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Fecal Fingerprints of Enteric Pathogen Contamination in Public Environments of Kisumu, Kenya, Associated with Human Sanitation Conditions and Domestic Animals

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Abstract

Young children are infected by a diverse range of enteric pathogens in high disease burden settings, suggesting pathogen contamination of the environment is equally diverse. This study aimed to characterize across- and within-neighborhood diversity in enteric pathogen contamination of public domains in urban informal settlements of Kisumu, Kenya, and to assess the relationship between pathogen detection patterns and human and domestic animal sanitation conditions. Microbial contamination of soil and surface water from 166 public sites in three Kisumu neighborhoods was measured by enterococcal assays and quantitative reverse transcription polymerase chain reaction (qRT-PCR) for 19 enteric pathogens. Regression was used to assess the association between observed sanitary indicators of contamination with enterococci and pathogen presence and concentration, and pathogen diversity. Seventeen types of pathogens were detected in Kisumu public domains. Enteric pathogens were codetected in 33% of soil and 65% of surface water samples. Greater pathogen diversity was associated with the presence of domestic animal feces but not with human open defecation, deteriorating latrines, flies, or disposal

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Author Contributions

K.K.B. and A.S.G. conceived of this study and acquired funding. K.K.B., R.S., O.C., and J.M. collected data. K.K.B., R.S., and J.M. designed the study or study tools. D.S., R.S., and K.K.B. provided statistical analysis. K.K.B. drafted the initial manuscript. All authors edited the manuscript for intellectual content and provided final approval of the version to be published.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01528.

Additional text describing RT-PCR inhibition and standard curves, back calculations of final concentrations, and microbial detection and diversity in Iowa watershed; two figures showing location of public domain sampling sites in Kisumu, Kenya, and detection of pathogens from an agricultural Iowa watershed; three tables listing soil and surface water samples from Kenya with evidence of inhibition of the QuantiFast Internal Control, source of reference DNA or RNA used for standard curves, and concentrations of enterococcal indicator bacteria and enteric pathogen DNA and RNA gene copies (PDF)

Notes

The authors declare no competing financial interest.

of human feces. Sanitary conditions were not associated with enterococcal bacteria, specific pathogen concentrations, or “any pathogen”. Young children played at 40% of observed sites. Managing domestic animal feces may be required to reduce enteric pathogen environmental contamination in high-burden settings.

Graphical Abstract

INTRODUCTION

Recent multicountry epidemiological studies of diarrheal disease etiology in high-burden settings have shown a wide diversity of enteric pathogen infections within and between individual children, and between populations and population subgroups (i.e., by age).[1,2] The diversity in pathogens causing childhood enteric infections shows that children under the age of five years (<5yr) in these settings are chronically exposed to a range of environmentally transmitted enteric pathogens. Relatively little is known about the extent of environmental enteric pathogen contamination in these settings and the patterns with which enteric pathogen contamination occurs over space and time. A handful of studies have reported high

frequencies of detection of different types of enteric pathogens in Indian and Tanzanian household environments, confirming that household exposure pathways pose a risk to children.[3–5] However, recent multimillion-dollar randomized, controlled trials of household water, sanitation, and hygiene (WASH) interventions found inconsistent impact of household WASH on pediatric diarrhea.[6–8] This suggests that children may be infected by non-household exposure pathways, such as through play in public areas near their household. In low-income settings where sanitation coverage is low, neighborhood public areas are often used for open defecation and disposal of untreated human waste by many households, as well as for domestic animal husbandry.[9] Thus, levels of pathogen contamination in public areas may far exceed contamination levels in privately owned domestic areas, and public areas could pose a disproportionally high risk of infection by enteric pathogens for exposed children.

The overarching goal of the Social Microbes Study is to examine enteric pathogen transmission patterns in high disease burden settings, from sources of fecal contamination through the environment to children, using pathogen distribution and diversity as indicators of transmission pathways. This is the first Social Microbes report to be released, which focuses on testing the hypothesis that public areas within low-income neighborhoods with high disease burden are contaminated by a diverse set of enteric pathogens, and testing whether observed indicators of human and animal feces contamination would be associated with increased presence and total diversity in pathogens. We also measured how frequently <5yr children are observed in public areas and whether we saw unsafe exposure behaviors to assess whether public domain exposures are viable pathways for <5yr enteric infection.

Environmental fecal contamination is usually measured by general fecal indicator bacteria, fecal source tracking markers, or focused detection of specific pathogens of interest as indicators of exposure risk. Fecal *Escherichia coli* and enterococcal bacteria are popular, low-cost indicators but typically correlate poorly with other infectious pathogens in sewage, ecological soils, surfaces, and waters.[10–13] They may be particularly unsuitable indicators for measuring risks from feces in ecological systems where gut bacteria become a naturalized part of soil, water, and surface microbial communities.[14] Host-specific fecal source tracking markers have improved capacity to distinguish human versus animal fecal contamination of the environment, but the reliability of fecal source tracking markers for predicting infectious pathogens is currently unclear.[10,15] Selection of one or a few specific types of enteric pathogens is often used to understand transmission patterns of specific types of pathogens.[16,17] However, dozens of enteric viruses, bacteria, protozoans, and helminthic species circulate at different times of the year in endemic settings, so reliance on any one to predict risks from all pathogens transmitted by fecal–oral exposure pathways is risky.[1,2] In this study, environmental samples were tested for a wide range of common enteric viruses, bacteria, protozoans, and helminths involved in fecal–oral disease transmission in high-burden settings to reduce the likelihood of exposure misclassification (classifying samples as uncontaminated on the basis of one indicator) or inaccurate concentration estimates for environmental exposure pathways.

Pathogen diversity was an important systems-level evaluation metric in this study that was adopted on the basis of the theory that diversity in enteric pathogen contamination would

occur not just across high disease burden neighborhoods but also at fine spatial scales. If environmental microbial contamination by human and animal feces is pervasive in high disease burden settings, with multiple pathogens circulating at any given time, then there is a strong chance that multiple enteric pathogens co-occur in some environments (e.g., open defecation sites) at the same time. Exposure of children to these uniquely hazardous environments could increase their risk of infection by multiple pathogen types. The risk of exposure to any given pathogen from soil or surface water is influenced by the recent presence of a fecal source but also by the pathogen's capacity to persist in specific environments, like soil or surface water, and overall infection rate in a host population. The evolutionary traits that govern persistence of microbes may be unique to members of a phylogenetic group (e.g., nonobligate bacterial replication in the environment) as well as reflecting species-specific ecological adaptations. Examining both the pathogen-specific and higher-order taxonomic differences in pathogens in the environment could provide insight as to the types of pathogens potentially transmitted via different child behaviors, for example, ingesting soil versus water. From a programming standpoint, capacity to identify high-risk areas or exposure pathways, in a milieu of existing elevated background contamination, could improve how well investments reduce <5yr enteric disease in high-burden settings. Pathogen diversity could improve the identification of high- versus low-risk areas or exposure pathways and the fecal sources contributing to environmental contamination.

