Environmental and behavioural exposure pathways associated with diarrhoea and enteric pathogen detection in 5-month-old, periurban Kenyan infants: a cross-sectional study

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ABSTRACT

Objectives The aim of this study was to test whether household environmental hygiene and behavioural conditions moderated associations between diarrhoea and enteric pathogen detection in infants 5 months of age in Kenya and pathogen sources, including latrine access, domestic animal co-habitation and public food sources.

Design Cross-sectional study utilising enrolment survey data of households participating in the Safe Start cluster-randomised controlled trial.

Setting Kisumu, Kenya.

Participants A total of 898 caregivers with 5-month (22 week ± 1 week) aged infants were enrolled in the study and completed the enrolment survey.

Primary and secondary outcome measures Outcomes were (1) caregiver-reported 7-day diarrhoea prevalence and (2) count of types of enteric viruses, bacteria and parasites in infant stool. Exposures and effect modifiers included water access and treatment, cohabitation with domestic animals, sanitation access, handwashing practices, supplemental feeding, access to refrigeration and flooring.

Results Reported handwashing after handling animals (adjusted odds ratio (aOR)=0.20; 95% CI=0.06 to 0.50) and before eating (aOR=0.44; 95% CI=0.26 to 0.73) were strongly associated with lower risk of caregiver-reported diarrhoea, while cohabitation with animals (aOR=1.54; 95% CI=1.01 to 2.34) living in a household with vinyl-covered dirt floors (aOR=0.60; 95% CI=0.45 to 0.87) were strongly associated with pathogen codetection in infants. Caregiver handwashing after child (p=0.02) or self-defaecalation (p=0.03) moderated the relationship between shared sanitation access and infant exposure to pathogens, specifically private latrine access was protective against pathogen exposure of infants in households, where caregivers washed hands after defaecation. In the absence of handwashing, access to private sanitation posed no benefits over shared latrines for protecting infants from exposure.

Conclusion Our evidence highlights eliminating animal cohabitation and improving flooring, postdefaecation and food-related handwashing, and safety and use of cow milk sources as interventions to prevent enteric pathogen exposure of young infants in Kenya.

STRENGTHS AND LIMITATIONS OF THIS STUDY

We rely on objective molecular-based outcome measures of infant exposure to enteric pathogens, a large sample size and documentation of many household environmental and behavioural living conditions.

We explicitly analyse household environmental and behavioural living conditions as intermediate factors that can modify the transmission of enteric pathogens between sources of human and animal in the household and infants.

We included supplemental foods given to the infants within the last 24 hours as a potential transmission pathway.

Our 7-day self-reported diarrhoea and pathogen detection exposure outcomes occurred in recent time prior to observation of household living conditions, which limits causal inference.

Our statistical power to examine effect modification hypotheses was limited in cases where living conditions varied little across the population, for example, primary water sources.

INTRODUCTION

More than a third of all children in low-income and middle-income countries experience infections by one or more pathogens within the first year of life.1 2 Longitudinal studies of enteric pathogen prevalence show that the proportion of children in low-income communities shedding pathogens climbs rapidly after birth and is sustained through at least 24 months of age.2 3 One enteric infection episode can increase susceptibility to re-infection or co-infection through...
pathogen–pathogen interactions in the gut, downregulation of protective immune or microbiome responses and enteric enteropathy of intestinal tissue. Infants who experience early onset of enteric infections (eg, before 6 months of age) may be more vulnerable to enteropathy and malnutrition conditions that increase enteric infection incidence rates.

Multiple inter-related environmental exposure pathways can contribute to pathogen transmission. Literature identifying targeted interventions that prevent exposure of infants in the first months of life, beyond breast feeding, is scarce. Evidence on impact of improving household safe drinking water sources and/or treatment and storage, access to basic latrines and handwashing with soap on overall enteric pathogen detection and diarrhoea in children under 2 years of age is mixed, with some rigorously controlled and well-powered trials of interventions reporting little impact, even with high levels of behavioural compliance. One explanation for the trial results is that interventions did not target the most important conditions resulting in pathogen transmission. Most handwashing interventions focus on infant caregivers, not on <5 year siblings and playmates. Infant food safety depends on handwashing, but also hygienic conditions of the food preparation and feeding environment and safety of ready-to-eat roadside and packaged foods. The presence of rodents, flies and domestic animals in the immediate household or compound can contaminate soil and surfaces in areas where infants dwell with faeces containing pathogens, and those pathogens could persist longer in the environment if household flooring is dirt or another type of permeable material. Similarly, the presence of older siblings who could transmit pathogens through child–child interaction or open defecation on floors could contaminate surfaces. The benefits of maintaining hygiene conditions in one’s own household can be derailed if hygiene conditions of communal compound living spaces and infrastructure (eg, shared latrines) are poor. Access to a latrine within a compound is not a guarantee that all compound residents will consistently use it for personal defecation or child faeces disposal. Sharing latrines is an established risk factor for childhood diarrhoea. At a community scale, hygiene of one’s household does not protect infants in daycare or heavily polluted community settings, or if pollution from the community is blown, flooded, or transported by feet into compound grounds.

