AEM
Journals.ASM.org

Environmental Factors Associated with High Fly Densities and Diarrhea in Vellore, India

Stefan Collinet-Adler,[a] Sudhir Babji,[b] Mark Francis,[b] Deepthi Kattula,[b] Prasanna Samuel Premkumar,[b] Rajiv Sarkar,[b] Venkat Ragava Mohan,[b] Honorine Ward,[a,b] Gagandeep Kang,[b] Vinohar Balraj,[b] Elena N. Naumova[b,c]

Tufts Medical Center, Boston, Massachusetts, USA[a]; Christian Medical College, Vellore, India[b]; Tufts University, Medford, Massachusetts, USA[c]

Diarrhea causes significant morbidity and mortality in Indian children under 5 years of age. Flies carry enteric pathogens and may mediate foodborne infections. In this study, we characterized fly densities as a determinant of infectious diarrhea in a longitudinal cohort of 160 urban and 80 rural households with 1,274 individuals (27% under 5 years of age) in Vellore, India. Household questionnaires on living conditions were completed at enrollment. Fly abundance was measured during the wet and dry seasons using fly ribbons placed in kitchens. PCRs for enteric bacteria, viruses, and protozoa were performed on 60 fly samples. Forty-three (72%) fly samples were positive for the following pathogens: norovirus (50%), *Salmonella* spp. (46.7%), rotavirus (6.7%), and *Escherichia coli* (6.7%). Ninety-one episodes of diarrhea occurred (89% in children under 5 years of age). Stool pathogens isolated in 24 of 77 (31%) samples included *E. coli*, *Shigella* spp., *Vibrio* spp., *Giardia*, *Cryptosporidium*, and rotavirus. Multivariate log-linear models were used to explore the relationships between diarrhea and fly densities, controlling for demographics, hygiene, and human-animal interactions. Fly abundance was 6 times higher in rural than urban sites ($P < 0.0001$). Disposal of garbage close to homes and rural living were significant risk factors for high fly densities. The presence of latrines was protective against high fly densities and diarrhea. The adjusted relative risks of diarrheal episodes and duration of diarrhea, associated with fly density at the 75th percentile, were 1.18 (95% confidence interval [CI], 1.03 to 1.34) and 1.15 (95% CI, 1.02 to 1.29), respectively. Flies harbored enteric pathogens, including norovirus, a poorly documented pathogen on flies.

Diarrhea has been shown to be the cause of death of over 10% of Indian children under the age of 5 (1). Transmission of infectious diarrhea is fecal to oral, with zoonotic and/or anthroponotic cycles depending on the pathogen. Environmental factors that appear to be associated with the epidemiology of enteric pathogens include a wide range of events and processes. These include strong seasonal patterns, such as rain events, temperature extremes, soil and water ammonia levels, soil characteristics and vegetation cover, proximity of infective feces to water and food supplies ultimately used for human consumption, type of water disinfection used and the use of untreated or undertreated wastewater for agricultural irrigation, predation and sequestration of pathogens by invertebrates, pathogen reservoir characteristics, such as number and age of animals in direct and indirect contact with water and food supplies, and pathogen transport by flies and other flying insects (2).

Flies are known to carry enteric pathogens, and it is thought that some foodborne transmission of enteric infections may be mediated by flies (3). Enteropathogens previously isolated from flies in field and laboratory experiments include *Campylobacter* spp. (4–6), *Salmonella* spp. (4, 5, 7), *Shigella* spp. (4, 5, 8–10), *Vibrio cholerae* (4, 9), *Escherichia coli*, including enterohemorrhagic *E. coli* (4, 5, 7, 8, 11–13), *Bacillus cereus* (8), *Cryptosporidium* spp. (3, 14–16), *Giardia lamblia* (3), and rotavirus (17). The three proposed mechanisms of potential pathogen transmission from flies to their environment include mechanical transfer from the exoskeleton, regurgitation, and fecal deposits (3, 4, 10, 12, 14–16, 18). It has been shown that enteric pathogens can survive on flies for up to 10 days (19). The role of flies as vectors of infectious disease in humans has been suggested by the results of many experimental and field studies (3–6, 8–11, 14–16, 18, 20–30).

Most transmission of infectious diarrhea is primarily fecal to oral, although the routes taken from feces to ingestion by a host can vary. Pathogenic microbes originating from human feces (e.g., rotavirus, adenovirus, astrovirus, *E. coli*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Vibrio* spp., *Giardia* spp., *Cryptosporidium* spp., and *Entamoeba histolytica*) are considered to be the major causes of infectious diarrhea. Animal feces, however, can harbor human pathogens (e.g., *Campylobacter* spp., *Salmonella* spp., *E. coli*, *Giardia* spp., and some *Cryptosporidium* spp.) and can therefore participate in transmission of disease to humans. Given environmental conditions, such as those found on food at ambient temperature, many bacteria continue to multiply with augmentation of their pathogenic potential.

