The larval habitat of *M. sorbens* is exposed faeces lying on the soil. A study from Egypt states that ..."*M. sorbens* breeds exclusively in human excrement defecated as isolated stools on borders of canals, in alleys, narrow streets etc."...(Hafez & Attia, 1958c), but other media have also been reported, such as dog faeces in Hawaii (Mau, 1978) and horse, cow and pig dung in Egypt (Hafez, 1941). Certainly human faeces appears to be the ideal medium and when the consistency, temperature and moisture content are optimal can support the development of several hundreds of adults per stool (Zimin, 1948; Hafez & Attia, 1958b). Catches of insects emerging from the drop holes of pit latrines in Botswana and Tanzania (Curtis & Hawkins, 1982) and The Gambia (Chapter 4) have never included *M. sorbens* suggesting strongly that these do not provide a suitable breeding site. Where there is restricted access to sanitation systems the provision of simple pit latrines may reduce the larval medium available for *M. sorbens* and therefore be a low cost tool suitable for inclusion as part of the E component of the SAFE strategy for trachoma control.

After controlling for other known risk factors the absence of a household latrine has been associated with a higher prevalence of active trachoma in risk factor analyses from Egypt (Courtright *et al.*, 1991), Malawi (Tielsch *et al.*, 1988), Ethiopia (Zerihun, 1997) and Brazil (Luna *et al.*, 1992) and the presence of a latrine associated with a lower prevalence of active trachoma in Tanzania (Taylor *et al.*, 1989b).

Study Design

The purpose of the project was to provide evidence to inform policy makers and planners of the relative merits of fly control and the provision of pit latrines in trachoma control programmes.

Aims

- To prove the concept that domestic flies are mechanical vectors of trachoma.
- To quantify the relative contribution of domestic flies in the transmission of trachoma.
- To test the provision of latrines as an intervention against *Musca sorbens* to reduce the transmission of trachoma.

Technical objectives

- To conduct a cluster randomised trial intervention against domestic flies that has an 80% power to detect a 35% reduction in trachoma at the 5% level of significance.
- To test the provision of latrines as a potential low cost sustainable intervention against *Musca sorbens* and trachoma transmission.

Summary of study design

The Flies and Eyes project was a cluster-randomised trial which had two intervention arms and a control arm running concurrently (Figure 6.2). A total of 21 clusters each composed of 300-550 people were recruited in seven groups of three. One cluster from each group of three was randomly allocated to receive insecticide spraying, one to receive pit latrines and the remaining cluster to act as a control. The seven groups of clusters were recruited on a step-wise basis separated by two months to aid logistics and allow all seasons to be covered. The clusters were enumerated and subsequently screened for trachoma at months 0 and 6. Biweekly fly surveillance was carried out in all clusters for two months before the baseline trachoma survey, throughout the six months of the intervention and for three months after the post-intervention trachoma survey. After the second survey all participating villages received pit latrines.

Identification and recruitment of Study Villages

The study was conducted in the Saloum and Nianija districts of Central River Division and the Sanjal district of North Bank Division in The Gambia. Each cluster was composed of one village or up to three neighbouring hamlets with a total population of between 300 and 550 individuals. Villages were selected on the basis of known trachoma endemicity and matched for size, but not ethnicity. All study villages had benefited from Government/NGO collaborations in water supply and had ready access to potable water from covered wells, but none had a formal system of rubbish disposal.

Pre-implementation sensitisation was conducted in a top down approach involving the local government hierarchy in addition to the Ministry of Health. In the local government sector the project was introduced to the Divisional Commissioners and the District Chiefs before going to the villages. In the health sector the Secretary of State was sensitised, followed by the Divisional Health Teams and staff of government and non-governmental clinics in the study area. When all relevant authorities were sensitised discussions were held with the village elders in candidate villages. In order to avoid any problems caused by the need for control clusters the allocation of interventions was conducted in a transparent manner by "lucky dip" in a public meeting of the village headmen presided over by the area chief. The headmen were then responsible for drawing the intervention for their cluster from the hat in the presence of the others.

Once agreement for participation had been given by the elders, a full sensitisation meeting was held. To ensure that all participants were sensitised in the same way the meetings followed a prescribed format. The script of the sensitisation was developed in English, translated to Wollof, Fula (Tukalor dialect) and Mandinka, then back translated by a third party. Nuances of particular words were discussed. Meetings were conducted in the relevant local language by a staff member of that ethnicity. In addition to information concerning the Flies and Eyes project the sensitisation included background information about MRC and NECP and gave ample opportunity for comments and questions from the audience.

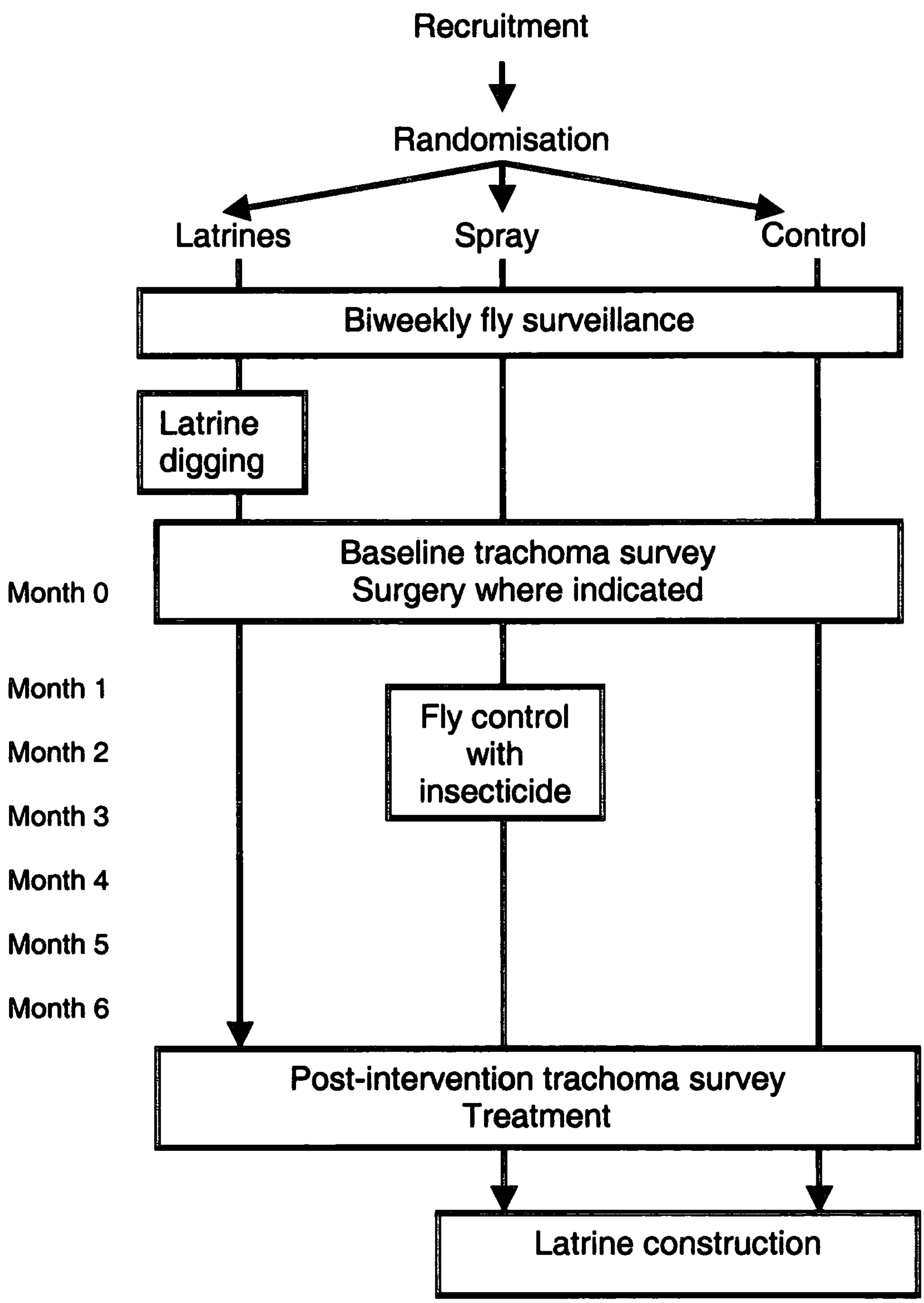

Figure 6.2 Simplified schematic of 'Flies and Eyes' project study design showing one of seven sets of three clusters

Recruitment and enumeration of participants

At enumeration all compounds were given a unique seven digit identifier in the form '12-3-4567' where the first two digits represented the cluster number; the third digit represented the village within a cluster; and fourth to seventh digits represented the compound number. Compound numbers were constructed by dividing the village into existing residential blocks (2 digits) and numbering clockwise within a block (2 digits). The four digit compound number was painted in a visible position on a wall. Within the compound the sleeping rooms were additionally numbered with a two digit number.