The goal of this study was to test for effect modification by intermediate household environmental (eg, flooring, refrigeration) and behavioural (eg, handwashing, water treatment) conditions in the relationship between human and animal faecal contamination sources and pathogen exposure of 5-month-old infants in Kisumu, Kenya. We utilised enrolment data for infants in the Safe Start Trial, a cluster-randomised trial of a food hygiene behaviour change intervention to estimate associations between water, animal, sanitation, hygiene, food and flooring conditions with caregiver-reported 7-day diarrhoea prevalence in infants, and with count of enteric pathogen types in infant stool as determined by molecular diagnostic assays. We then conducted a moderation analysis to examine hypotheses of proposed environmental conditions and human behaviours that could influence the size and strength of association between faecal sources and enteric pathogens in infants.

**METHODS**

**Study setting/ethics**

This cross-sectional study uses baseline data collected from 5-month-old (22 weeks ± 1 week) infants and their caregivers at the point of enrolment into Safe Start cluster-randomised controlled trial of an infant food hygiene behaviour change intervention in Kisumu, Kenya (Clinical Trials identifier: NCT03468114, Pre-results stage as of 18 Oct. 2022). The formative work and trial protocol, including the estimation of sample size for evaluating trial impact, are described elsewhere. Kisumu is a city of approximately 490,000 people (Kisumu county integrated development plan 2013–2017) in the western region of Kenya. The study site includes communities in two low-income periurban neighbourhoods in Kisumu. The study was approved by the scientific and ethical review committees at Great Lakes University of Kisumu (Ref. No. GREC/010/248/2016), London School of Hygiene and Tropical Medicine (Ref. No. 14695) and University of Iowa (IRB ID 201804204).

**Study design and participants**

Caregivers with 5-month-old infants living in the catchment area of participating Community Health Volunteers (CHVs) participated in a survey and provided a sample of infant stool for microbial analysis at enrolment into the trial. We defined eligibility of an infant as being 22 weeks (±1 week) of age, as verified by birth registration card, who resided in one of the two study neighbourhoods. We enrolled caregivers who were responsible for care of the infant during the day and were at least 18 years of age.

**Patient and public Involvement**

Study participants and CHVs provided input on the goals, design and implementation of the Safe Start Study through prestudy knowledge dissemination meetings and formative research.

**Outcomes**

The outcomes for this study are: (1) 7-day caregiver-reported diarrhoea prevalence prior to enrolment, where diarrhoea is defined as three or more loose, watery stools in the previous 24 hours; and (2) the sum count of enteric pathogens detected in infant stool.

**Data and sample collection**

The study was described in the caregiver’s natural language, and a signed copy of the consent form was left for their records. On verification of eligibility and consent, caregivers were interviewed to collect data...
about household socioeconomic conditions, access to water and sanitation infrastructure, animal ownership and hygiene practices. In anticipation that infant breast feeding and feeding practices may vary day to day and may be subject to response bias or recall bias, we asked caregivers about overall dietary history and foods given to infants in the last day. Caregivers were then provided several sterile commercially produced diapers and a sterile Ziploc bag, and asked to use these until the infant defecated. Diapers prevented cross-contamination via, for example, caregivers collecting infant faeces with dirt attached from the ground. Caregivers were instructed to fold the diaper, place it into the storage bag and store it in a cool dark place out of the reach of children and animals. The research team returned within 24 hours to collect the diaper, placed it in a cooler on ice packs and transported it to the laboratory within 5 hours of collection from the household. If the infant did not defecate in the first 24 hours, the team returned each day up to 5 days after enrolment to assess whether the infant had defecated. If no faeces could be collected, the infant was de-enrolled from the study.

**Nucleic acid extraction**

Lab technicians unwrapped diapers in biosafety cabinets and used sterile stool collection scoops to transfer 200 mg of stool into Zymobiomics Shield Collection tubes, which were vortexed on a bead beater for 20 min and then processed according to the manufacturer’s instructions for the ZymoBIOMICS DNA/RNA extraction mini-kit (Zymo Research, Irvine, California, USA). One molecular-grade water only sample was prepared each day of sample processing as a process contamination control. Approximately half (n=383) of samples were spiked with 3 µL of 1.8 × 10⁶ CFU/µL of live bacteriophage MS2 to serve as a process control to assess for inhibition and efficiency in DNA and RNA recovery. Samples were transported on dry ice to the University of Iowa and stored at −80°C until analysis.

**TaqMan Array card analysis**

A total of 23 gene targets of pathogen of interest in the TaqMan assays were used to assess pathogen presence in infants. Pathogen gene targets were Adenovirus 40–41 Fibre, Adenovirus broad species Hexon, Rotavirus NSP3, Norovirus GI ORF 1–2, Norovirus GII ORF 1–2, Aeromonas aerolysin toxin aerA, Campylobacter jejuni/C. coli (cadB), Enterohemorrhagic Escherichia coli (EHEC) 0157:rbd, Enteraggrevative E. coli (EAEC) aatA and aaiC, Enteropathogenic E. coli (EPEC) bfpA and eae, Enterotoxigenic E. coli (ETEC) elt and est, Clostridoides difficile tcdB, Salmonella enterica, Shigella spp virG, Vibrio cholerae hlyA, Giardia duodenalis Assemblage A triosephosphate isomerase (TPI), Giardia duodenalis Assemblage A triosephosphate isomerase (TPI), Cryptosporidium spp 18S, C. hominis LIB13 and C. parvum LIB13.26,27 For each sample, 40 µL of extract was mixed with 5 µL of nucleic acid-free water, 50 µL of 2X RT-buffer, 0.6 µL of 50 µL/µL bovine serum albumin (to reduce inhibition) and 4 µL of 25X AgPath enzyme from the AgPath-ID One-Step Reverse Transcription-Polymerase Chain Reaction (RT-PCR) kit (Thermo Fisher, Waltham, Massachusetts, USA) and pipetted into a well on a compartmentalised TaqMan card that included primer and probe assays in duplicate for each gene. TaqMan assays were completed in either a ViiA7 or QuantStudio8 instrument (Thermo Fisher, Waltham, Massachusetts, USA) for cycling conditions: 45°C for 20 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A subset of samples that included both low and high Cq results were analysed on both machines and compared to confirm that results did not vary between machines, before proceeding with further use of both machines. We defined a sample as positive if a gene target amplified within a 35 Cq threshold (Cq35). If multiple gene targets were used to detect one type of pathogen, amplification of either gene (EAEC aatC/aatA, EPEC bfpA/eae, ETEC elt/est) was considered positive for the general type of pathogen. The pathogen-specific detection patterns used to define the pathogen count variable for this analysis are described elsewhere.28