In order to explore the potential for mechanical transmission of diarrheal pathogens, we undertook a comprehensive description of living conditions in 240 recruited households in two urban slums and two rural areas of Vellore District, Tamil Nadu, India. The aims of the study were to examine the link between fly densities and diarrheal outcomes at the household level and to identify potential targets for public health interventions to decrease infectious diarrhea in resource-poor settings. We anticipated that en-

Received 23 April 2015 **Accepted** 22 June 2015

Accepted manuscript posted online 26 June 2015

Citation Collinet-Adler S, Babji S, Francis M, Kattula D, Premkumar PS, Sarkar R, Mohan VR, Ward H, Kang G, Balraj V, Naumova EN. 2015. Environmental factors associated with high fly densities and diarrhea in Vellore, India. Appl Environ Microbiol 81:6053–6058. doi:10.1128/AEM.01236-15.

Editor: H. L. Drake

Address correspondence to Elena N. Naumova, elena.naumova@tufts.edu.

doi:10.1128/AEM.01236-15

teric infection would be more frequent and clustered in households with compromised water and food supplies either at the source or at the point of use and associated with ineffective segregation of human and animal fecal material. Ineffective segregation of waste from products of consumptions would be characterized and predicted by detectable conditions, such as increased fly densities, specific hygiene patterns, and living conditions. In this pilot study, we found that flies harbored enteric pathogens, including norovirus, a poorly documented pathogen on flies.

MATERIALS AND METHODS

Study sites. This pilot study was embedded in a parent study on environmental drivers of diarrhea (a detailed description of which is presented by Kattula et al. [31]) and took place in two rural villages, Kattuputhur and A. Kattupudi in Kaniyambadi Block (a rural development block with an approximate population of 104,792 residents in 82 villages) and two urban slum areas, Ramanaickanpalayam (RNP) and Kaspa, with a combined population of approximately 24,843. All of these sites are in Vellore District in the Indian state of Tamil Nadu. The study protocol was reviewed and approved by both the Tufts Medical Center and Christian Medical College Institutional Review Boards. All participating study families signed an informed consent form.

Health outcomes. We recorded the numbers and durations of diarrheal episodes for families in the study sites in a cohort of 160 urban and 80 rural households with 1,274 individuals, 27% of whom were under 5 years of age, accumulating 198,795 person-days of follow-up from 6 August 2010 to 31 January 2011. Trained field workers visited all households weekly to inquire about illness. An episode of diarrhea was defined as 3 or more unformed or watery stools in a 24-h period (31). An interval of at least 48 h without diarrhea was used to define a new episode. Stool samples were collected from those who experienced a diarrheal episode and were screened for parasites by light microscopy using wet mounts and modified acid-fast staining. Stool culture was performed for *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. Multiplex PCR for diarrheagenic *Escherichia coli* (enteropathogenic, enterohemorrhagic, enteroinvasive, diffusely adherent, enteroaggregative, and enterotoxigenic *E. coli*) and enzyme-linked immunosorbent assay (ELISA) for group A rotavirus were performed according to previously described methods (32). Norovirus testing was not performed on stools.

Characterization of flies. From 13 to 30 October 2010, fly ribbons were placed in 234 households in 2 urban sites and 2 rural sites for 2.92 to 11 days (median 4.71 days). From 12 to 28 January 2011, fly ribbons were placed in 229 households at the same 4 sites for 0.83 to 15.08 days (median, 1.13 days). The sticky ribbons were nontoxic passive traps (Revenge Fly Catcher) placed in or as close to food preparation areas as was feasible by house geometry and as was acceptable to households, many of whom live in cramped quarters. Date and time (to the nearest hour) of placement were recorded. In order to increase the fly trapping surface, every attempt was made to place the ribbons vertically and away from walls, but in a minority of cases (11/234 in October and 6/229 in January), family preferences resulted in traps placed against the wall to decrease the spatial burden of the ribbons on day-to-day activities. In all cases, ribbons were placed away from direct sunlight and were sheltered from rain. House geometry and in some cases family preferences dictated the height of traps. The ribbons were placed such that the bottoms of the 61-cm traps were between 1 and 2 m off the ground, with the majority being between 1 m 70 cm and 2 m off the ground so as to be out of reach of children and pets. Collection of ribbons occurred several days after placement, with the date and time (to the nearest hour) of retrieval recorded. Two forceps were used to place the flies from each household ribbon into sterile 1.8-ml tubes. Several tubes were necessary for household ribbons with more than 30 flies. The forceps were thoroughly cleaned with 70% isopropyl alcohol and completely air dried between ribbons to decrease the likelihood of cross-contamination between household samples. The tubes were then placed 1 to 6 h after collection in a freezer at −70°C for microbiologic testing. Identification of flies was performed using morphological criteria to the family taxon level (33).

