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Pit Latrines: A Noninvasive Sampling Strategy to Assess Fecal Pathogen Occurrence in Low Resource Communities

Nathaniel LaHue[1] · Kathleen A. Alexander[1,2]

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Abstract

Limited understanding of disease in low resource communities continues to hamper improvements in health. We evaluated household pit latrine sampling as a non-invasive approach to investigate important fecal pathogens such as *Giardia lamblia* and *Cryptosporidium* spp. in impoverished communities where health-seeking behavior limits the sensitivity of health facility-based surveillance. Fecal samples were collected from pit latrines in randomly selected households and from patients presenting to the only hospital in the region during the same time periods. Samples were tested with a commercially available ELISA. *Giardia* household prevalence was 28.7% in 2016 and 48.4% in 2017, while individual samples from hospital submission had a *Giardia* prevalence of 2.4% in 2016 and 8.0% in 2017. *Cryptosporidium* was only found in one household. Results suggest that pit latrine surveillance for fecal-borne infections provide course estimates of community infection levels that are unbiased by health seeking behaviors and allow surveillance of vulnerable sectors of a population.

Keywords *Giardia* · Pit latrine · Low resource communities · Health seeking · Disease surveillance · Africa

Introduction

Diarrheal disease remains one of the largest causes of morbidity and mortality worldwide, responsible for an estimated 1.3 million deaths annually [1]. In Africa, efforts to reduce diarrhea associated mortality have had limited effect due to an array of environmental, socioeconomic and cultural barriers [2]. Diarrheal disease can lead to long-term effects, including malnutrition, stunted growth, and cognitive deficiencies, especially in children [3–7]. Our understanding of diarrheal disease causation is especially limited among poorer communities, where passive surveillance may fail to include more at-risk portions of the population, or may not accurately describe disease due to diagnostic limitations [8, 9]. Passive disease surveillance programs, which rely on information gathered from reporting medical facilities, may give biased results due to differences in health-seeking behavior that can arise from variation in socioeconomic status (i.e. affordability of care), knowledge of disease consequences, availability of health services, education level, culture and health belief systems [10–12]. Active surveillance is rarely an option to address these deficiencies as it is extremely labor intensive and costly compared to passive surveillance and is often not deemed worthwhile outside of outbreak investigations. In addition, active surveillance requires frequent community visits, with potentially invasive and unwanted sampling, and logistically reduces the number of communities that can be sampled, making extrapolation of the data dificult [13–15]. Affordable, non-intrusive active surveillance methodologies are needed to improve our understanding of disease burden in low resource regions where traditional passive surveillance systems might not be adequate to address the needs of more vulnerable sections of a population.

Among the many gastrointestinal diseases of importance in developing countries, waterborne and foodborne protozoal diseases that involve fecal-oral transmission, such as *Giardia* and *Cryptosporidium*, are a growing concern due to their association with poor sanitation and inadequate water treatment facilities, as well as their ability to cause inapparent to severe infections, both of which can result in

✉ Kathleen A. Alexander
kathyalx@vt.edu

[1] Department of Fish and Wildlife Conservation, Virginia Tech, Integrated Life Science Building, Blacksburg, VA 24060, USA

[2] Center for Conservation of African Resources, Animals, Communities, and Land Use (CARACAL), Kasane, Botswana

long-term health consequences [16–19]. *Giardia* especially, is one of the most common causes of childhood diarrhea [20, 21], and both are commonly found in asymptomatic children [17, 22]. Both protozoa, especially *Cryptosporidium*, can cause life-threatening infections in HIV patients [23], which is of grave importance in sub-Saharan Africa and more specifically Botswana, where HIV infection rates are among the highest in the world [24]. Cases of both protozoal infections are often misdiagnosed, particularly when asymptomatic or chronic, or when diagnostics are limited only to direct smear evaluation by light microscopy [3, 25]. In addition to misdiagnosis, the high number of chronic or apparently asymptomatic infections can lead to significant levels of under-reporting and testing for *Giardia* and *Cryptosporidium* infections [26, 27].

