Kristen Aiemjoy
Biostatistics 209
Spring 2014

Final Project: Design and chemical characteristics of latrines associated with exit trap fly densities in the Kilombero district of Tanzania

INTRODUCTION

Globally, diarrhea is the second leading cause of mortality among children under 5 [1]. Diarrheal disease has multiple transmission routes, and its control often requires multiple interventions, which can include improvements in access to water and sanitation, better food and hand hygiene, and drinking water treatment. Synanthropic flies may also play an important role in the transmission of diarrheal disease and randomized controlled trials on fly control have shown a reduction in diarrhea ranging from 22% to 26% [2,3]. Pit latrines if designed and managed well can play an important role in diarrheal disease control [4]. However, latrines can also provide a feeding and breeding ground for flies. Flies transmit enteric pathogens by landing on, or consuming fecal waste, and then transport this waste on body parts, regurgitate, or defecate on human food and fomites [5]. Despite the widespread availability of latrines and fairly standard contents, not all latrines produce equal numbers of flies [6,7]. Little is known about the reasons for these differences. The pilot study here presented aimed to investigate the association between modifiable latrine design characteristics, chemical characteristics of the pit contents and fly presence in latrines.

The goal of this analysis is the build the best regression model that accounts for the hierarchical nature of the data (clustered by village).

METHODS

Study Design: The study was conducted as part of a larger study investigating biodegradation in pit latrines in the Morogoro region of southern-central Tanzania. Fifty latrines representing different design and management practices were purposively selected after consultation with village leaders and homeowners in order to monitor latrine fill up and biodegradation.

Latrine characteristics were collected by direct inspection of the latrine and a questionnaire. Characteristics of the latrines recorded included: the age, type of pit-lining (presence and type), floor type, wall type, roof (presence and type), type of latrine use (family or communal, and number of people using the latrine), usage of decomposing additives (usage and type), water table level (flooding), and depth of a latrine (from drop hole to the surface of the pit latrine contents).

Chemical analysis was conducted by collecting approximately 150 ml of material from the top layer (0-15 cm) of each pit latrine. Samples were analyzed the same day for: total chemical oxygen demand (COD), ammonium, total phosphate, volatile fatty acids (VFA), carbohydrates, protein, moisture content, total and volatile solids.

Adult flies were collected using exit traps that were placed over the latrine drop-hole. The drop-hole was modified with heavy plastic so the trap was the only exit point. Families were instructed to remove and replace the trap when they needed to use the latrine. Of the 50 latrines examined, only 42 were suitable for fly trapping. Three had collapsed, three had flush/septic tank systems, and two were public latrines with a high number of users, making a 24-hour exit trap collection impossible.

Data Analysis: Four linear regression approaches were investigated to best fit the data: 1) a linear regression model without accounting for village, 2) a linear regression model accounting for clustering by village by including it as a variable in the model, 3) a generalized estimating equation with village as the group variable and 4) a random effects model (allowing random intercepts for village). All four models were then used with different distributions of the outcome variable total fly count. Three different approaches were attempted: normal/untransformed, log +1 transformed, and a gamma distribution.

Potential predictor variables were analyzed individually for their association with total fly count and included in the models if their significance level was less than or equal to 0.10. All potential predictor variables were analyzed in each of the four models (1) fixed, no village, 2) fixed with village included, 3) GEE and 4) mixed); if a variable was associated with total fly count in at least one model it was included in the analysis. If a predictor was significant at the 0.05 level it was retained in the model. When available, robust standard errors were calculated. The GEE models were corrected for the small sample size using the nmp option. The Gamma models all used a log link function. To fit the gamma distribution, which does not allow zero cells, 0.01 was added to total fly counts. All models were checked for basic assumptions, including the normality of the residuals. The final model was thoroughly checked for assumptions.

RESULTS

Fly counts and families: A total of 3,539 flies were collected for the study. Four latrines produce no flies while the top-producing latrine yielded 951 flies. This latrine produced nearly three times as many flies as the second leading latrine (333 flies). The mean fly count was 84.26 with a standard deviation of 167.26 and the median count was 19.5. Table 1 presents a breakdown of fly catches by family. The dominating families in the traps were Psychodidae and Calliphoridae, making up 99% of the total number of flies caught.