This paper describes enteric pathogen detection frequencies and diversity patterns in soil and surface water from public areas of three low-income, urban neighborhoods of Kisumu, Kenya, with low sanitation coverage. Then we examine the relationship between human and domestic animal fecal sources and pathogen detection and diversity patterns.

MATERIALS AND METHODS

Study Population.

Kisumu is the third largest city in Kenya and has a population of approximately 409 928 inhabitants.[18] Up to 60% of the city's population resides in peri-urban informal settlements, which have emerged due to economic migration and a lack of affordable housing.[19] Kisumu County has a high prevalence of diarrhea (18% two-week period prevalence) with most cases of diarrhea occurring in children less than three years of age.[20] The child mortality rate is 105 deaths per 1000 live births, and the prevalence of severe childhood stunting (>–2 standard deviations below the reference norm) is approximately 25%.[20] This study took place in three established informal settlements, Nyalenda A [population density (pd) = 8953 persons per square kilometer] Nyalenda B (pd = 6886/km^2), and Obunga (pd = 1913 km^2). Cohabitation with domestic animals is common, and most residents rely upon open defecation or sanitation facilities shared by eight or more households.[21,22]

Pilot of Environmental Sampling and Microbiology Methods.

Prior to the primary study in Kenya, a pilot sampling project was conducted in Iowa in June 2015 (similar climate conditions) to understand how often fecal indicators and enteric pathogens would be detected in a watershed impacted by agriculture, free-range animal management (cows), and concentrated animal feeding operations (pigs). This information is

presented in Supporting Information Sections G and H as a comparative baseline for understanding the importance of fecal indicator and pathogen contamination in Kisumu, Kenya.

Sample Site Selection.

A cross-sectional observational survey with environmental sampling was performed in Kenya in July 2015. The study was designed to ensure sampling sites were randomly distributed across special areas of interest to optimize measurement of variability in pathogen diversity and co-occurrence patterns and to prevent introduction of observer bias in selection of environmental sampling locations. Neighborhood boundary parameters were visualized utilizing Batchgeo (Google), and these spaces were defined geographically by the northernmost, westernmost, easternmost, and southernmost latitude or longitude values in rectangular form. For each neighborhood, 60 latitude and longitude pairs that fell within these specified ranges were randomly generated by utilizing the Web site geomidpoint.com, prior to field-based data collection (Figure S1). Global Positioning System (GPS) coordinates were entered into a Waytracker mobile phone app, and daily routes were identified to navigate between ~15 coordinates per day. Observers navigated to the coordinates (±3 m), which was considered the center of a site, defined as all areas falling within a 25 m radius around the central coordinates. If the coordinates fell within a private household yard or business, the nearest set of coordinates outside that private space but within 25 m of the random coordinates were identified. Sites were visited at various times during the day (morning and afternoon), with site visits lasting approximately 20 min.

Public Site Observation.

Study teams systematically documented sanitary conditions at each site using an observational survey implemented via mobile phone app (FieldLogs, Trekea Mobile Inc.). Observers documented landscape features such as surface waters, grasslands, and altitude. Development of infrastructure was noted, including roads, drains, dams, industry, housing, public water sources, and the presence, physical condition, and hygiene of public or communal latrines. Indicators of human open defecation or unsafe disposal of excreta included observed “flying toilets” (plastic bags containing excreta), used diapers, piles of human feces, emptying of latrines into drains or land around the latrine, septage emptied from a latrine next to the latrine, and visual confirmation of an adult or child actively defecating in the open. Presence and type of domestic animals and their feces were recorded. Observers recorded whether, during that ~10–15 min spot observation, children approximately <5 yrs were observed in the public area, the number present, and any behaviors that would result in hand or mouth contact with environmental fomites (touching soil, surface water, animals, or objects on the ground, swimming, eating food, eating dirt, mouthing hands). Additionally, enumerators documented whether any children were defecating in the open at the time of observation. Ecological conditions that could influence pathogen presence and persistence, specifically daily temperature and relative humidity, were extracted from National Oceanic and Atmospheric Administration (NOAA) data collected at the Kisumu Airport. Observers did not include areas within private housing or businesses adjoining or overlapping with the site area to avoid potential bias in conditions observed from privately held property. However, private conditions that impacted public

space, such as drains or sewage leaching from the household or animals roaming between public and private areas, were recorded.

Sample Collection.

Standard operating protocols were implemented to ensure standardized hygienic sampling and processing of environmental materials. Approximately 5 g of soil was collected at every site by inserting an alcohol-sterilized scoop into the ground at a 45° angle to a depth of 5 cm (half the length of the scoop) and transferring the soil into a sterile WhirlPak bag (Sigma–Aldrich, St. Louis, MO). Surface water was collected, if present, by skimming water into a WhirlPak bag. Collection bags were stored on ice packs in a cooler and transported to the laboratory within 6 h of collection. In Kenya, four samples were collected at seven randomized sites in each neighborhood to account for anticipated variance in pathogen distributions at public sites caused by the final spatial scale of sites per neighborhood.

Indicator Analysis.

One gram of soil was measured into 10 mL of phosphate-buffered saline (PBS), and the mixture was vortexed for 30 s and then mixed on a rotator for 20 min. Solid matter was allowed to settle for 5 min and elute was removed for enterococcal assays. Enterococci are recommended by the U.S. Environmental Protection Agency for identifying fecal material in fresh and marine recreational waters,[23] although both enterococci and *E. coli* are considered inadequate indicators in tropical settings like Hawaii.[15,24] There is no global recommendation on appropriate indicators for tropical waters, so enterococci were chosen based upon the EPA policy. Enterococci were enumerated by vacuum filtration of three serial dilutions of surface water (10, 1, and 0.1 mL) or soil (1, 0.1, and 0.01 mL) rinsing through a white gridded 0.45 μm mixed cellulose esters filter (product GSWP04700, Millipore, Billerica, MA), and culturing filters for 18–24 h at 37 °C on mEI agar (EPA Method 1600). Colony-forming units (cfu) of enterococci were counted according to the manufacturer's recommendations.

DNA and RNA Extraction.

DNA and RNA was extracted from 0.5 g of soil by use of the FastDNA and FastRNA SPIN kits for soil (MP Biomedicals, Solon, OH), including a bead beating step. A 10 mL volume of surface water was processed by adjusting water to 2.5 mM $MgCl_2$ and pH 10.0 and then vacuum-filtering through a 0.45 μm mixed cellulose esters filter. This volume was chosen to ensure we could systematically sample a wide range of surface waters (floodwater puddles, drains, rivers) representing potential exposure hazards in Kisumu. This method was chosen because it more efficiently removes inhibitors than ultrafiltration and is practical for laboratories with limited capacity and for isolation of many different types of pathogens.[25,26] Filters were frozen at –20 °C for 1–4 weeks and transported to the University of Iowa, where they were stored at –80 °C until extraction (1 week). Filters were cut in half and processed by use of the FastDNA and FastRNA for soil kits (equivalent to 5 mL of water).