Due to material limitations, household fly samples were selected for pathogen detection if the fly count was at least equal to or greater than 15. We identified 15 rural and 15 urban fly samples in October and in January, for a total of 60 fly samples for detailed analysis. Five hundred milligrams of the frozen flies was used to extract DNA and RNA using the QIAamp DNA minikit and QIAamp viral RNA minikit after the flies had been crushed in a sterile mortar and pestle. PCRs for *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp., *Yersinia* spp., diarrheagenic *Escherichia coli*, *Shigella* spp., *Cryptosporidium* spp., rotavirus, and norovirus (genogroups I and II) were performed using previously described primers and conditions (34–38).

Environmental, demographic, and hygiene characteristics. Trained field workers administered questionnaires a few days following recruitment in each recruited house. These questionnaires explored demographic and hygiene characteristics of the household through a total of 130 questions: the questions relevant for this study were selected for the statistical analyses.

Statistical analyses. (i) Predictors of fly density. Fly counts for 234 households were transformed into fly densities by dividing the crude count by the number of days the trap was up at a household level and are expressed as flies per day. The fly densities from October and January were then averaged for each household. These average values were used for all statistical models. The analysis of associations between the outcome and variables obtained through the questionnaires was performed using correlation measures. To examine environmental predictors of fly density, we implemented two models: linear and logistic regressions. For multivariable linear regression, average fly densities were log transformed to accommodate a right skew. The best-fitted model was chosen based on the value of coefficient of determination; a few nonsignificant variables (e.g., highest household educational level attained) were retained in the model as they were considered possible confounders or effect modifiers. The results of the linear model were presented as the estimates of relative risk (RR) with their 95% confidence intervals (95% CIs).

To better account for the high fraction of low values, we also applied a logistic regression model to the dichotomized log-transformed fly density using a breakpoint of 4.8 flies caught per day. This breakpoint separates well between low and high values estimated from a mixture of gamma and normal distributions. The gamma distribution was used to approximate low fly density values (<4.8 flies caught per day), while values above the cutoff were fitted by a normal distribution. We suspect that two underlying distinct sets of household groups were represented by this mixture. The results of the logistic models are presented as the estimates of odds ratio (OR) with their confidence intervals (95% CIs).

(ii) Predictors of diarrhea. Both the number of episodes and the duration of diarrhea for the household observed during a 6-month period (6 August 2010 to 31 January 2011) were considered the health outcomes for a multivariate analysis. Both outcomes were regressed against potential predictors. For a multivariate analysis, we fitted a generalized log-linear model with a negative binomial distributional assumption for an outcome. In this model, the relationships between the predicted mean and variance were modeled in a form that supports the right skew and a large fraction of zeros in the health outcome. A P value of 0.15 or lower was used as the threshold for including the variable in the final model. Most variables reflecting individual and household hygiene had a low impact on the model fit and thus were omitted from the final models. We also explored interactions among a number of terms to ensure detection of joint effects.

All statistical analyses were performed using R and SPSS 18.0.2 (SPSS, Inc., 2009, Chicago, IL).

RESULTS

In October 2010, 2,101 flies were captured, with 2,095 (99.7%) from the Muscidae family, 4 (2 per 1,000) from the Callidiphoridae family, and 2 (1 per 1,000) from the Sarcophagidae family. In

TABLE 1 Pathogens isolated from flies in rural and urban areas

Pathogen	No. (%) of samples with identified pathogen							
	October 2010			January 2011				
	Rural		Urban,	Rural		Urban		
	A. Kattupudi ($n = 8$)	Kattuputhur ($n = 7$)	Kaspa ($n = 15$)	A. Kattupudi ($n = 7$)	Kattuputhur ($n = 17$)	Kaspa ($n = 5$)	RNP ($n = 1$)	Total ($n = 60$)
Norovirus	4 (50.0)	3 (42.8)	10 (58.8)	4 (57.1)	9 (52.9)	0 (0)	0 (0)	30 (50.0)
Salmonella spp.	5 (62.5)	5 (71.4)	7 (46.7)	4 (57.1)	6 (35.3)	1 (20.0)	0 (0)	28 (46.7)
Rotavirus	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	2 (40.0)	0 (0)	4 (6.7)
Escherichia coli[a]	1 (12.5)	0 (0)	0 (0)	0 (0)	3 (17.6)	0 (0)	0 (0)	4 (6.7)

[a] Three enteroaggregative *E. coli* isolates and 1 enterohemorrhagic *E. coli* isolate.

January 2011, 4,212 flies were collected with 4,209 Muscidae (99.9%), 2 Callidiphoridae (5 per 1,000), and 1 Sarcophagidae (0.2 per 1,000) flies. Forty-three of 60 (72%) fly samples were positive for pathogens, including *Salmonella* spp., rotavirus, and *E. coli* (Table 1). Half of the samples were positive for norovirus.