All people resident in the compound were enumerated. Those over three months old, and who intended to stay for six months or more, were recruited to the study. The following details were taken for each person: Compound ID; name of head of compound; name of head of household; room number; first name; second name; alias; ethnicity; mother's name; sex; age and method of age determination. 'Compound Head' was a designation assigned by the residents and usually given to the oldest man (very occasionally a woman) to whom they reported. 'Household Head' was the man (or woman) who was in charge of the finances of a family within a compound. There were usually one to three households in a compound. Census taking was associated by some people with taxation, these people may have had a tendency to reduce the reported population of a compound. Others associated censuses with the distribution of aid and may have wished to inflate the population. To avoid this, each person enumerated was either seen in person or family members were asked to describe where missing people were, and what they were doing. Age of children was determined from the Infant Welfare Card; Clinic Card; Birth Certificate; mother's birth history or by relating the child to another of known age. For adults a local events history calendar was used. Each resident was assigned a five digit individual identifier with the form '1-23-45' where one represented the household; two and three represented the room number; and four and five the person within that room. When concatenated with the compound ID this gave a unique identifier which allowed analysis at the level of the cluster, village, compound, household or room.

Living conditions for each household (economic unit) within a compound were assessed by collecting data on the source of drinking and washing water; the time taken to collect water; the mode of waste disposal; the presence and type of a functioning latrine and by assigning each sleeping room a score from one (highest) to eight (lowest) based on the building materials used and condition of the structure and fabric. Room scores were combined to form a composite score for the household, compound or village.

Entomological Sampling

Indoor and outside fly populations were monitored for two months prior to starting an intervention, during the intervention and for three months after it. The collection period before the intervention allowed the determination of the baseline fly density and the post-intervention collections gave an indication of the duration of control achieved with insecticide spraying. Flies were collected using 245mm x 200mm yellow sticky traps (AgriSense, Pontypridd, MidGlamorgan, UK). Sixteen traps were set biweekly per cluster and collected one week later. Eight traps were hung inside rooms and eight outside. The number of traps set in a compound was limited to a maximum of one inside and one outside by random selection without replacement, of compound numbers from a hat. Flies were not distributed randomly in compounds or rooms therefore the exact location of the traps was not random. *M. sorbens* is a synanthropic fly, and is found in greatest densities where it has access to people. Indoor traps were set in rooms that are 'usually open' since flies could only enter through open doors and windows. To standardise the location in a room, traps were hung to the opening-side of the front door. Flies stuck to traps hanging outside were liable to desiccate, become brittle and the bodies break off when exposed to rain or strong wind. In order to maximise the potential catch and reduce the likelihood that desiccated flies would be dislodged, outside traps were set in a location that best met the following criteria: protected from rain and strong wind; under shade (to reduce desiccation) and close to a place where people gather. Once selected, trap sites were fixed for the period of the trial. Fly-eye contact was measured by direct capture of flies from the faces of children in hand-nets as described previously (Chapter 4). Eight hand-net collections were made per cluster bi-weekly. Flies were sorted in the laboratory using magnification and standard taxonomic keys (Zumpt, 1956; Pont, 1991; Crosskey & Lane, 1993) where necessary.

Trachoma Surveillance

At month 0 and 6 all participants were screened for clinical signs of trachoma by the same NECP-trained Community Ophthalmic Nurse. Both eyes were inspected for signs of trichiasis and the right eyelid was everted and examined with x2.5 magnification and torch light if required. When fewer than five follicles, or follicles <0.5mm were seen in the right eye the left eye was also examined. Clinical signs were graded according to the WHO simplified grading system (Thylefors *et al.*, 1987) (Figure

6.3). Inter-observer variation was avoided by using a single screener. Intra-observer variation was minimised, and consistency monitored, by frequent (every 6-9 months) standardisation exercises.

Participants identified in the baseline survey with symptomatic trachoma (grade TI) were offered treatment with 1% topical tetracycline or oral azithromycin according to national guidelines. Participants with any clinical sign of active trachoma were offered treatment after the second survey. Baseline treatment was likely to reduce the overall community prevalence of active disease in the second survey. However, this would be similar between intervention groups and outcome measures were in comparison to the control group. Study participants identified in the clinical surveys with trichiasis were offered free, community-based, lid surgery. Each affected individual was counselled in the presence of close family members, then a day arranged for surgery to be performed on everyone affected in the village about a week later. Surgery was carried out in a designated place, usually the headman's compound. Those who declined to attend were counselled again and offered surgery at the district hospital via the normal NECP routes.

Grade	Description
N	Normal eye, no evidence of current or past infection.
TF	Follicular trachoma: Presence of 5 or more follicles >0.5mm in diameter in central zone.
TI	Trachomatous inflammation- intense: pronounced inflammatory thickening that obscures more than half of the normal deep tarsal vessels.
TS	Trachomatous scarring: presence of easily visible scarring.
TT	Trachomatous trichiasis: at least one eyelash rubs on the eye ball. Evidence of recent removal of inturned lashes should also be graded as trichiasis.
CO	Corneal opacity: presence of easily visible corneal opacity over the pupil.

Figure 6.3 WHO simplified grading scale for trachoma

Fly Control with insecticide

After obtaining clearance from the National Environment Agency, flies were controlled by space-spraying with water soluble permethrin (Aqua-Resilin®, Agrevo Environmental, UK) at a rate of 3.75-5.0g active ingredient per hectare. All areas in the village were sprayed, with particular attention given to vegetation near houses, door ways, kitchens and rubbish heaps where flies congregated. Insecticide was administered with Hudson Portapac® (Hudson Manufacturing, Chicago, USA) or Micronair AU8000® (Micron, Bromyard, UK) spray equipment. Fly control was based on an 'attack phase' of spraying every two days for two weeks to kill the adult population followed by a 'maintenance phase' of a twice weekly spray round. Spraying was conducted by suitably trained and protected personnel in the relative cool of the morning when the normal temperature gradient was reversed – the air being warmer than the ground. This stopped the 25-40μm spray particles being carried away in convection currents and lead them to hang in an invisible mist in the target area. People were advised to cover food and water on spray days and leave their compounds, and not to re-enter for ten minutes after spraying.

Latrine construction

Pit latrines were constructed by the Department for Community Development (DCD), using their Rural Environmental Sanitation Programme infra-structure. Latrines were of the Gambian Improved Household Pit latrine type (Figures 6.4-6.7). They were constructed in two parts: the 'collar' and the 'slab'. The collar was composed of two courses of eight cement blocks and a cement base for the slab. When the collar was cured the latrine was dug straight down to a depth of 3-3.5m and the ferro-reinforced slab laid on top. The collar prevented rats from digging under the slab and stopped the top of the pit collapsing in the rainy season. Materials required per latrine were two 50Kg bags of cement, 6m of 4mm diameter ferro-reinforcing bar and 5½ wheel-barrows of sand. Labour was provided by the Village Development Committees. Latrines were allocated at the rate of one per compound or per twenty people, whichever was the greater. Compounds retained ownership of latrines by being responsible for the provision of any shelter around the slab. Latrines were dug within 6m of the nearest house and at least 30m away from wells and water sources.

Sample size calculations

Assuming a mean age-standardised community prevalence of active trachoma at baseline of 25%, and that this varies between 15% and 35%, a design with clusters matched for baseline trachoma prevalence and size, with 7 clusters in each group will give an 80% power to detect a 35% reduction in trachoma prevalence at the 5% level of significance.

Figure 6.4 Cement blocks with rounded front edges to make the latrine 'collar'

Figure 6.5 Completed latrine 'collar' ready for digging

Figure 6.6 Digging the latrine to 3.5 metres

Figure 6.7 The owner showing the shelter he has built around his completed latrine

Discussion

The intervention trial described in this paper is best suited to community level randomisation because of the need to reduce fly-eye contact through insecticide spraying as well as the use of latrines. Randomisation at the household level would not have worked for two reasons:

1) Households are not discrete geographical units within a village. They butt against each other and other households are always in sight. Flies are able to move these distances so there would be a constant threat of re-invasion of the spray households after each application of insecticide.

2) Children do not stay in their own households during the day. Movement of children between spray and control households would result in those from the spray households still being exposed to eye-seeking flies and visa versa.

If fly control proved effective at controlling trachoma, latrine interventions may be effective when implemented at the household level depending on local conditions such as distance between households, the level of environmental contamination with faeces, human behaviour and the ecology and density of eye-seeking flies.

For the Flies and Eyes project, and the SAFE strategy for trachoma control, to be a success both individual and community participation are needed. A climate in which there is community support, yet in which individuals give their own consent, can be fostered through careful sensitisation at all levels. This may be best achieved by going through established and trusted channels. Surgery, which requires individual decision making seems to be more acceptable where group participation can be used to allay individual fears (Bowman *et al.*, 2000b).

Problems were encountered during the Flies and Eyes project. In one instance, an individual was spreading false accusations about the project. The active support and endorsement of the district chief in an impromptu village meeting proved decisive in regaining community support. There remain some villages where difficulties such as local schisms render whole community support untenable. These difficulties may be directly associated with the disadvantage that puts these communities at risk for under-development and possibly higher risk for trachoma. It is these very communities where implementing the SAFE strategy is most difficult.