**Data analysis**

Independent variables representing point sources of faeces that could contaminate the environment with enteric pathogens included household latrine design and location of the latrine, sharing of a latrine, ownership of domestic animals, whether animals are typically kept inside the household and observation of rodents or their faeces in the household. Improved sanitation was defined as a flush, pour flush, ventilated pit latrine or pit latrine with an impermeable slab, according to WHO/UNICEF JMP criteria.29 Although they do not represent point sources of faeces, food ingredients and especially animal-based ones, as well as community drinking water sources could be sources of pathogens that originate outside the household. Thus, primary and secondary water sources and whether the primary water source is intermittent, and feeding the infant solid or liquid (other than breastmilk) foods were also defined as pathogen sources. A basic water source was defined as a piped tap to household or compound, a public tap, tube well, borehole, protected spring, protected hand dug well or rainwater, according to WHO/UNICEF Joint Monitoring Programme criteria, available within 30 min round trip.28 Independent variables representing intermediate environmental conditions or behaviours that could modify pathogen transmission pathways and prevent exposure included household flooring (soil/surfaces), treating drinking water after collection, prior and recent (in the last day) breastfeeding status, presence of a handwashing station with soap and water and self-reported handwashing at critical times. Three of these critical handwashing times are behaviours that could modify hand cleanliness after touching human faeces or animals, while
three were focused specifically on modification of food sources being prepared within the household.

Potential confounders included in this analysis are marital status of caregiver, maternal education, household wealth, presence of multiple children under 5years of age, infant preterm birth status, rotavirus vaccination status and prior or current breastfeeding practices. Principal components analysis with Promax rotation of 15 household assets (bicycle, motorbike, car, refrigerator, mobile phone, wrist or pocket watch, wall clock, radio, cassette or CD player, television, DVD player, microwave oven, presence of grates on the windows and doors, use of electricity for lighting and use of propane or electricity for cooking) resulted in a household wealth variable which was stratified into five quintiles.

All analysis were conducted with R software V.4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Associations between exposures and the binary indicator for 7-day caregiver-reported diarrhoea were evaluated using logistic regression. Bivariate associations were evaluated between each exposure and self-reported diarrhoea, and fully adjusted relationships between the exposures and the outcome was evaluated by running a single model with the confounder variables listed above and all exposure variables included. Associations between exposures and the ordinal categorical pathogen count variable were estimated using ordinal logistic regression. As with caregiver-reported diarrhoea, we evaluated bivariate and fully adjusted relationships.

In both fully adjusted models, multicollinearity among the exposure variables resulted in non-identifiable or nearly-non-identifiable model, resulting in the exclusion of some redundant exposure variables. In addition, some confounder variables had insufficient variability to have estimable effects and hence were removed. Finally, caregivers reporting a lack of breast feeding were so rare that this variable was excluded from the data. Logistic regression results are reported as ORs, with 95% CIs, for having diarrhoea, and ordinal logistic regression results are reported as ORs for having a higher count of pathogen types in stool versus fewer pathogen types. A random effect was initially included in models to adjust for spatial clustering, but was removed due to lack of variation in outcomes between villages.

Our moderation analyses tested whether hygiene of intermediate exposure pathways modified the relationships between point sources of pathogen contamination and infant health outcomes.

Hypothesis 1
Lack of access to a latrine with a barrier between users and excreta, and/or sharing unclean latrines with others can result in infant exposure to pathogens in human faeces through faeces being tracked by feet onto household floors, where infants play and place objects or hands that have been on the floor in their mouth. We hypothesise that floor type moderates the effect of latrine access on pathogens in infants such that permeable dirt floors that absorb liquids and sustain microbial growth, and that are harder to clean and disinfect, increase the association between human sanitation and pathogens in children compared with impermeable floors, like vinyl, concrete, or tile.

Hypothesis 2
Lack of or sharing latrine access can result in infant exposure to pathogens when caregivers do not wash hands after self-defecation or cleaning a child and then place hands in the infant’s mouth. We hypothesise that handwashing after defecation or child defecation moderates the effect of latrine access on infant health, such that human sanitation will be associated with pathogens in children among caregivers not washing hands after self or child defecation but not among caregivers who wash hands after self/child defecation.