High fly densities were observed in rural sites: 8.11 ± 14.8 flies per day versus 1.38 ± 4.14 flies per day in urban sites (Table 2). Rural villages and urban slums differed by the use of cow dung, garbage disposal in dug-out pits in and around the living compound, the use of indoor latrines. Regression models reveal that fly densities were on average 61% higher in households with garbage disposal in dug-out pits in and around the living compound and 31% higher in households that use firewood as fuel (Table 3). Fly densities were also high when the animals in and around living

TABLE 2 Summary of environmental, demographic, and hygiene characteristics by study site

Parameter[a]	Result by site				*P* value[b]
	Rural		Urban		
	A. Kattupudi	Kattuputhur	Kaspa	RNP	
Avg fly density, mean ± SD (IQR)	7.15 ± 9.37 (0.55–11.78)	9.06 ± 11.39 (1.55–12.31)	2.23 ± 3.95 (0–2.73)	0.52 ± 1.23 (0–0.55)	≤0.001
No. of diarrheal episodes, mean ± SD	0.36 ± 0.873	0.32 ± 0.69	0.38 ± 0.677	0.43 ± 0.869	NS
Duration of diarrhea, mean ± SD days	0.95 ± 2.2	0.68 ± 1.9	1 ± 2.386	1.34 ± 3.891	NS
Education, mean ± SD yr	11.44 ± 2.78	11.12 ± 3.00	9.04 ± 2.81	9.07 ± 3.2	≤0.001
Income in rupees, median (IQR)	3,000 (2,000–5,000)	3,500 (3,000–5,000)	3,500 (3,000–5,000)	3,000 (2,500–4,500)	NS
Family size, median (IQR)	5 (4–6)	5 (4–7)	5 (4–6)	5 (4–6)	NS
Crowding, mean ± SD[c]	2.75 ± 1.26	3.31 ± 1.54	3.44 ± 1.36	3.65 ± 1.56	NS
House type, no./total(%)					
Pucca	27/39 (69.2)	20/41 (48.8)	60/92 (65.2)	48/68 (70.6)	NS
Mixed	3/39 (7.7)	12/41 (29.3)	16/92 (17.4)	12/68 (11.8)	NS
Kutcha	9/39 (23.1)	9/41 (22)	16/92 (17.4)	8/68 (11.8)	NS
Religion, no./total (%)					
Hindu	20/39 (51.3)	38/41 (92.7)	50/92 (54.3)	2/68 (2.9)	≤0.001
Christian	19/39 (48.7)	3/41 (7.3)	3/92 (3.3)	0/68 (0)	≤0.001
Muslim	0/39 (0)	0/41 (0)	39/92 (42.4)	66/68 (97.1)	≤0.001
Cooking fuel, no./total (%)					
Firewood	24/39 (61.5)	17/41 (41.5)	15/92 (16.3)	3/68 (4.4)	≤0.001
Kerosene	2/39 (5.1)	2/41 (4.9)	27/92 (29.3)	23/68 (33.8)	≤0.001
Gas	13/39 (33.3)	22/41 (53.7)	43/92 (46.7)	41/68 (60.3)	0.05
Animals present in neighborhood, no./total (%)	26/39 (66.7)	32/41 (78)	21/92 (22.8)	7/68 (10.3)	≤0.001
Family uses cow dung, no./total (%)	34/39 (87.2)	35/41 (85.4)	6/92 (6.5)	0/68 (0)	≤0.001
Garbage disposal in dug-out pits in or around compound, no./total (%)	10/39 (25.6)	21/41 (51.2)	1/92 (1.1)	0/68 (0)	≤0.001
Use of indoor latrine, no./total (%)					
Family	11/39 (28.2)	13/41 (31.7)	89/92 (96.7)	67/68 (98.5)	≤0.001
Child	4/39 (10.3)	10/41 (24.4)	35/92 (38)	33/68 (48.5)	≤0.001

[a] SD, standard deviation; IQR, interquartile range.
[b] For continuous variables, significance was assessed by ANOVA with Bonferroni correction. For categorical variables, the χ^2 test was used. NS, not significant.
[c] Crowding was determined by the number of family members divided by the number of rooms.

TABLE 3 Major predictors of fly density

Predictor	Result by[a]: Linear model RR	95% CI	*P* value	Logistic model OR	95% CI	*P* value
Garbage disposed of in dug-out pits in and around living compound	1.61	1.13–2.28	0.008	3.81	1.36–10.73	0.011
Firewood used as fuel	1.31	0.99–1.73	0.056	6.06	2.56–14.32	<0.001
Absence of animals in home or neighborhood	0.59	0.46–0.75	<0.001	0.27	0.11–0.64	0.003
Residence in RNP	0.60	0.49–0.77	<0.001	0.15	0.02–1.21	0.074
Family uses indoor latrines	0.61	0.45–0.82	0.001			

[a] Both linear and logistic models included variables indicating the number of family members and the highest educational level attained by a member of the household.

quarters were observed. Fly densities were very low in urban households of RNP, where the use of indoor latrines is near 98.5%.