The building of latrines was co-ordinated by the Flies and Eyes team who allocated them on the basis of the enumeration data, demarcated suitable positions following guidelines from the health inspector and ensured that the necessary materials were available and delivered to the villages. Construction of the latrines was conducted by Department of Community Development extension workers using the village development committees to arrange labour, cast cement blocks and slabs and carry out the construction. If the project was successful in proving that latrines do reduce the prevalence of trachoma and provides an explanatory mechanism for this, then latrine building could be implemented as part of the SAFE strategy. Applications for funding will be strengthened because of the availability of existing technologies and the wider health benefits of improved sanitation. In many sites tools, skills and infra-structure for implementation are already available without modification.

The Flies and Eyes project has melded some of the health and development aims of the Gambian government with research questions and has, where possible, utilised skills and resources currently available in-country. It complements the National Eye Care Programme trachoma control activities but relies on NECP staff for trachoma screening and the provision of community-based lid surgeries, whilst contributing to capacity building by exposing staff to new techniques and research opportunities. It has also helped the Rural Sanitation Unit of the Department for Community Development exceed its targets for latrine construction in the study areas.

Chapter 7

Effect of fly spraying and pit latrines on trachoma transmission in The Gambia: results of the Flies and Eyes project

Figure 7.1 Alieu Tunkara laying a latrine slab

Effect of fly spraying and pit latrines on trachoma transmission in The Gambia: results of the Flies and Eyes project

Abstract

Background The Flies and Eyes project was a community-based, cluster-randomised, controlled trial designed to show whether eye-seeking flies are vectors of trachoma; to assess the scale of their role in trachoma transmission; and to test a method of sustainable fly control using pit latrines.

Methods Clusters were recruited in sets of three, and randomly allocated to receive insecticide spraying for six months, household latrines or to act as a control. Participants were screened for clinical signs of trachoma at baseline and after six months.

Findings Of 7080 people enrolled, 6087 (86%) were screened at follow-up. Baseline prevalence of active trachoma was 5.8%. Controlling for ethnicity, sex and household characteristics, and allowing for the effect of household clustering of cases, young age was strongly associated with active trachoma at baseline: age 0-4 years OR=6.42 (95% CI; 4.52-9.13), age 5-9 OR=4.72 (3.43-6.49) age 10-14 OR=1.85 (1.22-2.80). No other individual or household level factors predicted active trachoma at baseline. Fly spraying resulted in 91% fewer *Musca sorbens* contacting children's eyes and provision of latrines was associated with a 25% reduction in fly-eye contact compared with the controls. Analysis of age-standardised trachoma prevalence rates at the cluster level (N=14) showed that spraying was associated with a mean reduction in trachoma prevalence of 56% (19-93%; t=3.70, 6d.f. P=0.01) compared to controls and with 37% (4.3-69.6%; t=2.218, 6d.f. P=0.068) fewer new prevalent cases at follow-up. Provision of latrines was associated with a mean reduction in community prevalence of 30% (-21.9-80.8%; t=1.405, 6d.f. P=0.210) compared to controls and with 28% (-4.8-60.2%; t=1.671, 6d.f. P=0.146) fewer new prevalent cases at follow-up.

Interpretation These findings show that both the community prevalence of trachoma and the number of new prevalent cases of trachoma were substantially lower in clusters receiving fly control compared to control clusters. The effect of household level latrine provision on trachoma prevalence was encouraging, and further investigation of the use of simple latrines in trachoma control is warranted.

Introduction

Building on the pilot work presented in Chapter 3, which suggested that eye-seeking flies were important in the transmission of trachoma, a community-based, cluster-randomised, controlled trial to demonstrate whether flies were vectors of trachoma, and to assess the scale of their role was designed (Chapter 6). Studies on the ecology of eye-seeking flies have shown that the majority of fly-eye contacts were made by *Musca sorbens* (Chapter 4) and that this species of fly breeds most successfully in human faeces lying on the surface of the soil (Chapter 5). Therefore a community-based strategy to reduce the quantity of human faeces on the soil surface by providing latrines was simultaneously tested, which we hypothesised would have the effect of reducing the population of *M. sorbens*, and hence reduce fly-eye contact and trachoma transmission.

Methods

Recruitment

A detailed description of the study design is given in Chapter 6. Briefly, since fly control is best achieved at the whole community level, rather than at the household, or individual level, the trial was designed around a series of seven triplets of clusters, each cluster comprising one or more whole communities. Within each of the seven triplets the clusters were randomly allocated to receive either insecticide spraying to control flies, the provision of household level pit latrines or to act as a control. The triplets were introduced into the study on a step-wise basis separated by two months to aid logistics and to ensure that the trial covered all seasons. After six months of participation the project provided all households in the study communities with pit latrines.

Clinical surveys

Study participants were screened for clinical signs of trachoma at baseline and after six months of intervention by the same grader using standard methods as advocated by WHO (Thylefors *et al.*, 1987).

Household surveys

Trachoma is known to cluster by household. This observation has been linked with differences in socio-economic status, the presence of a latrine, distance from a water supply, time taken to collect water and the presence of refuse (reviewed in Chapter 2). Profiles of the distribution of these potential risk factors for trachoma were collected from each participating household. The building materials and condition of houses were used as an objective proxy for socio-economic status. The presence of latrines was validated by direct observation. As all villages had a well and there was little variation in distance to water the round-trip time taken to collect water was used as a measure of water accessibility.

Fly control and surveillance

Flies were controlled by ULV space-spraying with water soluble permethrin (Aqua-Resilin®, Agrevo Environmental, UK) at a rate of 3.75-5.0g active ingredient per hectare using Hudson Portapac® (Hudson Manufacturing, Chicago, USA) or Micronair AU8000® (Micron, Bromyard, UK) spray equipment. Fly control was based on an 'attack phase' of spraying every two days for two weeks to kill the adult population followed by a 'maintenance phase' of a twice weekly spray round. Fly populations were monitored in all clusters using standard biweekly sticky fly-traps and from catches of eye-seeking flies from the faces of volunteer children.

Sample size considerations

The study was designed to detect a 35% reduction in active trachoma cases between the control and intervention groups, at the 5% level of significance and with 80% power (Chapter 6).

Ethical approval

This study was approved by the joint Ethical Committee of the Gambian Government and Medical Research Council Laboratories. Children were recruited to the study after their parents or guardians had given their informed consent.

Statistical methods

Data were doubled entered by different entry clerks in EpiInfo (version 6.01). Validated data files were analysed using SPSS (version 10.0) and STATA (version 6.0). Clinical results and data for personal and household characteristics were normally distributed and described by the mean and 95% confidence intervals (95% CI). Categorical data were compared with chi-squared tests and continuous data with analysis of variance. Logistic regression was used to identify predictors of active disease at baseline for individuals, allowing for clustering at the household level using Huber/White standard errors. Aggregate household scores were constructed by taking the mean of the scores of the individual sleeping rooms per household. Households were then categorised into 'best' (top quartile), 'average' (middle quartiles) and 'worst' (bottom quartile) using the approach advocated by the Royal Australian College of

Ophthalmologists (1980). Hand-net and fly trap data were excessively skewed with many zero catches, particularly where there was fly control. They were described using an adjusted geometric mean (one added to all values and subtracted from the calculated geometric mean) and compared between two groups with the Wilcoxon sign-ranks test. Trachoma prevalence rates were age-standardised within each triplet using the direct method of standardisation, with four age categories; 0-4 years, 5-9 years, 10-14 years and ≥15 years. When calculating the rate of new prevalent trachoma cases the denominator was the cluster population who did not have signs of active trachoma at baseline. Rates were compared between treatment groups using paired t-tests (Hayes & Bennett, 1999). Quoted P values are two-tailed.

Results

Enrolment and loss to follow-up

After participation in the study had been agreed in a village meeting everybody was enumerated. Of the 8005 people enumerated from all clusters, 925 were not enrolled in the study (Figure 7.2). The majority (59.1%) of those not enrolled had travelled away from the study area between enumeration and screening; 34.3% were not eligible for enrolment because they were not available for screening, less than 3 months old, did not intend to stay in the village for the next six months or were mentally unwell; and 6.6% declined to participate.

Loss to follow-up was significantly greater in the control clusters (403/2606;15.5%) than the spray clusters (269/2244; 12.0%; χ^2= 11.9, P=0.001), although there was no difference between the control and latrine clusters (321/2230; 14.6%; χ^2=1.0, P=0.32). Most of those lost to follow-up (718/993, 72.3%), had travelled away from home, or were not available for screening on any of the three visits to each cluster for the second clinical survey (149/993, 15.0%). Others had died (36/993, 3.6%), withdrew their consent to participate (26/993, 2.6%), or were lost for other reasons (64/993, 6.4%).