Hypothesis 3
Like with latrines, an association between domestic animals or rodents and enteric infections in children could be caused by exposure of infants to floors contaminated with animal faeces. We hypothesise that floor type can moderate this risk such that a dirt floor increases the association between domestic animals kept in or near the household or the presence of rodents and pathogens in children compared with households with impermeable floors.

Hypothesis 4
Zoonotic transmission of pathogens to infants could also occur through hands of caregivers who touch domestic animals or their faeces, and then place hands in the infant mouth. We hypothesise that washing hands after handling animals moderates the effect of animal or rodent presence in the household on pathogens in children such that caregivers not washing hands after handling animals increases the association between animals or rodents and pathogens in infants compared with caregivers who wash hands after touching animals.

Hypothesis 5
Animal or human faeces contamination on hands can be introduced into infant food during preparation or feeding and ingested by the infant while handwashing after defecation and animal handling could prevent transmission. We hypothesise that food-related handwashing also moderates the association between faeces sources (latrine access and the presence of animals or rodents in the household) and pathogens in infants such that caregivers (1) washing hands before preparing food, (2) washing hands before eating or (3) washing hands before feeding the infant decreases the association between latrines, domestic animals and rodents with pathogens in infants, compared with caregivers who do not wash hands at these times.
Hypothesis 6
Infant supplemental foods include street foods prepared by vendors, raw fruits and prepackaged commercial products (eg, pasteurised milk) that could contain contamination. Cooking (eg, porridge), washing (eg, fruit) or storing these foods can mitigate or enhance these external food system-based pathogen transmission risks.31
We hypothesise that access to a refrigerator for food storage moderates the pathway between supplemental foods and pathogens in infants such that a lack of refrigeration increases the association between supplemental food and pathogens in infants compared with households with refrigeration.

Hypothesis 7
Similarly, reliance on unsafe water sources can increase the chances of pathogen infection through water, but water treatment can reduce or eliminate this contamination. We hypothesise that filtering, boiling or chlorinating water after collection moderates the association between type of water source and pathogens in infants such that not treating drinking water increases the association between household drinking water source and pathogens in infants compared with households who treat their drinking water.

These hypotheses were tested one at a time by adding an interaction term to the fully adjusted pathogen count model; the large number of potential confounders and exposure variables prohibited the simultaneous inclusion of all interaction terms being estimable. For each hypothesis listed above we ran an ordinal logistic regression model of the form

\[
\text{Pathogens} = \text{Confounder variables} + \text{Exposure variables} + \text{Hypothesis} - \text{specific interaction term.}
\]

Moderation effects were then tested using a likelihood ratio test comparing the additive model excluding the interaction term to the full model including the interaction term.

RESULTS
Characteristics of study participants
A total of 898 infant–caregiver pairs were enrolled in the study and completed the enrolment survey. The majority of caregivers were married, with secondary-level education, and had multiple young children (table 1). The preterm birth rate was high at 16% of infants. Most infants had received two doses of oral rotavirus vaccine, as verified by infant health registration cards. Access to a basic drinking water source and a latrine of improved design was common, although a third of caretakers used secondary water sources and nearly all households shared their latrine with multiple other families. Most households had cleanable (ceramic, tile, concrete or wood) floors or covered a dirt floor with rugs or vinyl. Of the 12.8% of households who owned animals, 82.5% (10.5% of all caregivers) kept their animals inside the household.

Table 1 Characteristics of caregivers and their 5-month-old infants enrolled into the Safe Start Study in Kisumu, Kenya between 2018 and 2019

<table>
<thead>
<tr>
<th>Socio-economic, environmental, and behavioural living conditions</th>
<th>% (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caregiver is single vs Married</td>
<td>11.8 (105)</td>
</tr>
<tr>
<td>Caregiver has primary or no education vs secondary or higher education</td>
<td>39.1 (348)</td>
</tr>
<tr>
<td>Household wealth Quintile</td>
<td></td>
</tr>
<tr>
<td>Poorest</td>
<td>19.6 (174)</td>
</tr>
<tr>
<td>Low-middle</td>
<td>19.7 (175)</td>
</tr>
<tr>
<td>Middle</td>
<td>20.7 (184)</td>
</tr>
<tr>
<td>Upper-middle</td>
<td>20.0 (178)</td>
</tr>
<tr>
<td>Highest quintile</td>
<td>20.0 (178)</td>
</tr>
<tr>
<td>More than one child under 5 years lives in household</td>
<td>35.6 (320)</td>
</tr>
<tr>
<td>Infant was born preterm (&lt;37 weeks gestational age) vs not preterm (&gt;=37 gestational weeks age)</td>
<td>16.0 (140)</td>
</tr>
<tr>
<td>Rotavirus vaccination series*</td>
<td></td>
</tr>
<tr>
<td>No vaccination</td>
<td>1.2 (10)</td>
</tr>
<tr>
<td>One dose</td>
<td>1.6 (13)</td>
</tr>
<tr>
<td>Both doses</td>
<td>97.2 (803)</td>
</tr>
<tr>
<td>Infant ever breastfed vs never breastfed</td>
<td>99.8 (885)</td>
</tr>
<tr>
<td>Infant still breastfed vs weaned</td>
<td>99.7 (884)</td>
</tr>
<tr>
<td>Breastfed in the last day vs not breastfed in last day</td>
<td>57.5 (516)</td>
</tr>
<tr>
<td>Exposure variables</td>
<td></td>
</tr>
<tr>
<td>Household relies on Improved latrine vs unimproved latrine or open defecation†</td>
<td>86.6 (770)</td>
</tr>
<tr>
<td>Sharing latrine</td>
<td></td>
</tr>
<tr>
<td>Private household latrine</td>
<td>10.9 (98)</td>
</tr>
<tr>
<td>1–5 households share</td>
<td>17.7 (159)</td>
</tr>
<tr>
<td>&gt;5 households Share latrine</td>
<td>71.4 (641)</td>
</tr>
<tr>
<td>Household owns animals vs does not own animals</td>
<td>12.8 (114)</td>
</tr>
<tr>
<td>Animals sleep inside household vs do not sleep in household</td>
<td>10.5 (94)</td>
</tr>
<tr>
<td>Rodents or rodent droppings observed vs no droppings</td>
<td>69.7 (620)</td>
</tr>
<tr>
<td>Live rodent, n=500</td>
<td></td>
</tr>
<tr>
<td>Droppings, n=93</td>
<td></td>
</tr>
<tr>
<td>Rodent tracks, n=25</td>
<td></td>
</tr>
<tr>
<td>Other, n=1</td>
<td></td>
</tr>
<tr>
<td>None, n=269</td>
<td></td>
</tr>
<tr>
<td>Household Flooring</td>
<td></td>
</tr>
<tr>
<td>Unfinished dirt floor</td>
<td>8.4 (75)</td>
</tr>
<tr>
<td>Unfinished floor covered with vinyl sheets or carpet</td>
<td>23.2 (206)</td>
</tr>
<tr>
<td>Finished floor (ceramic, concrete, tile, wood)</td>
<td>68.4 (608)</td>
</tr>
</tbody>
</table>