Table 1: Summary data of flies caught in all 42 traps

	Calliphoridae	**Psychodidae**	**Sarcophagidae**	**Stratiomyidae**	**Stratiomyidae**	**Unknown**	**Total**
Total caught (% of total)	1,004 (28.37%)	2,488 (70.30%)	2 (0.1%)	8 (0.2%)	18 (0.5%)	19 (0.5%)	3,539
Min-max per trap	0-324	0-625	0-1	0-2	0-8	0-4	0 - 951

Latrine Characteristics: The characteristics of the latrines are displayed in Table 2. The mean depth of the latrine from the drop-hole to the pit contents was 1.48 m (95% CI: 1.26-1.65 m), with a minimum depth of 0.46 m and a maximum depth of 2.8 m. The majority of latrines were used by a single household, with a mean number of users of 8 (ranging from 2 to 35). The mean age of latrines was 4 years (95% CI: 2.5-5.4 years).

Table 2: Latrine Characteristics

Characteristic		Number	Percentage
Roof	No	9	31%
	Yes	33	79%
Roof Material	Grass/palm leaves	22	67%
	Iron sheets	11	33%
Wall Material	Grass/ plastic sheeting	12	29%
	Mud	8	19%
	Brick/concrete	22	52%
Floor material	Concrete	15	36%
	Wood/soil	27	64%
Pit of Latrine	Unlined	31	74%
Pit	Lined with bricks	11	26%
Use of Additive	No	20	51%
	Yes	19	49%
Type of additive	Bleach	3	16%
	Ash	16	84%
Groundwater floods latrine	No	13	31%
	Yes	29	69%
Water stored inside	No	6	14%
	Yes	36	86%

Models: The following variables were found to have a univariate association with total fly count in at least 1 model: (the bracket indicate in which of the four models an association was found: 1) fixed, no village, 2) fixed with village included, 3) GEE and 4) mixed.

- Roof (presence or absence)[1,2,3,4]
- Wall material type (grass/plastic, mud or bricks) [1, 4]
- Volatile solids [3,4]
- Total phosphate [1, 4]
- Protein [1,3,4]

The five potential predictor variables were included in each of the models under all 3 transformations. If a predictor was significant at the 0.05 level it was retained in the final model. When included in the multivariate analyses, the chemical characteristics were no longer significantly associated with total fly count in any of the models or distributions. Wall type was significantly associated with fly counts in all of the GEE models, it was also significantly associated under all gamma distributions except when the mixed model was used. There is a lot of variation in the coefficients produced for wall type by the models. For example, in the fixed model with a gamma distribution mud walls produce more flies than brick walls but in fixed model including village and a gamma distribution brick walls produce for than mud walls. This significance of wall as a predictor also varies greatly throughout the models. For example in the fixed model with village and a gamma distribution mud walls have marginal significance ($p=0.06$) but in the GEE model also with a gamma distribution mud walls are highly statistically significant ($p<0.001$). Table 3 lists the final models with their coefficients, standard errors and p-values for each distribution.

Table 3: Final models with coefficients (standard errors) and p-values

		Normal (untransformed)	Log+1	Gamma
Fixed	Roof	-231.60(55.56) p<0.001	-2.78 (.59) p<0.001	.03(.02) p<0.001
	Wall (mud)			5.84(5.55) p=0.06
	Wall (bricks)			4.58 (3.37) p=0.039
Fixed +village	Roof	-221.89 (61.09) p=0.001	-2.80 (.64) p<0.001	0.009 (0.007) p<0.001
	Wall (mud)			7.0 (5.18) p=0.009
	Wall (bricks)			11.25 (10.22) p=0.008
GEE	Roof	-262.75 (108.67) p=0.016	-3.65 (1.01) p<0.001	.03 (0.03) p<0.001
	Wall (mud)	43.62 (22.64) p=0.05	1.31 (.59) p=0.03	5.84 (1.2) p<0.001
	Wall (bricks)	32.27 (10.98) p=0.003	.86 (.61) p=0.19	4.58 (2.63) p<0.008
Mixed	Roof	-231.6 (54.22) p<0.001	-2.78 (0.57) p<0.001	.14 (0.09) p=0.002

There is some concern over the distribution of the residuals with the models using untransformed total fly count as the outcome. In all four residual plots the residuals did not follow a normal distribution and were skewed to the right. The log+1 transformations improved the normality of the residuals. However, the log transformations are not appropriate to fit the aim of the project. The research question of interest is why some latrines produce tremendous numbers of flies while other latrines produce none, in other words to understand the determinants of the actual *number* of flies. We could try to back transform the log but the log of the mean is not the same as the mean of the log and will not produce results interpretable as mean fly counts. A solution is to leave the outcome untransformed and assume a sensible distribution of the data. In this case, the gamma distribution is reasonable because fly numbers are positive, continuous and the standard deviation is proportional to the mean.