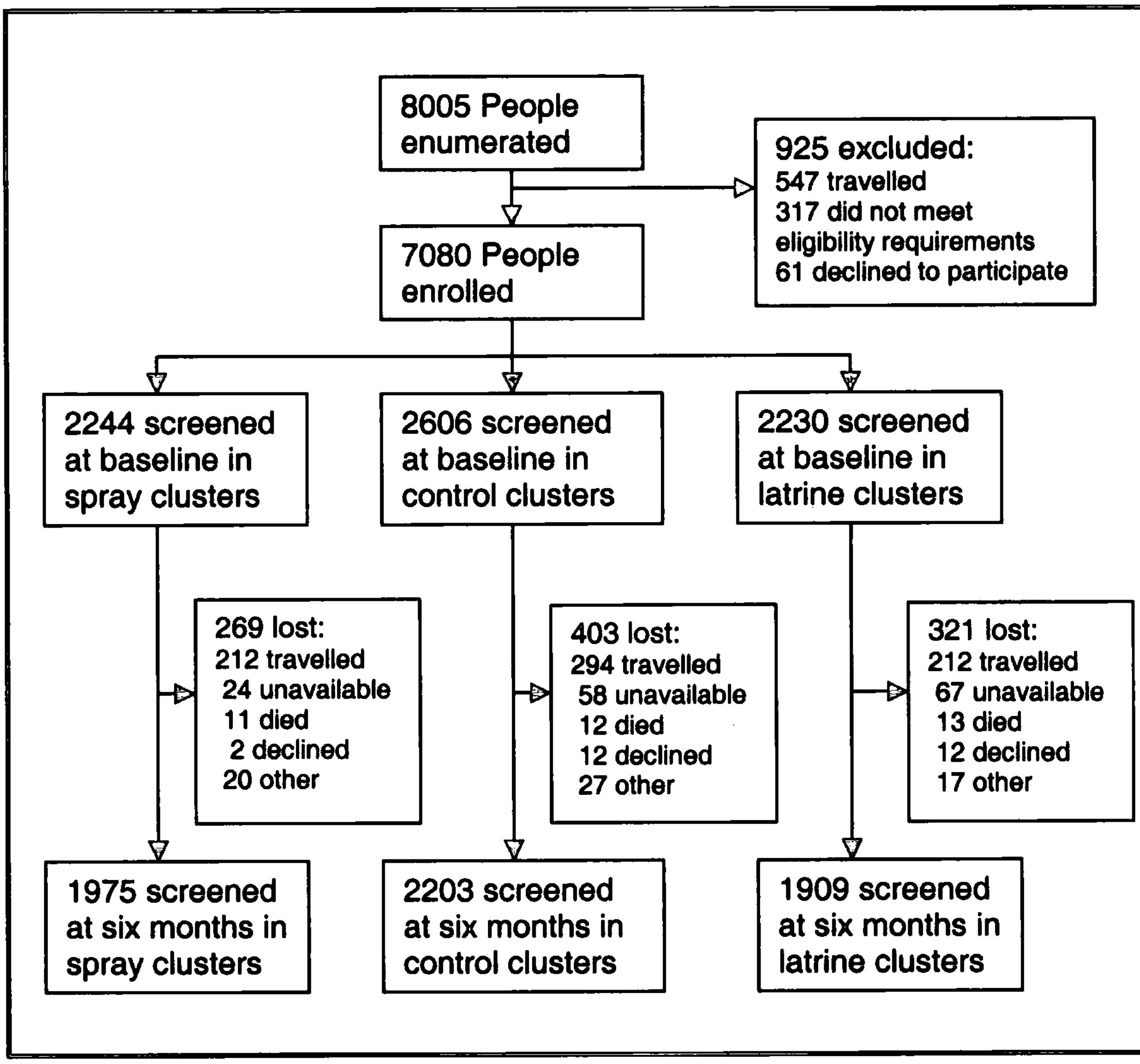

Figure 7.2 'Flies and Eyes' project trial profile

Baseline trachoma survey

The crude baseline trachoma survey results, for the 6087 study participants who were seen both at baseline and follow-up, are shown in Table 7.1. The overall baseline prevalence of clinically active trachoma (grades TF, TI or both) was 5.8%, evidence of scarring (without entropion) was visible in 14.8% of participants and trachomatous trichiasis was present in 1.2%. There were more active cases in the spray clusters (141/1975, 7.1%), than the control clusters (107/2203; 4.9%, χ^2=9.31, P=0.002), but no difference between the latrine (106/1909; 5.6%) and control clusters (χ^2= 0.87, P=0.35). Active trachoma was more commonly seen in children (Figure 7.3).

Table 7.1 Crude results of baseline trachoma survey using the World Health Organization Simplified Grading System for 6087 study participants seen in both surveys

Trachoma grade	Treatment Group Spray N (%)	Control N (%)	Latrine N (%)	Total
N	1483 (75.1)	1785 (81.0)	1518 (79.5)	4786 (78.6)
TF	121 (6.1)	92 (4.2)	92 (4.8)	305 (5.0)
TI*	20 (1.0)	15 (0.7)	14 (0.7)	49 (0.8)
TS	316 (16.0)	296 (13.4)	259 (13.6)	871 (14.3)
TT	33 (1.7)	13 (0.6)	25 (1.3)	71 (1.2)
CO	2 (0.1)	2 (0.1)	1 (0.1)	5 (0.1)
Total screened	1975	2203	1909	6087

*Includes grades TI, TF/TI and TS/TI

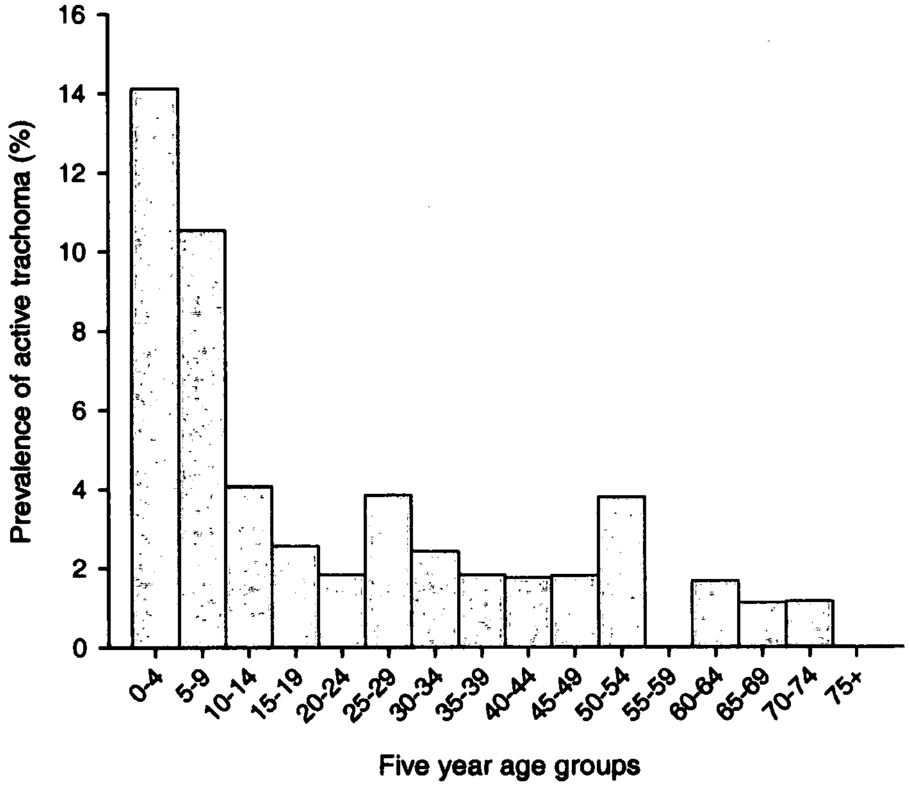

Figure 7.3 Graph of active trachoma prevalence against five year age group

Results of logistic regression analysis to identify potential individual and household level predictors for active disease are shown in Table 7.2. At the individual level, belonging to one of the young age groups (0-4, 5-9 and 10-14 years) was strongly associated with a greater risk of being an active case compared to those aged 15 years and above. Membership of any of the ethnic groups in the study population and sex were not associated with active trachoma at baseline.

At the household level the presence of any latrine (whether it be a 'local' or 'improved' type) was weakly associated with less active trachoma OR=0.77 (95% CI; 0.56-1.06) compared to the absence of a latrine. The time taken to collect water was not associated with active trachoma. The method of refuse disposal was the same for all participants and therefore not included. There was no association between housing score and risk of active trachoma.

Baseline characteristics of the study population by treatment group.

The proportion of children (under 15 year-olds) was similar between the spray clusters and the control clusters (spray 1049/1975 (53.1%), control 1155/2203 (52.4%) χ^2=0.17, P=0.68) and the latrine clusters and the control clusters (latrine, 1012/1909 (53.0%) χ^2=0.12, P=0.73). Clusters were not matched on ethnicity at recruitment, so there were differences in the ethnic composition of the treatment groups which were due to chance. However, since ethnicity was not associated with being an active case of trachoma, it was not controlled for in analyses.