Continued
Of the domestic species owned by households, chickens (73 of 89 households) and cats (45 of 49) represented the vast majority of animals kept in the household, although many other households kept animals in the compound yard (online supplemental table 1). Almost all caregivers reported that the infant was still breast fed, but only half reported giving the infant breastmilk in the last 24 hours.

Almost a third of infants consumed cow or goat milk by 5 months of age, with Long Life ultra-high temperature (UHT) pasteurised milk being the most common supplemental food. In the last day prior to the interview, a fifth of infants were given water and a third cow’s milk, with UHT being the most common. Only ~5% of caregivers reported feeding infants solid foods. While 332 caregivers indicated they had a handwashing station in the household, only 74 of these had water present at the time of observation and only 59 (6.6% of all caregivers) had soap and water. Yet handwashing at critical times was reported by over 60% of caregivers for after self-defecation or infant defecation, and for preparing food, feeding the infant and personal eating. In contrast, just 12.0% reported washing hands after handling animals.

Variables excluded from multivariable exposure models due to low variation in subgroups included rotavirus vaccination, infant ever breast fed, infant still breast fed, primary drinking water source, infant has been fed solid food before and ever feeding the infant local milk or other milk type ever (UHT and fresh packed milk were only types with sufficient data). Although nearly all caregivers reported prior or ongoing breast feeding of the infant, only 57.5% of caregivers reported breast feeding the infant within the last day, so this variable was used to control for confounding of exposure–outcome relationships by breast feeding. Variables excluded due to collinearity included animals kept inside the household (strongly predicted by animal ownership) and feeding the infant milk in the last day (proxy for history of milk feeding).

### Exposure pathways and 7-day caregiver-reported diarrhoea history

A total of 862 out of 898 caregivers interviewed at enrolment had complete data for all exposure variables of interest and self-reported 7-day diarrhoea in infants. There were no discernible differences between individuals with and without full exposure data. Diarrhoea prevalence among 5-month-old infants in Kisumu at enrolment was 14.9% (154). Associations with potential confounders are reported in online supplemental table 2. In the full multivariable model, washing hands after handling animals and before eating were strongly associated with a fivefold and 2.27-fold lower odds of reported diarrhoea, respectively (table 2). In bivariate models, use of improved latrine was also protective against diarrhoea, but this relationship was weakened after adjusting for other wash conditions. Odds of diarrhoea were higher for infants fed UHT and fresh packed milk, but these relationships were weakened after adjusting for other exposure pathways.

### Exposure pathways and enteric pathogens in infants

Data on both the exposures of interest and pathogen detection outcome were available for 773 infants. Genes for at least one of 23 assessed enteric pathogens were detected in stool in 88.9% (n=707) of infants, with two
or more pathogens detected in 66.9% (n=532) of infants (median: 2 (25%–75%, IQR: 1–3)), based on a Cq35 threshold. The ordinal categorical model outcome variable was categorised as one of 0, 1, 2, 3, 4, 5 or ≥6 types of pathogens detected, due to scarce data for higher pathogen counts. Associations with confounders are reported in online supplemental table 3. In the multivariable ordinal regression model, living in a household with a vinyl-covered dirt floor was associated with a 1.67-fold lower chance of having more pathogen types detected in infant stool compared with infants in households with cement or tile floors (table 3). Infants fed any type of milk had a 1.42-fold to 2.32-fold increased chance of a higher count in pathogen diversity in infant stool versus infants never given milk. Keeping animals in the house posed a 1.54-fold higher odds of higher pathogen count compared with not keeping animals, while washing hands after handling animals was counterintuitively associated with a 1.52-fold increase in odds compared with not washing hands after handling animals.