Quantitative PCR on Environmental Samples.

The concentration and quality of DNA and RNA was measured on a Nanodrop UV–vis spectrophotometer (Thermo Scientific). Duplicate 6 and 0.6 μL volumes of nucleic acid extract were tested for inhibition by the QuantiFast pathogen internal control kit (Qiagen, Germantown, MD). These methods effectively removed most amplification inhibitors (Table S1). Samples that were not inhibited were analyzed by combining 20 μL of DNA and RNA each (total 40 μL) with AgPath polymerase and then performing quantitative reverse transcription polymerase chain reaction (qRT-PCR) using a 19-pathogen microfluidic TaqMan array card (TAC) on a QuantiStudio 12K Flex real-time PCR system with array card block (ThermoFisher, Chicago, IL).[27] Inhibited samples were diluted 1:10 with deionized (di) H_2O before TAC analysis. The card format included five types of viruses (adenovirus 40/41, astrovirus, sapovirus, norovirus GII, and rotavirus), nine types of bacteria [enteroaggregative *E. coli* (EAEC), enter-opathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), shiga toxin-expressing *E. coli* (STEC), *Campylobacter jejuni, Shigella*, *Vibrio cholerae*, *Salmonella enterica*, and *Clostridium difficile],* three protozoans *(Cryptosporidium* spp., *Giardia lamblia*, and *Entamoeba histolytica*), and two helminths (*Ascaris* and *Trichuris).* Exponential curves and multicomponent plots were visually examined to validate positive amplification. Gene targets with real amplification in one well were reanalyzed and considered positive if amplification was detected again. Gene targets with cycle threshold (CT) values over 35 were cross-validated by performing a 14-cycle preamplification reaction with pathogen-specific primers (0.2 μM each), to increase the starting concentration of pathogen DNA/cDNA in a sample,[28] and then reanalyzed by qRT-PCR. Samples were classified as positive for a specific gene target if amplification was verified at a lower cycle threshold and negative if unverified. This resulted in presence and concentration data for 24 pathogen gene targets representing 19 types of pathogen taxa per sample (detection of either gene target for ETEC, EPEC, EAEC, and STEC was positive at a taxa level).

The concentration of each pathogen per reaction volume was estimated by comparing the CT for a pathogen gene target in a sample to a standard curve generated by qRT-PCR of a 6–7-fold serial dilution of a positive control of known concentration for each of the 19 qRT-PCR targets (Table S2). For samples that were repeated by use of the preamplification step, the initial concentration was estimated by comparison to a standard processed by preamplification. If samples determined to be positive still reflected signs of significant inhibition, concentrations were excluded from analysis to avoid biasing statistical estimates of mean and standard deviation. Otherwise, final concentrations of gene copy per gram of soil or per milliliter of surface water were generated by multiplying the concentration of each gene target by the dilution factors introduced by processing. Concentrations for samples with no detectable amplification were not transformed to avoid inflating statistics caused by relatively high methodological lower limit of detection (LLOD) for some pathogens.

Statistical Analysis.

Data were analyzed by use of SAS version 9.4 (SAS Institute, Cary, NC). Descriptive statistics of variables were reported as proportions or mean and standard deviation.

Microbial contamination outcomes of interest included the following: (l) presence of enterococcal fecal indicator bacteria, (2) $\log_{10}$ number of enterococcal cfu, (3) presence of any pathogen gene targets, (4) pathogen diversity, defined by the sum count of all unique types of enteric pathogens detected in an individual soil or water sample (either target for pathogenic bacteria defined as one group), and (5) $\log_{10}$ gene copy of pathogen gene targets.

Exposure variables representing potential sources of fecal contamination were any human or animal feces observed versus none observed; indicators of open defecation versus none observed; presence of any type of domestic animal present versus none observed; flies present versus none observed; and a categorical variable for latrines present in good condition, latrines present in bad condition, or no latrines present. Frequently latrines in both good and bad condition were present at a site. In this case the site was classified as latrines in bad condition, based upon the default assumption that just one deteriorating latrine could introduce contamination into soil and water. Potential confounders included type of sample (soil versus water), altitude in meters, relative humidity, temperature in Celsius, and landscape use (housing area versus undeveloped).

Generalized linear mixed models (glmm) with binary log link, robust standard errors, and random intercept for site ID and neighborhood with exchangeable correlation structure to account for spatial clustering at each level were used to test whether sites with any of the exposure variables were more likely to have at least one type of enteric pathogen detected. Identical glmm models with Poisson log link were used to test whether sites with any of the same potential fecal sources were more likely to have increased sum counts in pathogen diversity. Censored regression models were used to assess whether exposure variables were associated with increased concentration of enteric pathogen gene copy number to account for left-censoring of data. Each modeling process involved analyzing the effect of each exposure variable independently on the pathogen outcome, while adjusting for landscape type, daily temperature, relative humidity, altitude, and type of sample (soil vs water). Full models included all variables simultaneously to measure individual effects, accounting for co-occurrence of exposure variables at a site. On the basis of full model results, a subanalysis of association between specific types of domestic animals and pathogen diversity was performed by replacing the binary domestic animals variable with presence/absence variables for specific categories of animals. A one-sided Mantel test, using the geographic distances between sites, was used to test for spatial clustering of sites with compositional similarity between pairs of sites in terms of the number of pathogens detected at both sites.

RESULTS

Characteristics of Study Sites.

A total of 166 public sites were inspected for this study. Ecological conditions and the proportion of sites developed with housing were relatively similar in the three surveyed neighborhoods, with the exception of higher mean elevation in Obunga, more sites with indicators of open defecation or open feces disposal in Nyalenda B, fewer sites with shared latrines in Obunga, and more latrines in good structural condition in Nyalenda B (Table 1).

Children less than five years of age were observed at 40% (66 of 166) sites (median two children per site, range 1–10), including infants (defined as unable to stand and walk) at 9% (15 of 166) of sites. At least one <5yr child was observed crawling on the ground or sitting on the ground playing in dirt or mud at 24 sites, with a third of these observations ($n = 8$) recording infants crawling or sitting in the dirt. Children were observed playing with objects in the dirt at four sites. One infant and one child ~ 12–24 months were observed playing in a water puddle or drain water at two sites. Children were seen mouthing their hands at three sites (multiple ages), and one infant was eating soil at one site. Open defecation was not observed.

Microbial Detection, Concentration, and Neighborhood-Level Diversity in Kenya.

A total of 185 public site soil samples and 51 water samples were collected and tested for enterococci and enteric pathogens. Enterococcal colonies were isolated from 100% of surface water samples and 74.6% of soil samples (Figure 1). At least one type of enteric pathogen was detected in 92% of water samples and 82% of soil samples. This included 20 gene markers representing 16 types of enteric pathogens in water and 20 gene markers representing 17 types of pathogens in soil. The most common pathogens in soil were *Cryptosporidium* (67.0%), *Giardia* (17.8%), ETEC (13.5%), EAEC (10.8%), and EPEC (8.6%). The most common pathogens in water were *Cryptosporidium* (70.0%), EAEC (58.0%), ETEC (56.0%), EPEC (46%), and human adenovirus (36.0%). The relative abundance of different biological phyla and types of pathogens differed between pooled soil and water samples. Protozoan organisms accounted for 61.2% of microbial organisms in soil, followed by 30.6% pathogenic bacteria, 5.8% helminths, and 2.3% viruses (Figure 2). In contrast, pathogenic bacteria were more abundant in water (57.5%), followed by 26.9% protozoans, 5.8% viruses, and 2.3% helminths.