Table 7.2 Multivariate model of individual and household level risk factors predicting active trachoma at baseline adjusted for clustering by household

Risk factors for active trachoma	Active cases/N†	% active	Odds Ratio (95% CI)	P
Individual level risk factors				
Age				
Age 0-4 years	150/1209	12.4	6.42 (4.52-9.13)	<0.001
Age 5-9 years	108/1127	9.6	4.72 (3.43-6.49)	<0.001
Age 10-14 years	34/867	3.9	1.85 (1.22-2.80)	0.004
Age 15+	62/2855	2.2	1.0	-
Sex				
Female sex	178/3325	5.4	0.97 (0.79-1.20)	0.804
Male sex	176/2733	6.4	1.0	-
Ethnicity				
Fula	148/2475	6.0	1.00 (0.75-1.35)	0.990
Mandinka	14/307	4.6	0.81 (0.42-1.57)	0.525
Other ethnicity	5/38	13.2	3.14 (0.70-14.18)	0.136
Wollof	187/3238	5.8	1.0	-
Household level risk factors				
Sanitation				
Latrine	115/2221	5.2	0.77 (0.56-1.06)	0.106
No latrine	239/3837	6.2	1.0	-
Round-trip to water				
≤ 30 min	289/4781	6.0	1.19 (0.91-1.58)	0.201
> 30 min	65/1277	5.1	1.0	-
Housing conditions				
Best conditions	98/1568	6.3	1.18 (0.81-1.70)	0.386
Average conditions	145/2582	5.6	1.0	-
Worst conditions	111/1892	5.9	1.01 (0.72-1.41)	0.951

† Denominators add to 6058 (not 6087) because of missing data for some variables

Main activity of children aged 5-15 years

Table 7.3 shows the results of a multivariate model of the main daily activity of 1507 children (52% girls) and risk of active trachoma, adjusting for household clustering, compared to children who helped at home or in the farm. Where more than one activity category applied only the main one was recorded. A total of 470 children (31%) had been studying the Koran, of whom 46% were girls; 74 (16%) of the koranic scholars lived away from home with their teacher and 396 (84%) lived at home attending a nearby madrass (Islamic school). Government school, offering an academic curriculum, was attended by 278 children (18.4% of the total), of whom 45% were girls. Of the 516 children (34%) who helped their parents by working at home or in the farm, girls comprised 88% of those working at home and 29% of those working in the farm. Of the 228 children for whom the main daily activity was staying at home to play, 48% were girls. There were no differences in the prevalence of active trachoma by main activities that was not explained by differences in age.

Table 7.3 Multivariate model of main daily activity and risk of active trachoma for 1507 children aged 5-15 years adjusted for household clustering

Activity	Active cases/N	% active	OR (95% CI)	P
Age (each additional year)	-	-	0.85 (0.78-0.93)	<0.001
Koranic scholar living with teacher	2/74	2.7	0.47 (0.12-2.02)	0.316 n.s.
Koranic school	26/396	6.6	1.00 (0.58-1.70)	0.993 n.s.
Government school	10/278	3.6	0.60 (0.29-1.24)	0.167 n.s.
Herder	1/21	4.8	Insufficient data	
Home-helper	21/296	7.1	1.00	
Farm helper	12/199	6.0	1.00	
Stay at home	30/228	13.2	1.43 (0.82-2.51)	0.207 n.s.
Disabled	2/7	28.6	Insufficient data	
Formal employment	0/8	0	Insufficient data	

Household level characteristics between treatment groups

Household level characteristics were collected from 649 households. The overall mean housing score was 6.0 (95% CI 5.9-6.1) which did not vary between treatment groups (F=2.35, P=0.10). The mean number of people in a household was 12.3 (95%CI 11.7-12.9) and 2.8 (2.7-2.9) per sleeping room, and these did not vary between the treatment groups (number of people per household; F=2.63, P=0.15), number of people per sleeping room; F=0.13, P=0.87). Data on presence of a latrine were available for 99.5% of households; 207 (32%) had a functioning latrine of which 180 (87%) were the 'local' type and 27 (13%) the 'improved' type (i.e. one with a ferro-reinforced cement cover). There was no difference in the proportion of latrine ownership between spray clusters (33%) and control (32%; χ^2=0.01, P=0.908) or latrine clusters (31%) and control (χ^2=0.01, P=0.939). Data on refuse disposal was collected from 99% of households. All households reported throwing their domestic refuse onto a heap or into a hole in the ground (without covering it), no other method of waste disposal was reported. Refuse was almost always thrown onto a heap, or into a hole, outside the perimeter of the compound (98%), the remaining 2% disposed of it inside the compound. Almost all households (99.5%) provided data on water source. All reporting households got their water from a well; 549 (85%) from a covered one, and 97 (15%) from an open one. The majority (78%) of women reported that a round trip to collect water took less than 30 minutes. The proportion of women who reported that water collection took less than 30 minutes was similar between spray clusters (78%) and control (72%; χ^2=1.76, P=0.185), but was significantly greater in the latrine clusters (86%) compared to the control (χ^2=11.90, P<0.001). However, as this was not associated with the baseline prevalence of active trachoma this was not controlled for in analyses.

Entomology data

Hand-net collections

A total of 2009 hand-net collections of eye-seeking flies were conducted during the study period. All of the 3600 flies caught were either *M. sorbens* (87%) or *M. domestica* (13%). The ratio of female to male flies caught from eyes was approximately 4:1 (Table 7.4). Female *M. sorbens* comprised 70.1% of all flies caught from the eyes of volunteer children.

Table 7.4 Species and sex of flies caught in 2009 hand-net collections of flies from children's faces

Fly sex	*M. sorbens* N (%)	*M. domestica* N (%)	All flies N (%)
Male	613 (19.5)	99 (21.3)	712 (19.8)
Female	2523 (80.5)	365 (78.7)	2888 (80.2)
Total	3136 (100)	464 (100)	3600

The adjusted geometric mean number of *M. sorbens*, *M. domestica* and total flies caught per hand-net collection in each cluster, and the relative percentage difference in catch compared to controls is shown in table 7.5. The adjusted geometric mean number of *M. sorbens* and total flies was significantly lower in the spray cluster than the control cluster of every triplet, with a median reduction in *M. sorbens* and all flies of 91% compared to controls. A similar reduction with spraying was seen was *M. domestica*, although this did not reach statistical significance in cluster four, in which counts of *M. domestica* were low. Adjusted geometric mean counts of *M. sorbens* were lower in all the latrine clusters compared to the controls, the difference was statistically significant in triplets 1-3 and the overall median reduction was 25%. The change in the counts of *M. domestica* compared to the controls varied widely (range -74%, +302%) with an overall median reduction of 18%.

Table 7.5 Adjusted geometric mean number of *M. sorbens*, *M. domestica* and total flies caught per hand-net collection in each cluster, and relative percentage difference in fly numbers compared to controls

Triplet and treatment		*M. sorbens*		*M. domestica*		*Total flies*[1]	
		Geometric mean flies/child	Difference relative to control (%)	Geometric mean flies/child	Difference relative to control (%)	Geometric mean flies/child	Difference relative to control (%)
	Spray	0.27	-88.5*	0.00	-100.0*	0.27	-88.7*
1	Control	2.32	-	0.04	-	2.37	-
	Latrine	0.92	-60.5*	0.06	+67.6	1.01	-57.4*
	Spray	0.61	-79.2*	0.02	-94.2*	0.63	-81.1*
2	Control	2.92	-	0.33	-	3.34	-
	Latrine	1.78	-39.0*	0.10	-70.4*	1.88	-43.8*
	Spray	0.19	-90.9*	0.03	-95.1*	0.23	-92.2*
3	Control	2.10	-	0.64	-	2.93	-
	Latrine	1.23	-41.2*	1.09	+70.1†	2.81	-4.0
	Spray	0.11	-78.5*	0.01	-85.1	0.12	-78.9*
4	Control	0.50	-	0.05	-	0.55	-
	Latrine	0.48	-4.2	0.19	+302.1†	0.66	+20.0
	Spray	0.06	-96.2*	0.04	-81.8*	0.08	-95.6*
5	Control	1.55	-	0.22	-	1.86	-
	Latrine	1.16	-25.2	0.16	-27.3	1.38	-26.0*
	Spray	0.16	-91.3*	0.00	-100.0*	0.16	-92.1*
6	Control	1.85	-	0.11	-	2.03	-
	Latrine	1.85	-0.2	0.09	-18.2	1.96	-3.5
	Spray	0.17	-91.1*	0.02	-88.9*	0.19	-91.4*
7	Control	1.95	-	0.18	-	2.23	-
	Latrine	1.84	-5.9	0.13	-27.8	2.00	-10.2
Median % difference between spray & control			-90.9		-94.2		-91.4
Median % difference latrine & control			-25.2		-18.2		-10.2

[1] Adjusted geometric mean of total flies is not exactly equal to the sum of the adjusted geometric means of *M. sorbens* and *M. domestica*.