In Botswana, as with much of the rest of Africa, a large part of the population in certain regions still use simple or vented pit latrines [28]. Pit latrines have the potential to provide access to fecal samples allowing course estimation of disease burden for some important fecally transmitted diseases in a community. This may be especially important for inapparent or chronic infections, where medical attention is not pursued irrespective of health-seeking behavior [8]. Results of such studies can provide indication for further, more focused, public health investigations and intervention. We evaluate the utility of this sampling approach by investigating the prevalence of common water-borne parasites, *Giardia lamblia* and *Cryptosporidium* spp., in fecal samples collected from pit latrines in a low resource community. In order to develop an understanding of the disease burden of patients presenting to the local hospital, we also assessed the prevalence of *Giardia* and *Cryptosporidium* infection among patients presenting to the health facilities in the District during the same sampling period.

Methods

Study Area

This study was conducted in Kgaphamadi, a low-income residential area in the city of Kasane, in North-eastern Botswana. The region has a subtropical climate with annual wet and dry seasons from November to March and April to October respectively. Kasane borders Chobe National Park, a primary focus for tourism and is the district center, with a population of approximately 9000 people [29]. The Chobe River is the source of drinking water extraction for the District. Kgaphamadi is the lowest income area in Kasane and consists of small approximately ¼ acre plots, varying from 1 to 10 one-room households that usually share an outdoor single or double pit latrine (that may in some limited cases include a flush toilet) and, in most plots, an open kitchen area. The majority of households in the region use pit latrines (69%, 95% CI (Confidence interval) = 59–79%) [30]. Some properties included a tuck shop (small local shop) or a shebeen (small unregulated bar offering homemade alcohol), which may increase the number of people using the pit latrine at a particular time, especially in the case of the shebeens. In Kasane, there is a single laboratory located in the primary hospital that runs samples collected across health facilities in the District. Medical care is provided to the population by the government for a small fee.

Sample Collection

Pit latrine samples were taken from a subset of 27 randomly selected households that agreed to participate in our study, with sampling events conducted in March, May, July, and August of 2016 and March of 2017. During the first four sampling events a single sample was taken per pit latrine and three repeated samples were taken during the March 2017 sampling event in order to determine within latrine variation. The number of households sampled differed between sample dates; either because the occupants were not home, or because there was no one at home who had the authority to allow the team to sample the pit latrine. Fecal samples were taken from the pit latrine using a long stick with a sterile tongue depressor attached to the end. The depressor was inserted into the fecal mound and moved back and forth through the material in order to obtain the best representative sample of the contents. The fecal samples were then transferred to a sterile, empty Eppendorf tube, transported our laboratory on ice packs, and frozen at − 20 °C the same day. Data were also taken during the July 2016 sampling event regarding the number and age of occupants in the compound and the presence of an onsite shebeen or tuck shop. Fecal samples were collected from the local hospital during the same time frame as the household sampling events. When available, anonymized patient data were also collected including the age, sex, date, and reason for testing, along with any clinical diagnoses.

Testing

All fecal samples were tested using the *Giardia/Cryptosporidium Quik Chek* SNAP ELISA from Techlab Inc. (Blacksburg, VA). Test sensitivity and specificity for Giardia was 94% and 100% respectively and a sensitivity and specificity of 100% for *Cryptosporidium* [30]. The test recognizes a *Giardia* cyst antigen and a *Cryptosporidium* oocyst antigen that is conserved across *C. parvum* and *C. hominis*. No cross reaction with other material in stools was reported. Testing was performed as per the manufacturer's instructions (http://www.techlab.com), with modification for firm fecal samples where a 1:1 dilution of the sample with distilled water was added in order to reduce increased background coloration in the well.

Statistical analyses were undertaken with R-studio [31]. Prevalence rates were calculated for each sampling event using Bonferroni adjusted confidence intervals to account for multiple comparisons. Fisher's exact tests were used to compare the results with household factors [21]. For March 2017, a Cohen's kappa statistic was calculated to determine the amount of intra-house agreement between multiple samplings of pit latrine contents.