Enterococcal concentrations were about 3 log units higher in concentration in surface water than in soil (Table S3). Mean pathogen concentrations in positive samples were 2–5 log units higher than the limit of detection, although wide standard deviations for all pathogens reflected variability in concentration ranges (Table S3). Concentrations of pathogens in 1 g of soil were consistently 1–2 log units higher in concentration than those in 1 mL of surface water. In soil, the adenovirus 40/41 hexon, ETEC *eltB, Salmonella invA,* and STEC *stx2* genes were all detected at >7.0 $\log_{10}$ units/g, whereas *C. difficile tcdB* was the lowest concentration at 4.2 $\log_{10}$ units/g. In surface water, adenovirus 40/41 hexon was the highest at 6.6 $\log_{10}$ units/mL, followed by EAEC *aaiC,* ETEC *eltB,* and STEC *stx1* and *stx2* genes at 5.6 $\log_{10}$ units/mL, and astrovirus capsid, norovirus GI/GII ORF1–2, and *Campylobacter cadF* at <3 $\log_{10}$ units/mL.

Within-Site Enteric Pathogen Diversity.

Two or more enteric pathogens were codetected in 35% (64/185) of soil samples (median 1; range 0–7) and in 69% (35/51) of surface water samples (median 4; range 0–10) (Figure 3). Patterns of pathogen codetection were heterogeneous, with only two patterns (*Cryptosporidium* spp. + EPEC + ETEC + EAEC and *Cryptosporidium* spp. + *Giardia* spp. + ETEC + EAEC) reoccurring twice at a total of two sites each among the 35 water samples with ≥2 pathogens detected. Of the 64 soil samples with ≥2 pathogens codetected,

Cryptosporidium spp. + ETEC + EAEC occurred three times, *Cryptosporidium* spp. + *Giardia* spp. + and ETEC occurred twice, and the remainder were various combinations of *Cryptosporidium* spp. with other types of pathogens. The *Cryptosporidium* spp. assays might have detected species that do not typically infect humans.[29] If *Cryptosporidium* spp. results are excluded from the analysis, then 44% (82/185) of soil samples and 80% (41/51) of water samples contained at least one pathogen, while two or more enteric pathogens were codetected in 18% (33/185) of soil samples (median 0; range 0–6), and 65% (33/51) of surface water samples (median 3; range 0–9).

Association between Sanitary Conditions and Enterococcal Contamination.

Observation of feces, indicators of human open defecation, domestic animals, presence and poor condition of latrines, and flies were not associated with the presence (Table 2A) or increased concentration (Table 2B) of enterococcal indicators of bacterial fecal contamination in water and soil, after adjustment for development of the terrain, relative humidity, altitude, ambient temperature, and type of sample.

Association between Sanitation Conditions and Pathogen Diversity.

Observation of feces, indicators of human open defecation, domestic animals, presence and poor condition of latrines, and flies were not associated with the detection of any type of enteric pathogen, after adjustment for development of the terrain, relative humidity, altitude, ambient temperature, and type of sample (Table 3A). However, the presence of domestic animals was significantly associated with increased diversity in enteric pathogens in fully adjusted models, although observation of feces, indicators of human open defecation, presence and poor condition of latrines, and flies were not associated with increased diversity (Table 3B). Subanalysis of animal types in the fully adjusted model found that chickens (adjOR = 1.64; 95% CI 1.22, 2.22), cattle (adjOR = 1.36; 95% CI 1.08, 1.72), and goats and sheep (adjOR = 1.39; 95% CI 1.00, 1.94) were associated with increased pathogen diversity, while pigs (adjOR = 0.80; 95% CI 0.36, 1.77) and dogs (adjOR = 1.20; 95% CI 0.87, 1.65) were not significantly associated. No association was observed between different fecal sources and increased concentration of individual types of pathogens (data not shown).

Spatial Clustering of Sites with High Pathogen Diversity.

High pathogen diversity was not statistically associated with lower distance between pairs of sites (p-value = 0.22).

DISCUSSION

This study confirmed that neighborhood landscapes in Kisumu, Kenya, are contaminated by many enteric viral, bacterial, protozoan, and helminthic enteric pathogen species, at both neighborhood and localized (within 25 m radius) levels of spatial scale. At least one type of pathogen was detected at 80% of public sites, with a third of soil samples and three-quarters of surface water samples co-contaminated by multiple taxa of enteric pathogens. Even if *Cryptosporidium* detections are excluded, in the event that the species detected were not human-infective, multiple pathogens were still detected in a fifth of soil sampels and two-thirds of water samples. This evidence addresses major knowledge gaps about enteric

pathogen co-occurrence patterns in the environment, especially for public areas that are "ground zero" for contamination from open defecation and animal feces. The most abundant types of pathogen taxa varied for soil and surface water, with protozoans being most abundant in soil and pathogenic bacteria being most abundant in surface water. Domestic animals (specifically chickens, cows, and goats/sheep)—rather than human sources of fecal waste—were associated with increased pathogen diversity, whereas enterococcal bacteria, presence of any pathogen type, and concentrations of individual enteric pathogen taxa were not significantly associated with human and animal sources of fecal contamination. Finally, this study validated that <5yr old children play in these contaminated public settings, confirming that child exposure to and infection by pathogens in public areas is plausible. Therefore, interventions that prevent neighborhood-level animal fecal contamination may be necessary for reducing enteric disease burden in <5yr children in Kisumu. These interventions could include creation of protected child play spaces with finished, cleanable floors, promoting the safe disposal of animal waste, or animal penning designs that safely collect animal waste away from children.

These findings confirmed our hypothesis that pathogen diversity in Kisumu neighborhoods would mirror the leading etiologic causes of symptomatic and asymptomatic childhood infections in low-income, high-burden endemic countries in Asia and Africa.[1,2,27] Many of the most common causes of moderate to severe diarrhea in children in Kenya (*Cryptosporidium* spp., ETEC ST toxin-expressing *E. coli,* adenovirus 40/41, *C. jejuni*)[1,30] were the most frequently detected in the environment in Kisumu, although rotavirus and *Shigella*/EIEC were rare. This suggests that widespread contamination by those pathogens in the environment is responsible for causing enteric infection in children. Our findings also confirmed our hypothesis that multipathogen contamination of soil and water would be detected at locations where children play. This further suggests that children could ingest multiple pathogens during play in just one area outside the household. Pathogen diversity is a novel approach for characterizing environmental exposure risks in low-income countries and for identifying potential fecal sources associated with environmental contamination. However, microbial community characterization has been used for decades to understand ecological health and to monitor the impact of natural or man-made actions on system function.[31–33] In public health, microbiome community characterization has become a platform for understanding human susceptibility to enteric infection, symptomology and severity of disease, and vaccine failure.[34,35] A few studies have used the microbial community approach to understand the relationships between environmental microbiota, fecal indicator bacteria, and pathogenic bacteria in surface water and soil, although they did not examine infrastructure conditions driving this contamination.[36,37] Rather than sequencing, we used customized multipathogen qPCR tools for disease-targeted detection of the viral, bacterial, protozoan, and helminthic causes of pediatric diarrhea.[27] Additionally, we accounted for potential interrelated conditions underlying pathogen co-occurrence in our analysis rather than treating pathogen occurrence as an isolated, independent event.