*Significant decrease compared to control, P<0.05

†Significant increase compared to control, P<0.05

Sticky traps

A total of 3871 sticky traps were set during the study; 1937 were inside people's houses and 1934 outside. The traps caught 64388 flies of which 65% were *M. domestica*, 23% *Chrysomya albiceps*, 10% *M. sorbens* and 2% other species. Females accounted for 45% of *M. domestica* and 53% of *M. sorbens.*

Table 7.6 shows the adjusted geometric mean number of *M. sorbens*, *M. domestica* and *C. albiceps* caught per trap in each cluster for each triplet, and the relative percentage difference in fly numbers between the spray and control clusters, and the latrine and control clusters. The adjusted geometric mean number of *M. sorbens* and *M. domestica* was significantly lower in all the insecticide-spraying clusters than control clusters, except triplet three. The median percentage decrease relative to the controls was 74% for *M. sorbens* and 71% for *M. domestica*. The number of *C. albiceps* caught in the spray clusters was an average of 11% greater than that caught in the control clusters. The adjusted geometric mean number of *C. albiceps* caught was 153% higher in the latrine clusters than control clusters in all triplets. There was no overall difference in the adjusted geometric mean number of *M. sorbens* or *M. domestica* caught on the sticky traps in the latrine clusters relative to the control clusters.

Table 7.6 Adjusted geometric mean number of flies caught per sticky trap in each cluster, and the relative percentage difference in fly numbers compared to controls

Triplet and treatment		*M. sorbens* Geometric mean flies/trap	*M. sorbens* Difference relative to control (%)	*M. domestica* Geometric mean flies/trap	*M. domestica* Difference relative to control (%)	*C. albiceps* Geometric mean flies/trap	*C. albiceps* Difference relative to control (%)
1	Spray	0.16	-88.7*	0.48	-78.4*	0.40	+73.9†
	Control	1.42	-	2.22	-	0.23	-
	Latrine	0.44	-69.0*	0.76	-65.8*	0.43	+87.0†
2	Spray	0.22	-78.4*	0.48	-79.9*	0.15	-92.3*
	Control	1.02	-	2.39	-	1.94	-
	Latrine	1.54	+51.0	4.22	+76.6†	5.48	+182.5†
3	Spray	0.78	-29.1	5.78	-18.4	0.50	+11.1
	Control	1.10	-	7.08	-	0.45	-
	Latrine	2.85	+159.1†	17.94	+153.4†	3.85	+755.6†
4	Spray	0.23	-73.6*	3.00	-68.8*	1.80	+97.8†
	Control	0.87	-	9.61	-	0.91	-
	Latrine	0.98	+12.6	10.33	+7.5	1.37	+50.5†
5	Spray	0.23	-67.1*	1.56	-71.2*	0.68	+47.8
	Control	0.70	-	5.42	-	0.46	-
	Latrine	0.70	0.0	3.58	-33.9*	1.60	+247.8†
6	Spray	0.13	-81.2*	0.60	-70.6*	0.37	-17.8
	Control	0.69	-	2.04	-	0.45	-
	Latrine	0.36	-47.8*	2.37	+16.18	1.14	+153.3†
7	Spray	0.36	-60.9*	1.14	-58.7*	0.32	-61.9*
	Control	0.92	-	2.76	-	0.84	-
	Latrine	1.15	+25.0	3.67	+32.97†	1.78	+111.9†
Median % difference between spray & control			-73.6		-70.6		+11.1
Median % difference between latrine & control			+12.6		+16.2		+153.3

*Significant decrease compared to control, P<0.05

†Significant increase compared to control, P<0.05

Clinical data

The age-standardised community prevalence of trachoma at baseline and after six months, and the change in prevalence are shown in Table 7.7. Within each pair of spray and control clusters the change in prevalence was lower in the spray cluster, with a mean difference of 3.47 cases/100 population (t=4.265, 6d.f. P=0.005). The mean reduction in the age-standardised community prevalence of trachoma in the spray clusters compared to the control clusters was 55.8% (18.8-92.7%) (t=3.696, 6d.f. P=0.01).

There was a mean difference of 1.26 cases per hundred population between the latrine and control groups (t=1.328, 6d.f. P=0.232). Of the seven pairs of clusters the change in prevalence in the latrine cluster was lower than that in the control cluster on four occasions, higher on two and similar in one. The mean reduction in the age-adjusted community prevalence of trachoma between the latrine and control groups was 29.5% (-21.9-80.8%) (t=1.405, 6d.f. P=0.210).

Table 7.7 Age-standardised community prevalence of trachoma by treatment group per triplet at baseline and after six months with change in prevalence.

	Treatment								
	Spray			Control			Latrine		
Triplet	rate1	Rate2	Rate change	Rate1	rate2	Rate change	rate1	rate2	Rate change
1	5.39	4.33	-1.07	2.89	6.27	+3.38	3.57	4.35	+0.78
2	7.46	4.15	-3.31	4.93	5.60	+0.67	3.74	4.61	+0.87
3	11.23	4.16	-7.07	4.04	4.36	+0.33	5.11	2.25	-2.86
4	6.95	1.60	-5.34	8.31	3.66	-4.66	6.20	3.37	-2.83
5	7.26	3.28	-3.98	3.98	2.74	-1.24	9.64	6.53	-3.11
6	3.90	3.89	-0.01	4.43	6.19	+1.76	4.98	1.97	-3.01
7	8.08	4.40	-3.67	6.66	6.30	-0.36	4.21	5.42	+1.21
Mean	7.18	3.69	-3.49	5.03	5.02	-0.02	5.35	4.07	-1.28

Table 7.8 shows the age-standardised rates, per 100 people, of new prevalent cases of trachoma in each cluster at the six month survey. The mean rate difference between the spray and control groups was 1.24 (t=2.218, 6d.f. P=0.068). This equates to 36.9% (4.3-69.6%) fewer new prevalent cases in the spray clusters than in the controls. The mean rate difference between the latrine and control groups was 0.93 (t=1.671, 6d.f.

P=0.146), which equates to 27.7% (-4.8-60.2%) fewer new prevalent cases compared to controls.

Table 7.8 Age-standardised rates/100 people, of new prevalent cases of trachoma by treatment group per triplet at the six month survey

Triplet	Treatment		
	Spray	Control	Latrine
1	3.90	5.66	3.82
2	1.94	4.21	3.17
3	0.38	3.48	0.92
4	0.43	0.86	1.01
5	2.65	1.23	2.77
6	2.20	4.02	1.69
7	3.33	4.05	3.62
Mean	2.12	3.36	2.45
(95% CI)	(1.12-3.11)	(2.08-4.63)	(1.53-3.33)

Discussion

In this cluster-randomised trial, fly control with insecticide spraying resulted in a reduction in the community prevalence of trachoma of 56% (95% CI 19-93%, P=0.01), compared to the control clusters, and was associated with 37% (4-70%, P=0.068) fewer new prevalent cases of trachoma in the follow-up survey. These findings demonstrate that the presence of eye-seeking flies in Gambian villages is associated with a higher prevalence of trachoma and more new prevalent cases of trachoma compared to villages in which flies are controlled. This supports the hypothesis that eye-seeking flies are trachoma vectors.

The provision of basic pit latrines to every household in a village – without additional health education – had the effect of reducing the number of *M. sorbens* caught coming to the eyes of children, which was accompanied by a decrease in the community prevalence of trachoma by 30% and a 28% decrease in new prevalent cases. These reductions did not achieve statistical significance (P=0.21 and P=0.232 respectively) when analysed by cluster (N=14 in each comparison). However, they demonstrate an additional, and significant, public health benefit of the safe disposal of human faeces, which is consistent with the observation that the absence of latrines is linked with increased rates of trachoma, elegantly made by Courtright and colleagues in 1991.

The trial was designed to detect a difference in trachoma prevalence between intervention and control of 35% when analysed with paired t-tests, as advocated by Hayes *et al.* (1995). The unit of sample was the cluster with N=14 in each paired t-test. This form of design is robust and takes account of clustering of cases and season, but produces wide confidence intervals which may be considered conservative. Individual level analysis would increase sample size to over 4,000 in each comparison and narrow the confidence intervals, but would be inappropriate as it would assume that each new case of trachoma was an isolated and independent event. A less conservative analysis could be conducted using the method of Generalised Estimation Equations which makes no assumptions about the causal relations among the covariates beyond cause preceding effect (Rothman & Greenland, 1998).

The allocation of treatment to the clusters was by random selection by the village heads, so the higher baseline population seen in the control clusters compared to the latrine or spray clusters was by chance. Overall loss to follow-up after six months was higher in the control (16%) than the spray clusters (12%), but similar to that in the latrine clusters (14%). The small, but statistically significant difference between spray and control probably reflects the greater effort made to participate by the villagers who received spraying, who had a greater perceived benefit from the study. Having ones eyelid everted to screen for signs of trachoma produces a slightly strange sensation, but if no swab is taken, is not invasive and relatively unintrusive. Consequently, since there was little to fear by being screened few people (0.4%) overtly withdrew their consent to participate after the first survey and, predictably, this was lower in the spray clusters who valued the project more. A minimum of three visits were arranged with each cluster for the second clinical surveys. Despite this considerable effort 2% of the study population were not available for screening, having 'gone to the farm', or who were reported by family members to be 'out'. It is likely that some of these people were withdrawing their consent to participate, but wanted to do so covertly in the face of community support.

