

## CHLAMYDIA ON CHILDREN AND FLIES AFTER MASS ANTIBIOTIC TREATMENT FOR TRACHOMA

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**Abstract.** There are various approaches to control trachoma. These include the elimination of the ocular strains of *Chlamydia trachomatis* that cause the disease and to decrease the spread of infection by other measures such as fly control. Here, we examined how these two are related (i.e., how treating children with antibiotics affects carriage of *Chlamydia* by flies). Flies were collected in villages that had received mass oral azithromycin distribution and were compared with flies in untreated villages. Polymerase chain reaction (PCR) was performed to detect chlamydial DNA on the flies. Conjunctival swabs were also taken to assay for chlamydial prevalence in the children. *Chlamydia* was found on 23% of the flies in the untreated villages but only 0.3% in treated villages. Prevalence of trachoma in children proved to be an excellent predictor of the prevalence on flies (correlation coefficient, 0.89). Thus, treating children with antibiotics may drastically reduce the role of flies as a vector.

### INTRODUCTION

Trachoma is the leading cause of infectious blindness worldwide.<sup>1</sup> The World Health Organization (WHO) has initiated a program to eliminate infectious trachoma using the SAFE policy, a multitiered approach that includes surgery, antibiotics, face washing, and environmental controls, which include fly control.<sup>2</sup> In this paper, we focus on two of these factors. The first is the mass distribution of antibiotics to eliminate the *Chlamydia trachomatis* infection that causes trachoma. The second is the alteration of conditions so that infection is not as easily transmitted. Children are encouraged to improve hygiene, and attempts are being made to control the face flies (*Musca sorbens*) that are thought to play a role in spreading the infection.<sup>3</sup> Because *M. sorbens* breeds preferentially on human feces, some programs have focused on building household latrines.<sup>4,5</sup>

It is now possible to assay not just the rate of infection in a community but also the proportion of flies that harbor *Chlamydia*. A previous report documented that *Chlamydia* could be found on 15% of *M. sorbens* in villages in previously untreated Ethiopian villages.<sup>6</sup> In this report, we determine how this proportion depends on the prevalence of infection in children and how treating children with antibiotics affects carriage of *Chlamydia* by flies in the same community.

### MATERIALS AND METHODS

As part of a larger study, eight villages from the Enemore district of the Gurage Zone, Ethiopia, were randomly selected to receive biannual mass azithromycin treatment of all individuals  $\geq 1$  year of age. Eight other villages not yet enrolled in the trachoma program were randomly selected from the same area to serve as untreated controls; these villages subsequently received treatment as part of the Ethiopian national trachoma control program. Three of the eight treated

villages were randomly selected for fly collection, as were three of the eight untreated control villages. All six villages were monitored in March 2004, 6 months after the second distribution of antibiotics in the treated villages. All children 1–5 years of age were identified through the census and requested to come to a central location with a guardian. Using new gloves for each patient, the examiner everted the upper lid, assessed a clinical grade using the WHO simplified grading scale,<sup>7</sup> and firmly swabbed the upper lid in a horizontal motion three times, rotating the swab with each motion. A repeat control swab identical to the first swab was taken to assess reproducibility of the test in a randomly selected 10% of children (not to exceed five per village).<sup>8,9</sup> Another, negative control swab was passed within 1 in. of the conjunctiva without touching from a randomly selected 10% of children (not to exceed five per village). Samples and controls were immediately placed at 4°C in the field, at –20°C within 6 hours, and kept at –20°C until transported at 4°C to the University of California, San Francisco (UCSF), where they were frozen at –80°C. Amplicor polymerase chain reaction (PCR; Roche Diagnostics, Branchburg, NJ) was used for the detection of chlamydial DNA in these samples according to protocol. All samples were processed in a masked manner.

Flies were caught from the faces of these same 1- to 5-year-old children as previously described.<sup>6</sup> A target of 90 flies for each arm of the study was set (30 per village). In the control arm, one village only had 20 children with face flies; therefore, in the next, larger village, flies from 40 children were collected. Flies were caught from the peri-ocular area using Silva Sleeve Fly Trap paper (Knared, Sweden), avoiding any contact with the child's face. Flies identified as *M. sorbens* from their size and characteristic thorax were passed to a gloved assistant. Acme scissors were used to cut an ~1-cm<sup>2</sup> region around a single fly. The fly attached to the adhesive paper was placed in a sterile 2.0-mL microcentrifuge tube. Using the same scissors, an arbitrary region of similar size was cut from the same piece of paper to serve as a negative control and placed in a separate microcentrifuge tube. The scissors were sterilized with 70% isopropyl alcohol wipes between children, and gloves were replaced with new ones for the next child. Samples and matching controls were stored and transported

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in the same manner described above for conjunctival swabs. At UCSF, samples were thawed, and 375  $\mu$ L of sterile saline was added to each vial. After vortexing for 2 minutes, the resulting wash was placed into separate sterile vials. Because a previous study showed a 0% positive rate for negative controls,<sup>6</sup> we only processed a random 20% of the matched negative controls. Samples and controls were labeled with random numbers for processing by masked laboratory personnel. Inhibition in the PCR assay was reduced by heating specimens to 100°C. Amplicor PCR was used to detect the presence of chlamydial DNA.