The inclusion of pathogen diversity as an indicator of interrelated contamination conditions was important for understanding the relative intensity and determinants of contamination of public areas across Kisumu, as well as between Kenya and a reference site in Iowa. Consistent with prior studies, enterococci were omnipresent in Kenya *and* Iowa soil and

surface water, even though evidence of human fecal contamination was absent in Iowa and animal feces were less common.[15] Evidence on fecal microbes in soil is limited, but our detection rates were similar to at least one other study.[38] The lack of association between human and animal fecal source risk factors with enterococci in Kenya reinforces that enterococcal indicators are not optimal tools for predicting or quantifying exposure to enteric pathogens or for fecal source tracking. An “any pathogen” indicator was also not statistically associated with fecal sources. However, the stark differences in overall pathogen detection frequencies between Kenya and an Iowa watershed impacted by farm and agriculture helped calibrate our expectations as to what contamination patterns should look like in a setting where open defecation is absent, and they highlighted how alarming the pathogen detection levels in Kenya are by comparison. Sampling at different times, for example, different seasons, or sampling from agriculturally impacted areas in a tropical state (e.g., Florida), may have produced different results.

Neighborhood-level pathogen contamination in Kenya corresponded with the frequent observation of human and animal feces in public areas. These neighborhoods have low levels of household sanitation coverage, and domestic animal ownership is common in Kisumu.[21,22] We expected open defecation, dilapidated latrines, and domestic animals to be associated with pathogen diversity, given the evidence of their role in diarrhea in children.[39,40] In spite of widespread human feces in Kisumu, only domestic animals were associated with increased diversity in pathogens in the environment. Domestic animal reservoirs may contribute to pediatric disease burden by facilitating a rapid turnover in the types of pathogen species that children are exposed to, a situation where exposure-based immunity provides little benefit. We are unaware of evidence of this hypothesis, but theoretically domestic animals may be more important disease vectors than humans. First, animals (dogs, chickens, pigs, ducks) are more likely to be coprophagous (eat feces than humans) and therefore have higher risks of infection.[41] Second, animals are less likely to be treated for diarrheal symptoms of enteric infection than children[42] and therefore shed pathogens for longer times due to persistent infection.Third, dozens of animals may be kept by a household versus one or a few children,[21] therefore density of potential animal disease vectors is higher than child vectors. The lack of association between human sanitation and pathogen presence or diversity might reflect lower levels of pathogen infection rates in humans relative to animals. We think that the lack of association with all sanitary conditions and pathogen concentration is due to ecological conditions (humidity, UV, properties of soil) playing a strong mediating role on pathogen persistence and fate in the environment.

This study has several limitations. It is cross-sectional and cannot establish the direction of causality. Pathogen contamination could be a proxy for sites with more abundant animal food sources (feces, grassland), which thus attract domestic animals or flies to those sites. Even if that is the case, this is likely a circular relationship that involves animals defecating at the feeding site. Our observational criteria may not have distinguished well between unsafe latrines (those that are deteriorated and/or sometimes unused) and safe latrines (those that effectively contain excreta and prevent release into the environment). Without an in-depth inspection of the underground integrity of the latrine pits, the degree to which excreta are effectively contained cannot be assessed. Our sample size may have not provided sufficient power to detect important associations between fecal sources and microbial

outcomes. There was no prior source of information to predict frequencies of enteric pathogen co-occurrence, especially for 19 enteric pathogens.

Inherent heterogeneity in pathogen distributions in the environment and insufficient spacing scale between sites (sites too far apart) may explain why we did not find many repeat patterns of pathogen co-occurrence or spatial hot zones. Such patterns would provide even more precise metrics for linking widespread contamination to specific fecal sources, compared to the relatively simple approach of simply summing the number of pathogen types detected. In the handful of repeat patterns noted, the pathogens detected were the most common overall, suggesting presence is more a function of probability and sample size than impacts from types of fecal sources. In particular, the most common pathogen gene detected was *Cryptosporidium* spp. 18S, which could indicate contamination by species rarely detected in humans (*Cryptosporidium meleagridis, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium muris,* or *Cryptosporidium suis*) rather than the species (*Cryptosporidium hominus* and *Cryptosporidium parvum*) commonly linked with human infection.[29] This may overestimate the abundance of clinically relevant pathogens in public areas of Kisumu, although even after exclusion of *Cryptosporidium*-positive counts, pathogen detection and codetection was still very high. Research involving larger numbers of environmental samples is needed to understand whether co-occurrence patterns of specific groups of pathogens is common. Either way, the substantial amount of heterogeneity in pathogen detection patterns highlights the risk for misclassification of environmental exposures if the presence of an individual pathogen type is used as a proxy for the presence of any fecal pathogens or for fecal contamination overall. While some associations with trends toward significance may surpass significance cutoff thresholds with larger sample sizes, we are confident that it would not change the overall conclusion that domestic animals are important contributors to multipathogen contamination in public domains of Kisumu.

Finally, detection of pathogen DNA or RNA in soil and water by PCR does not confirm viability or infectivity of pathogens. PCR methods may detect extracellular DNA or intact but noninfectious cells. By filtering sample eluate through a 0.45 μm pore size filter, our sampling methods may have removed some extracellular material, although nonviable cellular DNA or RNA could still be trapped on the filter. PCR was chosen as the most systematic way to detect viral, bacterial, protozoan, and helminthic classes of fecal pathogens, many of which have no alternative detection methods. Culture-based assays could have been used for some bacteria but may have been equally inaccurate due to the presence of viable but nonrecoverable bacteria (e.g., *V. cholerae*, *Shigella dysenteriae*, *C. jejuni*, and enterotoxigenic *E.* coli).[17,43–45] Since we did not adopt a pre-enrichment step, it is possible that our enterococcal concentration data underestimate actual live concentrations of enterococci in soil or water. This also would introduce intermethod variability in detection methods across viral, bacterial, protozoan, and helminthic taxa. Adjusting concentrations for pathogen decay under these ecological conditions may improve the accuracy of estimated PCR concentrations. However, there is no such information for many enteric pathogens included in our assay, so such an approach could not be systematic. Also problematic is that feces contamination by animals and humans in Kisumu is ongoing. Failing to counteradjust pathogen concentration estimates for periodic reintroduction of pathogens in the environment would underestimate final concentration estimates. While some pathogen DNA

detected in Kisumu may be nonviable, it is more likely to be relatively recent contamination because free and cellular DNA/RNA is typically degraded by native soil or water microbiota within a matter of days or weeks.[46–51]