Results

Sampled residential units had an average of three adults (n = 25, standard deviation (SD): 3.9 range = 2–15) and three children (n = 25, SD: 2.6 range = 0–9). Three households had shebeens and five had tuck shops. Where age information was available from hospital staff, most appeared to come from adults, with an average age of 29.1 years (n = 140, 95% CI 27.4–30.8) with 61.3% being female (n = 140, 95% CI 53.2–69.3%). In 2016, all stool samples taken at the hospital were tested. However, in 2017, due to limited testing supplies and an increased number of submissions, we randomly tested a subsample of all submission made between January 1st and March 14th, 2017 (n = 87, out of 114 submitted to the laboratory). Of fecal samples tested, 82.5% were obtained from individuals submitting samples for routine medical examinations required for food workers in restaurants and lodges.

Giardia was found in pit latrines and hospital fecal samples during all 2016 and 2017 testing dates (Tables 1, 2). Pit latrines during the 2016 sampling events had an average, non-weighted, *Giardia* prevalence of 28.7%. The likelihood of any household being positive on July 22nd (when the demographic data was collected) was independent of the presence shebeens (p = 1.000), the presence of tuck shops (p = 1.000), the number of household occupants (p = .669), and the number of children in the household (p = .932). For March 2017, a Cohen's kappa statistic showed moderate intra-household agreement between repeated samples at 0.56 (n = 27, p ≤ .005). Only one household tested positive for *Cryptosporidium* (March 2017) across all household sampling events. *Cryptosporidium* was not detected in any of the 171 samples obtained from the hospital during the entire scope of the study.

Table 1 Prevalence and confidence intervals of samples testing positive for *Giardia* in households, represented by event dates, and hospital samples in 2016

	N	Prevalence (%)	BACI (%)
Household sampling dates			
March 8th, 2016	10 households	20.0	0.0–51.6
May 10th, 2016	10 households	40.0	1.3–78.7
July 22nd, 2016	25 households	16.0	0.0–34.3
August 8th, 2016	18 households	38.9	10.2–67.6
Hospital dates			
February–August 2016	84 individuals	2.4	0.0–5.6

BACI 95% Bonferroni adjusted Confidence Interval

Table 2 Prevalence and Bonferroni-adjusted confidence intervals for the sampling in March 2017, where 3 repeated samples were taken from each pit latrine

	N	Prevalence (%)	BACI (%)
Household samples positive			
Any number positive (1–3)	27 households	48.1	29.3–67.0
All 3 samples	27 households	18.5	0.0–37.2
2 samples	27 households	14.9	0.0–31.9
1 sample	27 households	14.9	0.0–31.9
Hospital sampling			
January–March 2017	87 individuals	8.0	2.3–13.8

Samples positive refers the number of samples per pit latrine that were positive (0–3)

BACI 95% Bonferroni adjusted Confidence Interval

Discussion

Intestinal protozoal infections are recognized globally as critically important neglected pathogens requiring increased effort to improve detection and control, particularly in low resource communities [32]. In this study, we demonstrated the usefulness of utilizing pit latrine sampling as a novel method to perform broad-based, low-cost fecal pathogen surveillance in an at-risk community. To the authors' knowledge, this method of sampling has not been previously applied. Previous studies have utilized sewage for detection of pathogens in a community [33, 34], however the method in this study provides a mechanism of assessing enteric infections in extremely poor communities where community sewage sampling techniques would be inadequate given preponderance of pit latrine use. The approach allows public health staff to identify sectors of a community that might need more focused health interventions at minimal costs, both in terms of financial and human resource investments.

In this study, *Giardia lamblia* was found commonly in pit latrine samples collected from sampled households in Kgaphamadi, with prevalence varying over time (range 16.0–48.1%). As we cannot determine how many individuals were screened with the pit latrine approach, it is not possible to directly compare the household prevalence to the individual prevalence in hospital samples (Giardia 2.4% N:84

CI:0.0–5.6% and 8.0% N:87 CI:2.3–13.8%; Crypto 0.0%) for this study or our previous study [35]. However, the high percentage of positive households suggests *Giardia* may be an important health problem in this community and that more focused health investigations and interventions might be warranted. Given that *Giardia* can cause long term detrimental effects in children [36, 37], there is important public health value in identifying at risk households in a community, testing and treating affected individuals. We found evidence of cryptosporidium infections in only one study households and this may relate to the tendency for infections in Africa to occur dominantly in children < 2 years of age [38]. Similarly, our previous work in this population identified cryptosporidium infection only in this age group [35], suggesting that pit latrine sampling may have limited utility for surveillance of this pathogen.