**Moderation of pathogen exposure pathways**

After adjusting for confounders, moderation hypothesis tests for exposures and pathogen infections revealed:

1. There was no evidence that floor type modified the relationship between basic latrine access (p=0.93) or latrine sharing (p=0.49) and pathogen count in infants.
2. There was no evidence that handwashing after personal defecation (p=0.91) or after cleaning a child (p=0.96) modified the relationship between basic latrine access and pathogen count. There was moderate evidence for the hypothesis that handwashing after self-defecation (p=0.03) or after child defecation (p=0.02) modified the relationship between latrine sharing and pathogen count.

**Table 2** ORs and CIs from logistic regression models of caregiver-reported 7-day diarrhoea in 5-month infants and potential faeces sources and exposure pathways

<table>
<thead>
<tr>
<th>Exposure pathways</th>
<th>Bivariate OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved latrine vs unimproved latrine/open defecation</td>
<td>0.52 (0.32 to 0.84)</td>
<td>0.66 (0.38 to 1.17)</td>
</tr>
<tr>
<td>Sharing latrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private household latrine</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>1–5 Households share latrine</td>
<td>1.64 (0.77 to 3.74)</td>
<td>1.71 (0.69 to 4.53)</td>
</tr>
<tr>
<td>&gt;5 Households share latrine</td>
<td>1.51 (0.84 to 3.39)</td>
<td>1.62 (0.70 to 4.10)</td>
</tr>
<tr>
<td>Owns animals vs do not own animals</td>
<td>1.23 (0.71 to 2.04)</td>
<td>–</td>
</tr>
<tr>
<td>Animals sleep inside household vs do not sleep in household</td>
<td>1.40 (0.79 to 2.39)</td>
<td>1.64 (0.84 to 3.08)</td>
</tr>
<tr>
<td>Rodents or rodent droppings present vs not present</td>
<td>1.46 (0.96 to 2.26)</td>
<td>1.14 (0.69 to 1.92)</td>
</tr>
<tr>
<td>Household flooring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfinished dirt floor</td>
<td>1.22 (0.62 to 2.25)</td>
<td>0.76 (0.35 to 1.55)</td>
</tr>
<tr>
<td>Covered unfinished floor</td>
<td>1.07 (0.68 to 1.65)</td>
<td>0.86 (0.52 to 1.39)</td>
</tr>
<tr>
<td>Finished floor</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Limited/unimproved secondary water source vs improved or no secondary source</td>
<td>0.69 (0.45 to 1.03)</td>
<td>0.65 (0.40 to 1.03)</td>
</tr>
<tr>
<td>Drinking water is not treated in the household vs water is treated</td>
<td>1.34 (0.93 to 1.92)</td>
<td>1.24 (0.82 to 1.88)</td>
</tr>
<tr>
<td>Infant fed water in the last day vs no water</td>
<td>1.51 (0.97 to 2.31)</td>
<td>1.23 (0.69 to 2.15)</td>
</tr>
<tr>
<td>Infant fed milk in the last day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UHT vs no UHT</td>
<td>2.08 (1.25 to 3.37)</td>
<td>–</td>
</tr>
<tr>
<td>Fresh packed vs no fresh packed milk</td>
<td>1.14 (0.26 to 3.52)</td>
<td>–</td>
</tr>
<tr>
<td>Local cow milk vs no local milk</td>
<td>0.95 (0.05 to 5.62)</td>
<td>–</td>
</tr>
<tr>
<td>Automated Milk Dispenser (ATM) vs not ATM milk</td>
<td>0.84 (0.25 to 2.19)</td>
<td>–</td>
</tr>
<tr>
<td>Milk bar vs no milk bar</td>
<td>1.77 (0.97 to 3.08)</td>
<td>–</td>
</tr>
<tr>
<td>Owns refrigerator for food storage vs no refrigerator</td>
<td>0.73 (0.40 to 1.26)</td>
<td>0.96 (0.44 to 2.00)</td>
</tr>
<tr>
<td>Handwashing area with soap and water vs no area or area with water only</td>
<td>0.89 (0.38 to 1.81)</td>
<td>1.24 (0.45 to 3.17)</td>
</tr>
<tr>
<td>Reported handwashing at critical times vs no handwashing at these times</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After cleaning an infant that defecated</td>
<td>0.78 (0.54 to 1.14)</td>
<td>1.13 (0.72 to 1.78)</td>
</tr>
<tr>
<td>After self-defecation</td>
<td>0.60 (0.37 to 1.02)</td>
<td>0.80 (0.45 to 1.46)</td>
</tr>
<tr>
<td>After handling animals</td>
<td>0.25 (0.09 to 0.56)</td>
<td>0.20 (0.06 to 0.50)</td>
</tr>
<tr>
<td>Before preparing food</td>
<td>0.77 (0.53 to 1.12)</td>
<td>1.33 (0.82 to 2.19)</td>
</tr>
<tr>
<td>Before feeding child</td>
<td>0.70 (0.48 to 1.01)</td>
<td>0.81 (0.51 to 1.28)</td>
</tr>
<tr>
<td>Before eating</td>
<td>0.51 (0.35 to 0.74)</td>
<td>0.44 (0.26 to 0.73)</td>
</tr>
</tbody>
</table>