Over the 6 months of the study period, 91 episodes of diarrhea, with 248 total days of diarrhea (range per household, 0 to 22 days; mean, 1.03 days; interquartile range, 0 to 1 days) were recorded. Eighty-one episodes (89%) occurred in children under the age of 5. Stool pathogens isolated in 24 of 77 (31%) of samples included *E. coli*, *Shigella* spp., *Vibrio* spp., *Giardia*, *Cryptosporidium* spp., and rotavirus.

After being adjusted for socioeconomic, demographic, and behavioral characteristics, the average fly density consistently predicted duration of diarrhea at the household level (Table 4). Similar results were found for episodes of diarrhea (data not shown). With an increase in average fly density to the 75th percentile in a given household, the risk of diarrhea increased by 15% (RR, 1.15; 95% CI, 1.02 to 1.29; $P = 0.024$) for duration of diarrhea and by 18% (RR, 1.18; 95% CI, 1.03 to 1.34; $P = 0.014$) for number of diarrheal episodes. The use of latrines by children was protective against duration of diarrhea (RR, 0.45; 95% CI, 0.27 to 0.75; $P = 0.002$).

DISCUSSION

Several enteric pathogens were identified on captured flies, including norovirus, which was detected in 50% of the tested samples. Although the isolation of norovirus on flies is not surprising, given the well-documented presence of other enteric pathogens on flies and the putative role of flies in the transmission of enteric infections (3–6, 8–18, 20–28, 39–42), this common diarrheal pathogen has heretofore been poorly documented on flies. Although norovirus testing was not performed on stool in our pilot project due to financial constraints, a recent case-control study of children under 5 with diarrhea in Vellore, India using conventional and molecular methods revealed norovirus (genogroups 1 and 2) in 15.8% of diarrheal stool samples versus 7% of controls ($P = 0.051$), making it the third most common pathogen isolated in diarrheal samples (32).

TABLE 4 Relative risks estimated for duration of diarrhea

Predictor	RR	95% CI	*P* value
Avg fly density[a]	1.15	1.02–1.29	0.024
Highest educational level attained by a member of household[a]	0.33	0.14–0.81	0.015
No. of family members[a]	3.53	1.81–6.86	<0.001
Use of private well	18.25	3.92–84.94	<0.001
Use of water taps	3.04	1.72–5.39	<0.001
Use of latrines by children	0.45	0.27–0.75	0.002
Absence of animals in home or neighborhood	0.55	0.32–0.97	0.038
Rural living	0.40	0.20–0.83	0.014

[a] Increase to 75th percentile.

In the parent study (31), we demonstrated that the rate of diarrhea was lower in rural areas than in urban slums. The present study, covering the first 6 months of the observational period, also shows rural living to be protective against diarrhea. At the same time, fly densities are higher in rural villages than in urban slams. We observed a relationship between the households' fly densities and diarrheal events; however, our ability to demonstrate a causal link between diarrhea and fly abundance is limited. Previous studies attempted to demonstrate such a relationship (10, 39), and this task remains difficult.

Undeniable proof of transmission of enteric pathogens from flies to food to humans outside experimental situations may not be truly feasible. The exclusion of all other possible routes of enteric pathogen transmission is difficult in human field studies. Several human pathogens were isolated on flies in our study by PCR. Although a statistically significant association between fly densities and diarrhea was found, flies may acquire these pathogens from humans who are infected from another source and therefore serve as markers rather than causes of human disease. In our model with interaction terms, it would appear that the presence of high fly densities as a risk factor for diarrhea is partially mitigated in the absence of animals in and around living quarters and in rural settings where there is less crowding. This might suggest a possible role for fly vectors in zoonotic and person-to-person transmission.

There are several limitations inherent in our data collection methods. Homes within each site and among sites were so variable in indoor layout as to preclude true standardization of fly trapping. Very high fly densities were noted around the open sewers present immediately next to home entries. In RNP, the traps were placed in food preparation areas often found deep inside the homes away from the feeding and breeding areas represented by the open sewers. This may explain to a large extent the extremely low fly densities measured by fly trapping in food preparation areas in RNP. The other three sites generally did not have such flagrant visualized fly gradients within homes and had fewer separated kitchens. This finding could suggest that flies may be more important vectors at sites other than kitchens (e.g., children eating in the entry close to the open sewers and food covered by flies sold by food vendors in the street and not cooked prior to consumption). Placing several traps per home and measuring fly activity at food vending sites would have improved the quality of our con-

clusions, but such extensive trapping was beyond the scope of this study. Ideally, fly trapping would have been performed in a continuous manner over the course of the study. Unfortunately, this did not occur because of material and personnel limitations. In addition, it is questionable if the study participants would have accepted flytraps over extended periods. In the end, we opted for the average of data at two time points in two seasons in the hope that this would improve the relevance of cross-sectional data to a longitudinal collection of health outcomes.