While this method does not allow for characterization of within household prevalence or identification of infected individuals, it is much less invasive, easier to implement, and as a pooled household level sample, it may be more cost effective than individual screening. This method may also eliminate health-seeking bias inherent in passive surveillance systems. Health facility data may significantly underestimate the burden of diseases like *Giardia*, where chronically and/or asymptomatically infected individuals may not seek health care [36, 39]. Higher prevalence rates of *Giardia* have been associated with lower socioeconomic status [20, 40], which in turn is associated with decreased health-seeking behavior [11, 41], making areas with low socioeconomic status more likely to be underrepresented in passive surveillance approaches. Aside from socioeconomic status, other factors may also influence health-seeking behavior such as education level, ages of children in the household, and underlying health beliefs systems [8, 11, 42]. Households may also be more amenable to pit latrine sampling than individual sampling.

Besides the protozoal species sampled in this study, this methodology also can be used to evaluate a larger number of fecal pathogens in underserved communities, such as rotavirus, cholera, *Esherichia coli, Salmonella, Shigella*, parasitic worms, as well as other protozoa. Data may also be useful in understanding potential sources of pathogen contamination in ground water and the local environment. Identification of the occurrence of a particular pathogen of concern in a community may also increase clinician awareness and diagnoses. This may be especially useful in low-resource environments where diagnostic resources are often limited and syndromic approaches to case management are often the primary approach to patient care.

A limitation with this approach is the inability to determine the number of individuals in the composite sample. Additionally, there may be unknown numbers of non-household users (neighbors without toilets, visitors), especially in the case of shebeens. The repeated sampling in 2017 (three samples per latrine) seems to have increased the sensitivity of the testing, most likely due to more users being included; as a single sample appears to miss some individuals. Thus, it may be useful to collect more than one fecal sample from a household's pit latrine (2–3), feasibility of this dependent on existing human and financial resource limitations. This decision will be driven by the objectives of the study, which might be focused on estimating community infection levels or developing a more sensitive assessment of household infection. Additionally, pit latrine sampling also may not be appropriate for diseases affecting very young children as fecal inputs into the pit latrine might be limited or absent depending on sanitation practices in the household. Expected age prevalence data will be important in determining the appropriateness of this sampling method by pathogen. It was also dificult to compare consistency of stools. All stools were used from the hospital so a focus on diarrheal stools may have yielded a higher prevalence. The consistency of stools from the pit latrine was unknown. However, our method is more likely to show the overall true prevalence of the disease in the community rather than focusing on detecting individuals with symptomatic *Giardia* or *Cryptosporidium*.

Further research is needed to demonstrate the large-scale effectiveness of pit latrine sampling over time as an integrated part of a public health surveillance strategy. This should be done, where possible, with individual level studies directed at improving understanding of infection dynamics, including transmission and persistence in the community. Our data collected here suggest that further public health efforts should be directed at controlling *Giardia* infections in this community.

Conclusions

Pit latrines sampling may provide an important foundation for community health surveillance of fecal borne pathogens and act as a first step towards identifying areas where further investigation and/or public health interventions are needed. This may be particularly important in areas where health belief systems and health seeking behavior may affect passive surveillance accuracy. This approach may also provide a mechanism for identifying vulnerable populations in the community in need of more focused health interventions.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All human associated data were anonymized. As part of a large study informed written consent was obtained from each household prior to sampling and informed verbal consent was obtained and repeated at each sampling event. The research was conducted under a permit from the Ministry of Health in Botswana (HPDME-13/18/1 Vol IX (495)) and approved by the Virginia Tech Institutional Review Board (IRB# 11–573).

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