In conclusion, this study addresses two neglected realms in the WASH sector. First, we demonstrate that public domains are highly contaminated and may pose a substantial risk of enteric pathogen exposure for children. Young children will continue to play in soil and surface water in the public domain because they live in crowded conditions and at a certain age are drawn to engage in social play with their peers. In light of this, WASH interventions should invest in improving public sanitary conditions to prevent child exposure to enteric pathogens and possibly to reduce the childhood enteric disease burden that persists in many low- and middle-income countries. Second, even if high levels of household WASH coverage are achieved and human open defecation is eliminated, a high baseline level of enteric disease caused by contact with animal feces will persist. Domestic animal management must be included in the WASH agenda to reduce pathogen contamination at the neighborhood level, and potentially within households as well. Forthcoming research by this group is examining the generalizability of these findings in other geographical settings using improved assessment methods. Yet, the conditions in Kisumu—low-income urban settlements with high population density and limited public health infrastructure—are common in low-income settlements around the world, suggesting pervasive pathogen contamination of the environment in such settings is a universal problem.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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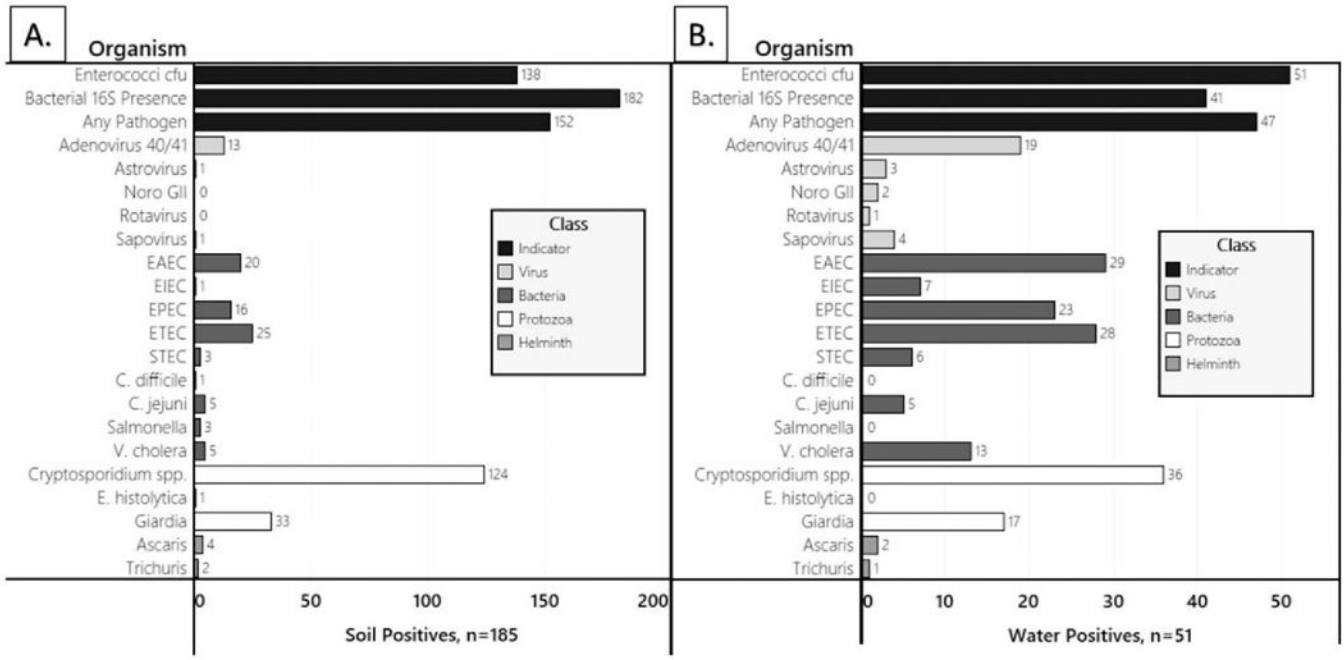

Figure 1.

Detection frequency for enterococcal bacteria, bacterial 16S DNA, and enteric virus, bacteria, protozoan, or helminth pathogens in (A) soil and (B) surface water from Kisumu neighborhoods.