For binary independent variables, the reference category is specified after ‘vs’. ‘–’ refers to a variable not estimated in multivariate model due to limited subgroup data or correlation with another exposure variable. Adjusted model includes independent variables of interest and confounders. Statistically significant results (p≤0.05) are in bold type. UHT, ultra-high temperature.
count (Figure 1A and B, respectively). Sharing a latrine with 2 to 5 (OR=1.35; 95% CI: 0.75 to 2.43) or >5 other households (OR=1.72; 95% CI: 0.99 to 2.98) was associated with higher pathogen count in infants relative to households with private latrines among caregivers who washed hands postdefecation. Sharing a latrine with 2 to 5 (OR=1.79; 95% CI: 0.92 to 3.51) or >5 households (OR=2.11; 95% CI: 1.13 to 3.94) versus private latrines was also associated with higher pathogen detection in infants among caregivers who washed hands after child defecation. However, pathogen count was lower in households sharing a latrine with 2 to 5 (OR=0.26; 95% CI: 0.04 to 1.62) or >5 other households (OR=0.18; 95% CI: 0.04 to 0.90) among caregivers who did not wash after defecation, or after child defecation (OR=0.47; 95% CI: 0.18 to 1.21; and OR=0.58; 95% CI: 0.30 to 1.10).
0.24 to 1.36, respectively). If sanitation is viewed as the effect modifier, then higher pathogen count was associated with not washing hands after defecation (OR=8.43; 95% CI: 1.71 to 41.56) or child defecation (OR=3.59; 95% CI: 1.35 to 9.51) in households with private latrines. The association between pathogen count and not washing hands after defecation or after child defecation was weaker in households sharing latrines with 2 to 5 (OR=2.18; 95% CI: 0.71 to 6.72 and OR=1.67; 95% CI: 0.78 to 3.57, respectively) or >5 (OR=1.54; 95% CI: 0.77 to 3.09, and 2.06; 95% CI: 1.07 to 3.97, respectively) other households.

3. There was no evidence that floor type modified the relationship between owning domestic animals, and by proxy keeping them in the household (p=0.60) and pathogen count in infants.

4. There was no evidence that handwashing after handling animals modified the relationship between keeping animals inside and pathogen count (p=0.28).

5. There was no evidence that washing hands before food preparation modified the association between pathogen count and latrine access (p=0.74), sharing a latrine (p=0.49), or animals sleeping inside (p=0.24). Similarly, there was no evidence that washing hands be-

Figure 1  Relationship between latrine sharing and pathogen count for handwashing after personal defecation and after child defecation (figure 1A and B, respectively). The OR estimate is indicated by a circle, with lines indicating the 95% CI in that estimate. The ORs shown in grey are from the main model with no interaction terms. The ORs in black are from the interaction model.
before feeding a child modified the association between pathogen count and latrine access ($p=0.54$), sharing a latrine ($p=0.61$) or owning animals ($p=0.94$). And, there was no evidence that washing hands before self-eating modified the association between pathogen count and latrine access ($p=0.15$), sharing a latrine ($p=0.47$), or owning animals ($p=0.63$).

6. There was no evidence that access to household refrigeration modified the effect of milk type on pathogen count ($p=0.17$).

7. We could not investigate modification of treating water on unsafe primary water sources and pathogens, but among the 99.8% of households using a basic water source, there was no evidence that treating water by chlorination or boiling affected pathogen count in children ($p=0.97$).

Figure 2 summarises the relationships identified between human sanitation and animal presence conditions that could introduce pathogens into the household, the housing conditions that can transmit pathogens, and higher versus lower pathogen count in infants.

**DISCUSSION**

The goal of this study was to identify risk factors linked to diarrhoea prevalence and enteric pathogen detection among young infants (<6 months old) in Kisumu, Kenya, and examine whether improved household hygiene environments (eg, flooring, refrigeration for safe food storage) and behaviours (eg, handwashing, water treatment) modified infant exposure to enteric pathogens from human and animal vectors. The very high prevalence...
of enteric pathogen detection and codetection in Kisumu infants at 5 months of age highlighted the critical need for exposure prevention interventions in early infancy, similar to other studies in low-income countries. Our evidence implicated handwashing after handling animals and before eating and living in a household with vinyl-covered dirt floors (vs finished floors) as strong factors reducing the risk of self-reported diarrhoea at 5 months of age, while owning and cohabitation with animals and feeding infants processed cow milk were associated with increased risk of exposure to pathogens. Additionally, our evidence suggested that the benefits of private sanitation are limited if post-defaecation handwashing is not practiced.

Latrine access was not a strong risk factor for diarrhoea or pathogens in infants after adjusting for other environmental and behavioural conditions, although sharing latrines was implicated in pathogen exposure when examined in the context of mitigating post-defaecation handwashing behaviours. The interaction between these two conditions was influenced by strong benefits from post-defaecation handwashing in households with private sanitation, and sustained pathogen exposure risks to infants from shared latrines, even when caregivers were washing hands after touching faeces. Infants usually have no direct contact with latrines designed for able-bodied children and adults, although shared latrines are a risk factor for moderate and severe diarrhoea in children across countries and studies. Pathogen transmission from shared latrines could occur through pathways not examined in this study, like fly density, flooding, or soil on shoes or feet moving between the latrine and the compound.