## RESULTS

Treated villages had a prevalence of ocular chlamydial infection of 42% before treatment (66 of 156) in children 1–5 years old. Twelve months later, after two mass antibiotic treatments, only 1.2% of children (2 of 170) had evidence of infection (Table 1). At the same 12-month time-point, untreated control villages had a prevalence of *Chlamydia* of 30% (56 of 185). After two rounds of azithromycin treatment, infection in children had decreased significantly in treated villages compared with pre-treatment ( $P < 0.001$ ) and compared with untreated villages ( $P < 0.001$ ). All negative controls (air swabs) were negative, and all positive controls (duplicate swabs) confirmed the results of the initial swab, although it should be noted that, in our previous studies, 99.1% of air swabs were negative and > 95% of duplicate swabs were concordant.<sup>8,9</sup>

Evidence of chlamydial DNA was found on 23% of flies (21 of 90) in the untreated (control) villages but only 1% (1 of 90) of flies in the treated villages ( $P < 0.001$ , Fisher exact). Paper controls for both arms of the study were all negative (0 of 72). The prevalence of trachoma in children proved to be an excellent predictor of the prevalence on flies in that village (Pearson correlation coefficient,  $r = 0.89$ ).

## DISCUSSION

Face flies are ubiquitous in trachoma-endemic areas of Ethiopia. In this arid climate, they seek moisture on the eyes

and mucosal membranes of children.<sup>3</sup> The potential of flies as a vector for trachoma has been recognized for hundreds of years.<sup>10</sup> The WHO has included efforts to reduce the fly population in its trachoma control programs since 1997.<sup>2</sup> Until recently, there had been minimal evidence that *Chlamydia* could even be found on face flies, in part because of the difficulty in transporting and culturing *Chlamydia*. Evidence of chlamydial DNA can now be readily detected by PCR of flies, particularly in hyperendemic communities such as those studied in this report.<sup>6</sup>

Our results confirm the presence of *C. trachomatis* on 23% of flies from untreated villages. This is even higher than the 15% found by Miller and others<sup>6</sup> in a nearby area. Mass azithromycin treatment is well known to reduce the prevalence of infection in children, even 6 months after the last treatment.<sup>8</sup> Perhaps it is not surprising that treatment also dramatically reduces the proportion of flies that harbor *Chlamydia*. While characteristics of a village such as altitude, water supply, and animal stock may all contribute to the number of flies present, it seems that most of the variance in the proportion of flies that harbor *Chlamydia* may be explained by the prevalence of infection in children ( $r^2 = 0.79$ ). Our results show that 6 months after treatment, the prevalence of *Chlamydia* on flies is significantly lower in treated villages than in untreated villages.

Contamination between *Chlamydia* populations in adjacent settlements does not seem to have occurred. This could be because flies do not fly very far or live very long or that chlamydial transmission is inefficient and low between flies. The fact that *Chlamydia* does not persist among flies after it has been eliminated from children suggests that flies may not be a significant reservoir in re-infecting a community.

Great efforts are being made to reduce the prevalence of flies in trachoma-endemic regions. Any synergistic effect that fly control has with antibiotics would be welcome to control programs, particularly in the most severely affected areas. However, it has been difficult to show that any specific sustainable measure such as latrine construction has had a dramatic effect on the transmission of ocular *Chlamydia* by *M. sorbens*.<sup>4,11,12</sup> Regardless, treating children with antibiotics drastically reduces the role of flies as a vector and is further evidence of the short-term effectiveness of mass antibiotics.

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TABLE 1  
PCR positivity and number of samples by village

Villages	PCR positivity	
	Flies	Children
Treated		
Imam Seman	3.3% (1/30)	1.6% (1/61)
Layginaw Ferejetiyie	0.0% (0/30)	0.0% (0/65)
Rahmeto Lencha	0.0% (0/30)	2.3% (1/44)
Totals	0.01% (1/90)	1.2% (2/170)
Control		
Darena Bota	40.0% (16/40)	37.0% (34/92)
Getosefere	0.0% (0/20)	0.0% (0/33)
Tache Sedie	16.7% (5/30)	36.7% (22/60)
Totals	23.3% (21/90)	30.3% (56/185)

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