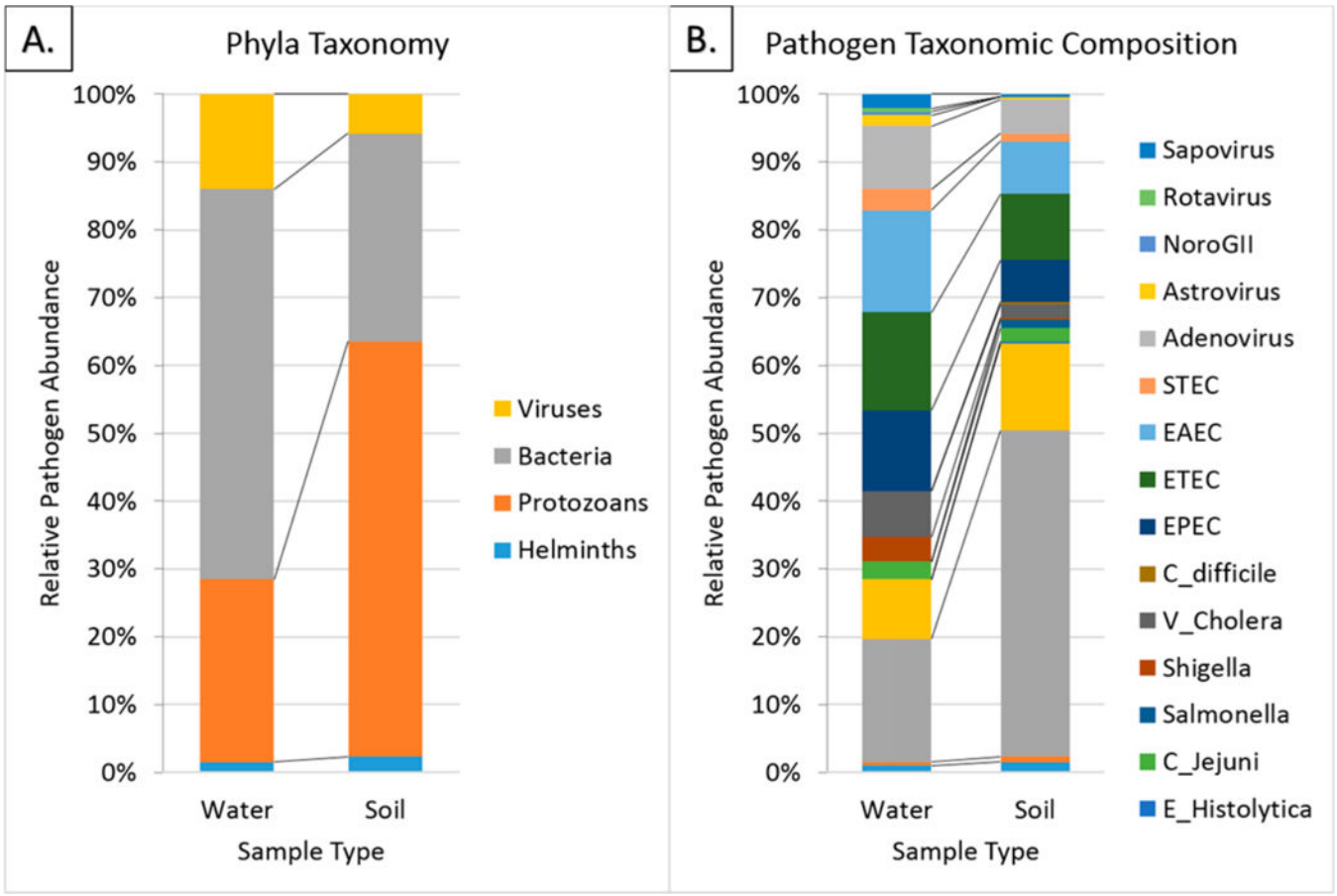

Figure 2.

Relative abundance of pathogen gene copies for (A) phylogenetic groups and (B) categories of enteric pathogens in soil and surface water from Kisumu neighborhoods.

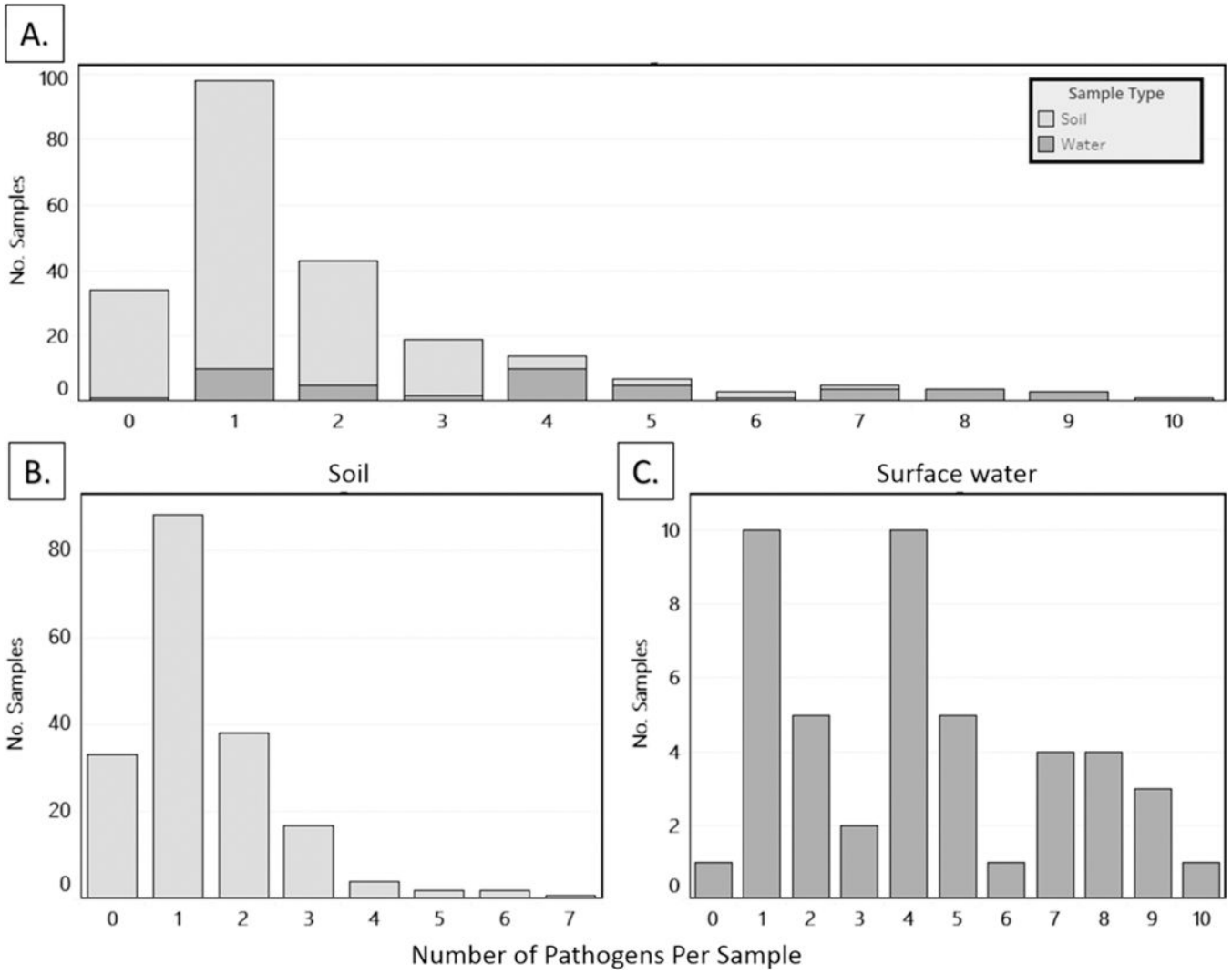

Figure 3.

Histogram of diversity in enteric pathogens detected in (A) soil and surface water combined, (B) soil, and (C) surface water samples from Kisumu neighborhoods.

Table 1.

Ecological and Sanitation Characteristics of Public Domains of Three Kisumu Neighborhoods[a]

observed sanitary indicators	Nya-A, *N* = 55	Nya-B, *N* = 53	Obu, *N* = 58	total, *N* = 166
Ecological Factors				
landscape developed with housing, *n* (%)	40 (72.7)	39 (73.6)	45 (77.6)	124 (74.7)
landscape with open lots/fields, *n* (%)	15 (27.3)	14 (26.4)	13 (22.4)	42 (75.3)
relative humidity, % (SD)	61.6 (3.1)	60.1 (0.4)	62.8 (1.1)	61.6 (2.2)
altitude, m (SD)	1147.3 (3.9)	1145.8 (4.7)	1162.1 (8.8)	1152 (9.7)
temperature, °C (SD)	24.4 (1.7)	23.4 (1.1)	22.1 (0.99)	23.3 (1.4)
surface water present, n (%)	15 (27.3)	11 (20.8)	14 (26.4)	40 (24.1)
Potential Fecal Sources				
human/animal feces on ground, *n* (%)	37 (67.3)	36 (67.9)	44 (75.9)	117 (70.5)
human open defecation indicators, *n* (%)	13 (23.6)	19 (35.8)	15 (25.9)	47 (28.3)
domestic animals present, *n* (%)	41 (75.6)	42 (79.3)	45 (77.6)	128 (77.1)
pig	2 (3.6)	2 (3.8)	2 (3.5)	6 (3.6)
chicken	22 (39.3)	23 (43.4)	34 (58.6)	79 (47.3)
cattle	8 (14.3)	11 (20.8)	12 (20.7)	31 (18.6)
dog	9 (16.1)	13 (24.5)	6 (10.3)	28 (16.8)
sheep/goat	5 (8.9)	12 (22.6)	13 (22.4)	30 (17.9)
flies present, *n* (%)	51 (96.2)[b]	50 (94.3)	56 (96.6)	157 (95.7)[b]
shared or public latrine on-site, *n* (%)	45 (81.8)	44 (83)	39 (67.2)	130 (78.3)
functional	0 (0)	9 (17.0)	0 (0)	9 (5.4)
dilapidated	44 (100)	36 (67.9)	39 (100)	121 (72.9)
children under 5 present, *n* (%)	24 (43.6)	17 (32.1)	31 (53.5)	72 (43.4)

[a]Total of 145 single sample sites and 21 multisample (more than one soil sample) sites. Nya-A, Nyalenda A neighborhood; Nya-B, Nyalenda Bneighborhood; Obu, Obunga neighborhood.