This pilot cohort study identified several modifiable risk factors for high fly densities and duration of diarrhea that could potentially improve the incidence of infectious diarrhea in resource-poor settings. To reflect the effect of average fly density on the number of episodes and the duration of diarrhea, we used the 75th percentile in the estimation of relative risk. This selection was driven by an attempt to set up an attainable goal of reducing average fly density.

Latrines may aid in the effective segregation of feces from water and food as well as decrease fly populations compared to the use of fecal fields and have been described as potentially effective public health interventions to reduce fly densities and diarrheal incidence (43). In our study, latrine use by adults was associated with lower fly densities (Table 2), and their use by children was associated with less diarrhea (Table 3). In urban communities, most latrines are located indoors. In rural areas, open defection is common (31). The presence of garbage in open pits in and around living compounds was found to be a risk factor for high fly densities but not for diarrhea. Transmission of diarrhea is typically fecal to oral and would not be expected to be increased by the accumulation of nonfecal matter, even if this represents high-quality food that attracts flies.

The presence of animals was a risk factor for both fly densities and diarrhea. The zoonotic cycle of transmission of several enteric pathogens supports the plausibility of this finding. Animals are prone to haphazard defecation, providing food sources and reproductive sites for flies and many opportunities for direct contamination of human water and food supplies. The distancing of animals from living quarters could be an appropriate, albeit difficult, public health goal.

As in previous studies (43–45), high fly densities and diarrhea were shown to be associated with limited education in the studied areas. The relationship between education and other variables is complex, and it certainly acts as a confounder and effect modifier. The total number of family members in a household was found to be a risk factor for both high fly densities and diarrhea. Household size has been previously linked to diarrhea (43), but the simple fact of having more family members increases the odds of one member having diarrhea over a given period of time. Crowding (number of family members/number of rooms) was also found to be an important risk factor for diarrhea in the analysis of the whole cohort (31).

In conclusion, studies that seek to elucidate the environmental conditions that predispose to diarrhea are important for the formulation of public health targets to reduce diarrhea in resource-poor settings; however, the complex and intricate interplay of the identified factors precludes easy interpretation, and standard regression techniques may not be the most appropriate statistical analysis methods for this type of data. In addition, sampling techniques and the use of questionnaires are fraught with inconsistencies and inaccuracies that add to the uncertainty of conclusions. Notwithstanding the foregoing, we identified several modifiable factors, such as the use of indoor latrines and the proximity of animals to living quarters, that seem to influence both fly densities and diarrhea. Effective fly mitigation through improved waste and food management likely protects against infectious diarrhea. Alongside improvements in water quality and sanitation, these modifiable factors are undoubtedly appropriate public health targets to combat infectious diarrhea in resource-poor settings.

ACKNOWLEDGMENTS

We thank J. Senthil, A. Bhuvaneshwari, G. Suganthi, D. Srinivasan, S. Dayalan, and S. Karunakaran for help with data collection and entry. We also thank the team at Wellcome Research Laboratory for conducting the laboratory testing. We are indebted to the families of the urban slums of Ramnaickapalayam and Kaspa and the families of the rural villages of A. Kattupadi and Katuputur for their participation and support.

This study was supported by the Indo US CDC/ICMR grant (IRIS ID 2008-07980). S.C.-A. was supported by NIH 5T32AI007329 and a grant from the Tufts Institute of the Environment.