The single most important risk factor for being an active trachoma case at baseline was young age, with children in the youngest age group being most likely to have signs of active trachoma. This finding corresponds with many other trachoma surveys e.g. (West *et al.*, 1996; Zerihun, 1997; Lansingh *et al.*, 2001).

There are differences in custom, housing style and animal husbandry techniques between the three dominant ethnic groups in The Gambia; Wollof, Fula and Mandinka, which may predispose one group to have a greater or lesser likelihood of infection with trachoma than another. We did not detect any difference in the risk of active trachoma among these groups at baseline. Other work has shown that sanitation and access to a regular water supply appear to be of primary importance in the community control of trachoma (Prost & Négrel, 1989). Here, there was a weak association between household latrine ownership with lower risk of active trachoma, but no association between active trachoma and time taken to collect water. In this part of The Gambia there is good access to water with every village having at least one well, most of which are fitted with hand-pumps. In contrast, access to latrines is poor, with only 31% of people belonging to a household with a latrine at baseline. Most of the latrines seen at baseline were of the 'local' type, which were often precarious and consequently children were barred from using them, so even this figure probably over-estimates the true proportion of people who actually had regular access to a latrine. The enthusiastic uptake of latrines by the participants revealed a felt need for sanitation. In informal discussion, household heads reported a desire for latrines, but were not able to build them because Ironwood, the only termite resistant timber, had become extremely scarce and it was difficult to obtain a permit to cut it. They were afraid that using poor quality timber would result in the latrine collapsing during the rainy season leaving the uncovered pit as a danger to children and livestock.

To avoid potential bias introduced by self-reporting about socio-economic status an objective scoring system to rank households on the basis of the materials used in house construction and the up-keep of their houses was developed. The risk of being an active trachoma case did not differ between broad categories of housing rank, suggesting that living conditions were basically homogenous.

The main activity conducted by children during the day may be associated with risk of trachoma (Chapter 2). Primary school attendance is often anecdotally linked with better attention to personal hygiene and it has been suggested that school attendance may be linked to a lower prevalence of active trachoma. Conversely looking after cattle and goats (Taylor *et al.*, 1989b) or looking after younger siblings at home has been linked to higher rates of active trachoma (Tielsch *et al.*, 1988; West *et al.*, 1991c). In rural parts of The Gambia the Islamic scholars (Talibes) living with a teacher away from home often appear dirty and neglected, and there is a perception that they are at increased risk of trachoma. However, among the 1507 children aged between five and 15 years for whom we obtained data on normal daily activity we found no differences in the risk of being an active trachoma case that could not be explained by age.

In the pilot study *M. sorbens* accounted for 9% of flies from the fish-baited traps and here it accounted for 10% caught on sticky traps: the true proportion of domestic flies that are *M. sorbens* is probably no greater than10%. *M. sorbens* was therefore greatly over-represented in the hand-net catches from the eyes of children in both studies, accounting for 92% in the pilot study and 87% here. In both studies females were caught roughly four times more frequently than males. Human eyes appear to be extremely attractive to female *M. sorbens* and catches of flies from eyes, or observations of flies on eyes are likely to be the most sensitive method of measuring their abundance. Catching the eye-seeking flies in hand-nets has the disadvantage of sampling without replacement. This give two potential problems; firstly that fly-eye contact will be under-estimated as a single fly may make multiple contacts in the catch time – the level of under-estimation being greater when there are more flies; and secondly it does not give any indication of the duration of time that flies are on the eyes. The sticky traps used here do not appear to reflect feeding behaviour of *M. sorbens*.

Whilst the provision of latrines leads to a reduction in *M. sorbens*, the population of *C. albiceps* was an average of 153% higher in the latrine clusters than the control clusters. However, since *C. albiceps* breeds in the latrine contents this increase was expected, and can be used as evidence that the latrines were being used by the recipients. *C. albiceps* was never caught from children's eyes during the study, and therefore can not be a vector of trachoma. It is possible that the increase in *C. albiceps* may be of public health significance as a mechanical vector of diarrhoea, although observations in the field suggest that it is more associated with raw fish, meat and ripe fruit than with cooked food, so although diarrhoea-causing pathogens may be found on it, it is not very likely to be a major transmission route, except possibly during fruit seasons.

This study improves on the pilot study presented in Chapter 3 by including all seasons, basing analysis on the cluster and not the individual, and including a sustainable method to reduce fly-eye contact. In this study the insecticide spray arm has incriminated *M. sorbens* as a vector of trachoma. The provision of latrines to each household reduced the number of *M. sorbens* caught from eyes by a quarter compared to controls, with a concomitant reduction in the prevalence of trachoma of 28%. This encouraging finding suggests that provision of sanitation could be added to trachoma control programmes using the SAFE strategy but further investigation of the 'value added' to trachoma control by latrine provision in the context of the full strategy should be conducted. Access to sanitation and the safe disposal of human faeces, is a fundamental part of the development process and has other health benefits such as a reduction in diarrhoeal disease.

Chapter 8

The wider context: How knowledge of the ecology and control of *Musca sorbens* relates to trachoma control

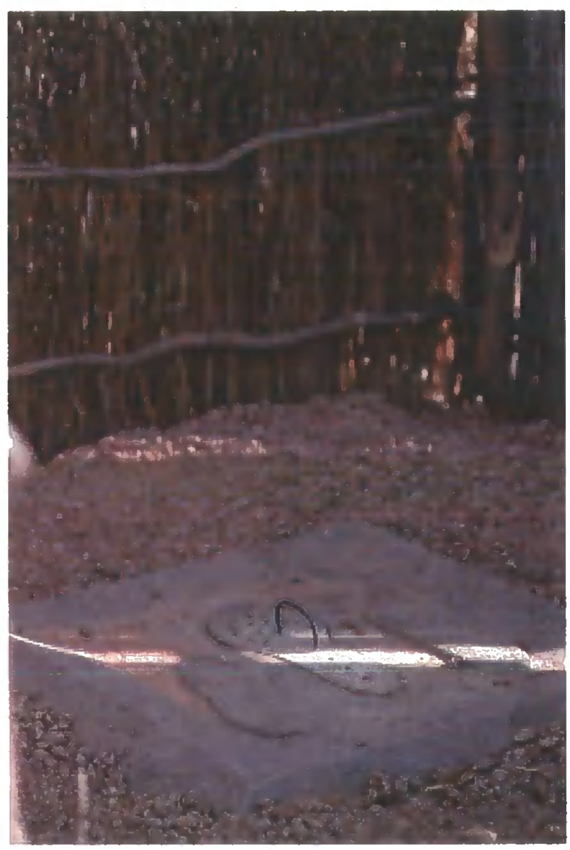

Figure 8.1 One of 677 latrines built during the course of the project

The wider context: How knowledge of the ecology and control of *Musca sorbens* relates to trachoma control

The SAFE strategy for trachoma control

With the formation of the WHO alliance for the Global Elimination of Trachoma in 1996, and the International Trachoma Initiative in 1998, the prospect of major advances in the control of trachoma have never been better (Bailey & Lietman, 2001). Working through the ITI, which acts as a clearing house for the drug donation, the pharmaceutical company Pfizer has already donated over two million doses of azithromycin (Zithromax®) for use in Ghana, Mali, Morocco, Sudan, Tanzania and Vietnam, and recently announced an expansion of their donation programme to include Ethiopia, Nepal and Niger. Pending a satisfactory evaluation of the first three years of activity, the Pfizer donation programme looks set to continue.

The control of blinding trachoma as advocated by the GET alliance and ITI is more than a drug donation programme. Based on the SAFE strategy of surgery, antibiotics, facial cleanliness and environmental improvement its aim is to have a long-lasting impact not only on blindness, but on the health infrastructure of some of the poorest communities of the world (WHO, 1996).

As this is a global strategy the WHO co-ordinators have produced a series of guides on the implementation of the SAFE strategy with the aim of harmonising practise and technique between affected countries. There are guides on screening (Thylefors *et al.*, 1987), community level trachoma control (Francis & Turner, 1995); primary health care level management of trachoma (WHO, 1995); surgical procedure (Reacher *et al.*, 1998); environmental sanitation and improved hygiene (Mariotti & Prüss, 2000); and trachoma rapid assessment (Négrel *et al.*, 2001). However, WHO are not able to support individual country programmes and in order for managers to justify expenditure or resource allocation on each aspect of the SAFE strategy there is a need for an evidence base on which to make rational decisions. There is good evidence showing that surgical correction of distorted lids is effective at stopping the progression to blindness (Reacher *et al.*, 1992; Bowman *et al.*, 2000a), although the length of time it is effective varies widely between surgeons from 2-20 years. Both topical tetracycline and oral azithromycin have been shown to be effective at clearing active infections

(Schachter *et al.*, 1999, Mabey & Fraser-Hurt, 2001) and face-washing on its own gave a modest degree of control (West *et al.*, 1995). However, the lack of compelling evidence for the E component of the SAFE strategy presents a barrier to implementation, which has lead to it being side-lined in almost all country programmes in favour of antibiotic distribution and surgery. The work described in Chapters 1-7 on the ecology and control of *Musca sorbens* provides essential evidence that *M. sorbens* is an important vector of trachoma which will allow programme managers to justify the inclusion of fly control as part of the SAFE strategy. There is encouraging evidence that the provision of latrines will offer a sustainable method to control *M. sorbens.* As such it is likely that this body of work will have an immediate impact on the form of trachoma control in countries where *M. sorbens* is present.