[b]Denominator = 163.

Table 2.

Association between Human Sanitation and Animal Feces Sources and the Presence and Concentration of Enterococci in Soil from Kisumu Neighborhood Public Domains[a]

	(A) Presence of Enterococci			(B) Log_{10} Concentration of Enterococci			
exposure	**enterococci present, % (*n* positive/N)**	**unadj OR (95% CI)**	**adj OR (95% CI)**	**exposure**	**mean log_{10} concn (SD)**	**unadj RR (95% CI)**	**adj RR (95% CI)**
observed feces on ground	80.7 (125/155)	2.67 (0.41, 17.44)	2.42 (0.62, 9.45)	observed feces on ground	2.5 (2.0)	1.65 (0.92, 2.96)	1.25 (0.66, 2.37)
no feces on ground	76.1 (54/71)	ref	ref	no feces on ground	2.4 (2.1)	ref	ref
observed open defecation indicators	71.0 (44/62)	1.42 (0.31, 6.55)[b]	0.86 (0.22, 3.28)	observed open defecation indicators	2.1 (2.0)	1.63 (0.87, 3.05)[b]	1.18 (0.60, 2.33)
no open defecation indicators	82.3 (135/164)	ref	ref	no open defecation indicators	2.6 (2.0)	ref	ref
domestic animals	83.0 (146/176)	2.88 (0.35, 24.03)	3.42 (0.69, 17.03)	domestic animals	2.6 (2.0)	1.82 (0.86, 3.85)	1.50 (0.63, 3.56)
no domestic animals	66.0 (33/50)	ref	ref	no domestic animals	2.0 (2.1)	ref	ref
flies	79.9 (17½14)	1.73 (0.11, 27.31)	0.72 (0.05, 11.39)	flies	2.5 (2.0)	3.02 (1.39, 6.56)	1.88 (0.74, 4.76)
no flies	57.1 (4/7)	ref	ref	no flies	0.6 (0.9)	ref	ref
latrine condition: none	71.1 (32/45)	ref	ref	latrine condition: none	1.9 (2.0)	ref	ref
latrine condition: good	66.7 (8/12)	0.78 (0.10, 5.85)	0.59 (0.05, 7.40)	latrine condition: good	1.2 (1.6)	0.67 (0.23, 1.94)	0.67 (0.22, 2.09)
latrine condition: bad	82.5 (139/169)	1.07 (0.19, 6.03)	1.03 (0.19, 5.56)	latrine condition: bad	2.7 (2.0)	1.50 (0.70, 3.22)	1.31 (0.57, 3.01)

[a] All models are adjusted for landscape features, relative humidity, altitude, and ambient temperature. Adjusted models also include all five exposure variables at the same time. SD, standard deviation; OR, odds ratio; RR, risk ratio; CI, confidence interval; ref, reference value. Surface water is not included because all samples were positive. Statistical significance ($P < 0.05$) is indicated by boldface type.

[b] The unadjusted OR and RR for this model reflect significant confounding by an ecological variable. Bivariate association for open defecation indicators and presence of enterococci is unadj OR = 0.50 (95% CI 0.19, 1.34), and the bivariate model for open defecation indicators and log_{10} concentration of enterococci is unadj RR = 0.92 (95% CI 0.48, 1.76).

Table 3.

Association between Human Sanitation and Animal Feces Sources and Presence and Diversity in Enteric Pathogens from Kisumu Neighborhood Public Domains[a]

	(A) Presence of Any Pathogen				(B) Pathogen Diversity		
exposure	**any pathogen, % (*n* positive/N)**	**inadj OR (95% CI)**	**adj OR (95% CI)**	**exposure**	**mean diversity (SD)**	**unadj RR (95% CI)**	**adj RR (95% CI)**
observed feces on ground	83.9 (130/155)	1.41 (0.41, 4.87)	1.01 (0.25, 4.04)	observed feces on ground	1.9 (1.9)	1.04 (0.80, 1.34)	1.0 (0.75, 1.33)
no feces on ground	83.1 (59/71)	ref	1.02 ref	no feces on ground	1.8 (2.0)	ref	2.0 ref
observed open defecation indicators	82.3 (51/62)	1.46 (0.41, 5.19)	0.98 (0.22, 4.52)	observed open defecation indicators	1.4 (1.5)	0.95 (0.70, 1.29)	0.88 (0.63, 1.22)
no open defecation indicators	84.2 (138/164)	ref	ref	no open defecation indicators	2.0 (2.1)	ref	ref
domestic animals	85.3 (151/177)	1.60 (0.43, 5.91)	1.53 (0.28, 8.54)	domestic animals	2.0 (2.1)	**1.39 (1.03, 1.89)**	**1.44 (1.01, 2.06)**
no domestic animals	77.6% (38/49)	ref	ref	no domestic animals	1.3 (1.2)	ref	ref
flies	84.7 (182/215)	17.1 (0.70, 418.0)	5.15 (0.20, 130.1)	flies	1.9 (2.0)	2.35 (1.00, 5.56)	2.00 (0.84, 4.79)
no flies	42.9 (3/7)	ref	ref	no flies	0.6 (0.8)	ref	ref
latrine condition: none	84.4 (38/45)	ref	ref	latrine condition: none	1.5 (1.7)	ref	ref
latrine condition: good	66.7 (8/12)	0.16 (0.01, 2.72)	0.70 (0.10, 4.99)	latrine condition: good	0.8 (0.8)	0.77 (0.39, 1.51)	0.78 (0.40, 1.53)
latrine condition: bad	84.6 (143/169)	0.78 (0.17, 3.54)	0.98 (0.21, 4.52)	latrine condition: bad	2.0 (2.0)	1.12 (0.79, 1.58)	1.08 (0.74, 1.57)

[a]Adjusted for landscape features, relative humidity, altitude, and ambient temperature. SD, standard deviation; OR, odds ratio; RR, risk ratio; CI,confidence interval; ref, reference value. Statistical significance ($P < 0.05$) is indicated by boldface type.