REFERENCES

1. **Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE.** 2015. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet **385:**430–440. http://dx.doi.org/10.1016/S0140-6736(14)61698-6.
2. **Collinet-Adler S, Ward HD.** 2010. Cryptosporidiosis: environmental, therapeutic, and preventive challenges. Eur J Clin Microbiol Infect Dis **29:**927–935. http://dx.doi.org/10.1007/s10096-010-0960-9.
3. **Graczyk TK, Grimes BH, Knight R, Da Silva AJ, Pieniazek NJ, Veal DA.** 2003. Detection of *Cryptosporidium parvum* and *Giardia lamblia* carried by synanthropic flies by combined fluorescent in situ hybridization and a monoclonal antibody. Am J Trop Med Hyg **68:**228–232.
4. **Graczyk TK, Knight R, Gilman RH, Cranfield MR.** 2001. The role of non-biting flies in the epidemiology of human infectious diseases. Microbes Infect **3:**231–235. http://dx.doi.org/10.1016/S1286-4579(01)01371-5.
5. **Khalil K, Lindblom GB, Mazhar K, Kaijser B.** 1994. Flies and water as reservoirs for bacterial enteropathogens in urban and rural areas in and around Lahore, Pakistan. Epidemiol Infect **113:**435–444. http://dx.doi.org/10.1017/S0950268800068448.
6. **Rosef O, Kapperud G.** 1983. House flies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. Appl Environ Microbiol **45:**381–383.
7. **Nmorsi OP, Agbozele G, Ukwandu NC.** 2007. Some aspects of epidemiology of filth flies: *Musca domestica*, *Musca domestica vicina*, *Drosophila melanogaster* and associated bacteria pathogens in Ekpoma, Nigeria. Vector Borne Zoonotic Dis **7:**107–117. http://dx.doi.org/10.1089/vbz.2006.0539.
8. **Butler JF, Garcia-Maruniak A, Meek F, Maruniak JE.** 2010. Wild Florida house flies (*Musca domestica*) as carriers of pathogenic bacteria. Fla Entomol **93:**218–223. http://dx.doi.org/10.1653/024.093.0211.
9. **Echeverria P, Harrison BA, Tirapat C, McFarland A.** 1983. Flies as a source of enteric pathogens in a rural village in Thailand. Appl Environ Microbiol **46:**32–36.
10. **Levine OS, Levine MM.** 1991. Houseflies (*Musca domestica*) as mechanical vectors of shigellosis. Rev Infect Dis **13:**688–696. http://dx.doi.org/10.1093/clinids/13.4.688.
11. **De Jesus AJ, Olsen AR, Bryce JR, Whiting RC.** 2004. Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). Int J Food Microbiol **93:**259–262. http://dx.doi.org/10.1016/j.ijfoodmicro.2003.12.003.
12. **Kobayashi M, Sasaki T, Saito N, Tamura K, Suzuki K, Watanabe H, Agui N.** 1999. Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. Am J Trop Med Hyg **61:**625–629.
13. **Sasaki T, Kobayashi M, Agui N.** 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. J Med Entomol **37:**945–949. http://dx.doi.org/10.1603/0022-2585-37.6.945.