The standard errors for the roof coefficients were slightly higher in the normal and log transformed fixed models including village compared to without including village. However, under the gamma distribution, the estimate and standard errors were much smaller. The GEE models produced much larger standard errors for the coefficients than the other models, with the exception being the standard errors for the wall estimate in the GEE model under a gamma distribution, which were smaller than the wall estimates for gamma fixed with or without village.

Final Model: Mixed effects, Gamma distribution: The mixed effect model is the most appropriate model for the data as it allows for random intercepts for village, the residuals are normally distributed it preserves the measure of effect of interest. The results of this model are as follows:

Latrines with roofs produced on average 14.78% (95% CI: 4.33% - 50.41%) of the flies produced in latrines without roofs (p=0.002), accounting for any clustering by village. No other physical characteristics of the latrine or chemical characteristics were significantly associated with total fly counts in a multivariate model and thus were not included in the final model.

The main finding was robust to influential points. When the top-producing latrine (951 flies) was dropped from the analysis the model still found a comparable effect: latrines with roofs produced on average 22.98% (95% CI: 8.7% - 60.9%) of the flies produced in latrines without roofs (p=0.003).

CONCLUSION
The most appropriate model for this data was found to be a mixed effects model with a gamma distribution. The key finding of this model is that latrines with roofs have far fewer flies than latrine without roofs.

The absence of a roof may provide easier access to flies to enter the latrine. Another factor to consider is the amount of light that is entering a latrine, which may also affect flies entering and exiting through the drop hole. The amount of light in the latrine could also influence the effectiveness of the exit traps (more light could have drawn more flies to the traps). Light entering the latrine might also result in important differences of temperature. To investigate these possibilities, this study will be repeated this summer at the University of Venda in South Africa. Temperature and light readings will by taken inside the latrine. Data on diarrheal disease among household members will also be collected.

Flies play an important role in the cycle of diarrheal disease transmission. While latrines are promoted to reduce diarrheal disease by minimizing open defecation, they may themselves increase transmission by providing a breeding site for flies. As insecticide control is not a viable option due to resistance and cost, other mechanisms need to be explored to reduce the contact between flies and human fecal waste. Modifying latrine designs to provide unfavorable environments for flies may be one such option.

References

1. Boschi-Pinto C, Velebit L, Shibuya K (2008) Estimating child mortality due to diarrhoea in developing countries. Bull World Health Organ 86: 710-717. doi:10.2471/BLT.07.050054. PubMed: 18797647

2. Chavasse DC, Shier RP, Murphy OA, Huttly SR, Cousens SN et al. (1999) Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. Lancet. 353: 22-25. doi:10.1016/
S0140-6736(98)03366-2. PubMed: 10023946.

3. Emerson PM, Lindsay SW, Walraven GE, Faal H, Bøgh C et al. (1999) Effect of fly control on trachoma and diarrhoea. Lancet. 353: 1401-1403. doi:10.1016/S0140-6736(98)09158-2. PubMed: 10227221

4. Clasen TF, Bostoen K, Schmidt WP, Boisson S, Fung ICH et al. (2009) Interventions to improve disposal of human excreta for preventing diarrhoea. Cochrane Database Syst Rev 16: CD007180: CD007180. PubMed: 20556776

5. 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. doi:10.1016/S1286-4579(01)01371-5.
PubMed: 11358717.

6. Curtis CF, Hawkins PM (1982) Entomological studies of on-site sanitation systems in Botswana and Tanzania. T Roy Soc Trop Med Hyg.76: 99-108.

7. Lindsay SW, Lindsay TC, Duprez J, Hall MJ, Kwambana BA et al. (2012) Chrysomya Putoria, a putative vector of diarrheal diseases. (2012a) PLoS Neglect Trop Dis.6 e1895.