A programme to control blinding trachoma based solely on the control of *M. sorbens* would be unlikely to be successful since there are several routes of transmission. Fly control was only conducted in isolation in this work to identify whether flies were important in trachoma transmission, having several interventions running concurrently would make it difficult to ascribe any observed effect to a particular intervention. The next step will be to investigate whether the addition of fly control to current active programmes can improve the level of trachoma control achieved by surgery, antibiotic distribution and the promotion of facial cleanliness. This could be done by adding fly control with insecticide to antibiotic distribution, and monitoring if resurgence of active disease can be delayed in comparison to areas where there is only antibiotic; or, by assessing the 'value added' to antibiotic distribution by the provision of latrines. These projects would lend themselves to being conducted in locations hyper-endemic for trachoma, with low levels of sanitation and where *M. sorbens* is present, such as Ethiopia, Niger, Mali or Tanzania.

The Flies and Eyes project database has extensive information on 8,500 participants, all of whom have benefited from three components of the SAFE strategy: Surgery has been offered where indicated and conducted where possible; all clinically active cases seen have been offered antibiotics; and every person has access to environmental change in the form of pit latrines. This cohort can be followed over time to assess the effect of these components of the SAFE strategy applied together. Using the enumeration database it may be possible to conduct a long-term follow-up to assess the relationship between resources spent and disability-adjusted life years averted. The project provides a model framework for assessing the effect of these three aspects of the SAFE strategy applied together.

Knowledge of trachoma transmission

The geographical distribution of trachoma is neither static nor constant. It was brought to Europe from Egypt at the end of the Napoleonic wars, but disappeared by the 1950s, and was one of the diseases checked for among would-be immigrants to the USA arriving at Ellis Island in the 1920s (Taylor, 2001). From being a world-wide problem it is currently confined to parts of Africa, with other pockets in South America, Asia and the interior of Australia. Trachoma disappeared from most countries in the absence of a major programme of drug distribution, and in those countries in which it remains it is patchily distributed. The prevalence of active trachoma in a particular village or area is liable to decline markedly within a few years without a specific treatment programme (Dawson *et al.*, 1976; Dolin *et al.*, 1997). Moreover, in areas where it is highly endemic, mass antibiotic treatment reduces the prevalence abruptly, only for it to re-emerge to pre-treatment levels again within a year or two (West *et al.*, 1993; West *et al.*, 1995). This suggests that there is a narrow range of environmental conditions that sustain transmission, with relatively minor perturbations putting the disease into decline. But if the underlying environment does not change it will be difficult to dislodge the disease, even in the presence of antibiotic treatment. Reducing transmission by environmental change will be essential if the GET alliance and ITI are to meet their targets.

The potential for traps in fly control

Concerns about the effect on non-target organisms and the evolution of insecticide resistance by flies mean that frequent or prolonged use of insecticide on a large scale is not considered practical for fly control (Esrey, 1991). Consequently fly traps are frequently proposed as an environmentally sound alternative (Curtis, 1984; Pickens & Mills, 1993; Chavasse *et al.*, 1994; Pickens *et al.*, 1994; Curtis, 1998). However, flies have evolved to react swiftly to exploit environmental opportunities by having a tremendous reproductive output; hence the limiting factor for the size of a population is usually the availability of suitable breeding media. While breeding opportunities exist it is extremely difficult to control fly populations by killing adults, even with insecticide spraying, and adequate control would require a large number of extremely efficient traps. Fly traps have been tested for large scale public health use in Israel (Cohen *et al.*, 1991), Pakistan (Chavasse *et al.*, 1999) and Kenya (reported in WHO, 2001). The Israeli study investigated the transmission of *Shigella* and was conducted in two army camps in the desert. Fly control (*M. domestica*) was achieved using traps baited with fermenting yeast sited near latrines, mess tents and kitchens, in conjunction to the application of permethrin from ...'High-capacity spraying machines [and] for routine fly control measures, 5 litre spraying devices were used'...(Cohen *et al.*, 1991). As it is unclear what proportion of the 64% reduction in fly numbers reported was attributable to the traps compared to the spraying, caution should be used when describing this project as a successful example of the use of fly traps in disease control. In Pakistan commercial fly traps on their own failed to have any effect on either fly numbers or diarrhoea incidence compared to controls (Chavasse *et al.*, 1999). The Kenyan study investigated the use of fly traps made locally from discarded plastic bottles to control trachoma, and has received wide media publicity for their claimed success e.g. (*New Scientist*, 22nd July 2000; Dobson, 2000). The study had serious methodological flaws making interpretation very difficult and should be considered as anecdotal until the full details have been published in a peer-reviewed journal.

Whilst there is little evidence to suggest that controlling domestic flies by attacking adults with traps will be successful, there is a role for fly traps in closed areas such as shops and restaurants, and as a tool for monitoring the effectiveness of interventions.

Now there is good evidence that *M. sorbens* is a vector of trachoma, attempts to control it can be justified in trachoma control programmes. Environmental change interventions for the control of trachoma, such as the provision of latrines, can be expected to reduce the population of *M. sorbens*, frequency of fly-eye contacts, active trachoma infections and, ultimately, blindness. The effectiveness of such interventions could be monitored with traps specifically designed to catch *M. sorbens*, perhaps baited with a pheromone or the chemical signature of children's eyes, which are so attractive to *M. sorbens*.

Fly movement and clustering of trachoma cases

Flies are able to move between compounds with ease, but new cases of trachoma do not occur independently, rather clustering by compound and sleeping room (Mabey *et al.*, 1992a; Muñoz & West, 1997). This observation does not appear to concur with flies being trachoma vectors. Dispersal by flight is expensive in terms of energy use, and risky in that it exposes the flies to predators such as birds and spiders. In the presence of abundant sources of food and potential breeding sites there will be little pressure on the flies to disperse. Natural selection may favour the strategy of not dispersing, which would lead to a patchy distribution of flies in the villages. Compounds rich in *M. sorbens* would be expected to have sufficient people to provide food and breeding media, in the form of inadequately disposed of human faeces.

Compounds poor in *M. sorbens* would have few people and proper disposal of human faeces. This hypothesis is consistent with the observation that latrine ownership is a protective factor for trachoma (Taylor *et al.*, 1989b), and lack of a latrine is a predictor for active trachoma at the compound level (Courtright *et al.*, 1991). There is a need to conduct investigations on the movement of *M. sorbens* within a village, and it would be instructive to ascertain whether the distribution of *M. sorbens* correlates with the distribution of active trachoma.

Genetics and control of Musca sorbens

This work incriminates *M. sorbens* as a trachoma vector and proposes that fly control be included in trachoma control programmes. Insecticide spraying is effective at controlling flies, but is unlikely to be included as a routine control measure. Promotion of sanitation and the safe disposal of human faeces relies on restricting breeding opportunities to be successful. This knowledge is currently based on work restricted to The Gambia, and we know that *M. sorbens* is considered to be a complex of three species (Paterson & Norris, 1970), two of which, *M. sorbens* and *M. biseta*, are sympatric in Africa. Paterson and Norris present good evidence to show that the African species are reproductively isolated from the Australia form (*M. vetustissima*), but did not attempt to cross-breed the African narrow-fronsed and broad-fronsed forms. It is not absolutely clear whether *M sorbens* and *M. biseta* are separate species, nor the degree of separation of ecology and behaviour. It may be necessary to characterise differences between the two forms with respect to eye-seeking behaviour and choice of breeding media before area-specific control tools can be developed. For example control of *M. sorbens* in Australia using the provision of latrines is unlikely to be successful since *M. vetustissima* predominately breeds in the faeces of cattle (Hughes *et al.*,1972).

Community participation in fly control

Flies are considered to be a nuisance and attempts to control them usually gain community support (Chavasse *et al.*, 1996). However, informal discussion with study participants suggests that flies are not linked in people's minds with the transmission of trachoma, and that childhood eye infections are not associated with trichiasis in later life. Therefore, since flies are not associated with blindness from trachoma and *M. sorbens* comprises only about 10% of the flies in a village, it may be difficult to get community support for control measures that only target *M. sorbens* (such as a species specific trap) without any other perceived benefit.

Reducing transmission of *C. trachomatis* through fly control should not be used in isolation for trachoma control. For maximum benefit, context specific control measures for *M. sorbens* should be integrated into the SAFE strategy for trachoma control, where they will enhance the effect of antibiotic distribution and complement hygiene promotion.

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