14. **Graczyk TK, Cranfield MR, Fayer R, Bixler H.** 1999. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. Am J Trop Med Hyg **61:**500–504.
15. **Graczyk TK, Fayer R, Cranfield MR, Mhangami-Ruwende B, Knight R, Trout JM, Bixler H.** 1999. Filth flies are transport hosts of *Cryptosporidium parvum*. Emerg Infect Dis **5:**726–727. http://dx.doi.org/10.3201/eid0505.990520.
16. **Graczyk TK, Grimes BH, Knight R, Szostakowska B, Kruminis-Lozowska W, Racewicz M, Tamang L, Dasilva AJ, Myjak P.** 2004. Mechanical transmission of *Cryptosporidium parvum* oocysts by flies. Wiad Parazytol **50:**243–247.
17. **Tan SW, Yap KL, Lee HL.** 1997. Mechanical transport of rotavirus by the legs and wings of *Musca domestica* (Diptera: Muscidae). J Med Entomol **34:**527–531. http://dx.doi.org/10.1093/jmedent/34.5.527.
18. **Graczyk TK, Fayer R, Knight R, Mhangami-Ruwende B, Trout JM, Da Silva AJ, Pieniazek NJ.** 2000. Mechanical transport and transmission of *Cryptosporidium parvum* oocysts by wild filth flies. Am J Trop Med Hyg **63:**178–183.
19. **Motarjemi Y, Kaferstein F, Moy G, Quevedo F.** 1993. Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. Bull World Health Organ **71:**79–92.
20. **Bidawid SP, Edeson JF, Ibrahim J, Matossian RM.** 1978. The role of non-biting flies in the transmission of enteric pathogens (*Salmonella* species and *Shigella* species) in Beirut, Lebanon. Ann Trop Med Parasitol **72:**117–121.
21. **Chavasse DC, Shier RP, Murphy OA, Huttly SR, Cousens SN, Akhtar T.** 1999. Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. Lancet **353:**22–25. http://dx.doi.org/10.1016/S0140-6736(98)03366-2.
22. **Cohen D, Green M, Block C, Slepon R, Ambar R, Wasserman SS, Levine MM.** 1991. Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). Lancet **337:**993–997. http://dx.doi.org/10.1016/0140-6736(91)92657-N.
23. **Lindsay DR, Stewart WH, Watt J.** 1953. Effect of fly control on diarrheal disease in an area of moderate morbidity. Public Health Rep **68:**361–367. http://dx.doi.org/10.2307/4588424.
24. **Sukontason K, Bunchoo M, Khantawa B, Piangjai S, Sukontason K, Methanitikorn R, Rongsriyam Y.** 2000. Mechanical carrier of bacterial enteric pathogens by *Chrysomya megacephala* (Diptera: Calliphoridae) in Chiang Mai, Thailand. Southeast Asian J Trop Med Public Health **31**(Suppl 1)**:**S157–S161.
25. **Sukontason K, Bunchoo M, Khantawa B, Sukontason K, Piangjai S, Choochote W.** 2000. *Musca domestica* as a mechanical carrier of bacteria in Chiang Mai, north Thailand. J Vector Ecol **25:**114–117.
26. **Sukontason KL, Bunchoo M, Khantawa B, Piangjai S, Rongsriyam Y, Sukontason K.** 2007. Comparison between *Musca domestica* and *Chrysomya megacephala* as carriers of bacteria in northern Thailand. Southeast Asian J Trop Med Public Health **38:**38–44.
27. **Szostakowska B, Kruminis-Lozowska W, Racewicz M, Knight R, Tamang L, Myjak P, Graczyk TK.** 2004. *Cryptosporidium parvum* and *Giardia lamblia* recovered from flies on a cattle farm and in a landfill. Appl Environ Microbiol **70:**3742–3744. http://dx.doi.org/10.1128/AEM.70.6.3742-3744.2004.
28. **Watt J, Lindsay DR.** 1948. Diarrheal disease control studies; effect of fly control in a high morbidity area. Public Health Rep **63:**1319–1333. http://dx.doi.org/10.2307/4586723.
29. **Holt PS, Geden CJ, Moore RW, Gast RK.** 2007. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella serovar* Enteritidis-challenged hens. Appl Environ Microbiol **73:**6030–6035. http://dx.doi.org/10.1128/AEM.00803-07.
30. **Farag TH, Faruque AS, Wu Y, Das SK, Hossain A, Ahmed S, Ahmed D, Nasrin D, Kotloff KL, Panchilangam S, Nataro JP, Cohen D, Blackwelder WC, Levine MM.** 2013. Housefly population density correlates with shigellosis among children in Mirzapur, Bangladesh: a time series analysis. PLoS Negl Trop Dis **7:**e2280. http://dx.doi.org/10.1371/journal.pntd.0002280.
31. **Kattula D, Francis MR, Kulinkina A, Sarkar R, Mohan VR, Babji S, Ward HD, Kang G, Balraj V, Naumova EN.** 18 February 2015. Environmental drivers of diarrheal infection in rural and urban communities in southern India in children and adults. Epidemiol Infect http://dx.doi.org/10.1017/S0950268814003562.
32. **Ajjampur SS, Rajendran P, Ramani S, Banerjee I, Monica B, Sankaran P, Rosario V, Arumugam R, Sarkar R, Ward H, Kang G.** 2008. Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. J Med Microbiol **57:**1364–1368. http://dx.doi.org/10.1099/jmm.0.2008/003319-0.
33. **Borror DJ, DeLong DM.** 1964. An introduction to the study of insects. Holt, Rinehart, and Winston, Austin, TX.
34. **Gomez-Duarte OG, Bai J, Newell E.** 2009. Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacter* spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. Diagn Microbiol Infect Dis **63:**1–9. http://dx.doi.org/10.1016/j.diagmicrobio.2008.09.006.
35. **Girish R, Broor S, Dar L, Ghosh D.** 2002. Foodborne outbreak caused by a Norwalk-like virus in India. J Med Virol **67:**603–607. http://dx.doi.org/10.1002/jmv.10145.
36. **Iturriza Gomara M, Wong C, Blome S, Desselberger U, Gray J.** 2002. Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. J Virol **76:**6596–6601. http://dx.doi.org/10.1128/JVI.76.13.6596-6601.2002.
37. **Nguyen TV, Le Van P, Le Huy C, Gia KN, Weintraub A.** 2005. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. J Clin Microbiol **43:**755–760. http://dx.doi.org/10.1128/JCM.43.2.755-760.2005.
38. **Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA.** 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol **65:**3386–3391.
39. **Greenberg B.** 1964. Experimental transmission of *Salmonella Typhimurium* by houseflies to man. Am J Hyg (Lond) **80:**149–156.
40. **Greenberg B.** 1973. Flies and disease. Vol II. Biology and disease transmission. Princeton University Press, Princeton, NJ.
41. **Khin Nwe O, Sebastian AA, Aye T.** 1989. Carriage of enteric bacterial pathogens by house flies in Yangon, Myanmar. J Diarrhoeal Dis Res **7:**81–84.
42. **Levine MM, Cohen D, Green M, Levine OS, Mintz ED.** 1999. Fly control and shigellosis. Lancet **353:**1020.
43. **Manun'ebo MN, Haggerty PA, Kalengaie M, Ashworth A, Kirkwood BR.** 1994. Influence of demographic, socioeconomic and environmental variables on childhood diarrhoea in a rural area of Zaire. J Trop Med Hyg **97:**31–38.
44. **Hashizume M, Wagatsuma Y, Faruque AS, Hayashi T, Hunter PR, Armstrong B, Sack DA.** 2008. Factors determining vulnerability to diarrhoea during and after severe floods in Bangladesh. J Water Health **6:**323–332. http://dx.doi.org/10.2166/wh.2008.062.
45. **U KM, Khin M, Wai NN, Hman NW, Myint TT, Butler T.** 1992. Risk factors for the development of persistent diarrhoea and malnutrition in Burmese children. Int J Epidemiol **21:**1021–1029. http://dx.doi.org/10.1093/ije/21.5.1021.