Household flooring type has been minimally explored in enteric disease literature, but finished flooring has been linked to decreased risk from diarrhoea in Egypt, and lower prevalence of Ascaris lumbricoides and soil-transmitted helminths in Kenya and Bangladesh in children under 5 years. In our study, we used household flooring construction as a proxy for floor hygiene, based on the premise that covered or finished floors are easier to clean and less absorbent for sustaining pathogens. Type of household flooring did not modify the relationship between shared sanitation and pathogens in infants in this study. In fact, we counterintuitively observed higher pathogen counts in infants in households with the highest standard of finished flooring (mostly concrete) versus vinyl-covered dirt floors. This could mean concrete, which is porous, can sustain pathogen contamination similar to dirt floors or that floor type was a poor indicator of floor hygiene. Caregivers who cover dirt floors with vinyl or carpet may have been more concerned about hygiene issues posed by living in undeveloped household structures, and clean them more often than caregivers who are not concerned about dirt floors or who have finished floors that they perceive to be safe. We did not ask caregivers about floor cleaning practices, which might be a better indicator on the role of floors in transmission.

Owning and sharing living spaces with animals was associated with higher pathogen counts. Cohabitation between infants and animals in domestic settings makes infant contact with animals or their faeces likely, such as from ingestion of contaminated household drinking water. Additionally, domestic animals belonging to one’s neighbours often wander through spaces of households who do not own animals, meaning ownership is not a comprehensive indicator of animal exposure. While handwashing after animal handling was not associated with pathogen detection count and did not moderate the strong association between owning animals and pathogen count, it was protective against infant diarrhoea. Owning animals may influence infant exposure via multiple pathways, with caregiver hands having relatively little importance as a pathway. We did not assess caregiver washing of infant hands, but infants place their own hands into their mouth frequently. Touching of animals, animal faeces or contaminated floors followed by placing hands, dirt or faeces in the mouth may be a more important mechanism linking animal presence and pathogens in infants.

Information on the role of supplemental foods in infant infection in the first months of life is also scarce, possibly due to the assumption that breast feeding is the only or primary source of infant food at this stage. Like many self-reported behaviours where respondents are aware of sanctioned and disapproved behaviours, caregivers may over report breast feeding to avoid censure. While nearly all caregivers said they still breast feed 5-month infants, only 57% reported breast feeding within the last day, reinforcing that supplemental foods are important exposure pathways for Kisumu infants at ages when exclusive breast feeding is encouraged. Those not breast feeding relied mostly on cow’s milk, especially Long Life UHT milk. Urbanisation of low-income cities has led to increased numbers of people living in low-income neighbourhoods and more women working outside of the home. More women working outside the home has led to increased demand for convenient supplemental foods, like packaged milk, that are palatable to very young infants and can be used by secondary caregivers for feeding. Our data indicated packaged milk-based foods were sources of pathogen exposure, prompting the question as to whether the risks from UHT milk consumption derive from food supply chains or unhygienic household food management.

Animal-based foods, like cow milk, can pose a risk for zoonotic enteric pathogen transmission although pasteurisation is an effective means for making milk safer. In a parallel study, we showed enteric pathogens do occur in processed milk products in Kenya, but households contribute more to infant food contamination than food products. Pasteurised milk products may be risk factors in this study due to postpurchasing contamination from household surfaces, hands or utensils or storing milk for prolonged periods after opening. Food-related handwashing did not modify human and animal source-to-infant transmission, although washing before eating was
generally protective for infants. Other practices related to the boiling, feeding and storage of food were not studied here, but may explain or moderate food-borne pathogen exposure. Forthcoming manuscripts from the Safe Start Trial will reveal whether promotion of handwashing during food preparation and feeding, boiling food, storing foods in closed containers and feeding infants from dedicated containers can reduce diarrhoea and enteric pathogen detection rates in this study population.

Additional limitations of the study include inability to assess the causal relationships between exposure conditions and outcomes, although it is unlikely the pathogen status of infants—to which caregivers were unaware—caused a change in household environments or behaviours. We also relied on self-reported response about breast feeding, handwashing and feeding behaviours and 7-day diarrhoea symptoms in infants, which are all vulnerable to reporting bias. Self-reported handwashing was reported at much higher frequencies than observations of functional handwash stations would indicate. Enumerators visually verified as many conditions, such as water sources, latrines, handwashing station and flooring as feasible. In the case of handwashing, we may have undermeasured functional handwashing stations due to households storing soap and water in other secure locations. Postdefecation handwashing, especially among households relying on shared latrines, may occur outside the premises that were inspected. Additional strengths include the use of an ordinal distribution representing pathogens codetection patterns for identifying many exposure risk factors that would have remained masked by using a simplistic pathogen presence/absence outcome. The utility of using microbial diversity in pathogen contamination for distinguishing between high and low risk conditions in settings where exposure is the norm has also been demonstrated in environmental studies of soil, water and food.\18\31\ We spiked around half of all stool specimens with MS2 virus as a sample transport and extraction process control. This was adequate for surveillance of cold chain failures that would affect batches of specimens transported en masse, but limited our ability to detect poor extraction efficiency among individual specimens. This study was performed among low-income periurban and urban Kenya households and the results may not be generalisable to rural households in Kenya, to higher-income households, and to low-income households in other countries. For example, animal ownership was relatively low in our study population compared with rural areas, where zoonotic transmission may be a more important source of pathogen transmission. Middle-income households are more likely to own a clean private latrine, a plentiful water supply and soap, as well as other indicators of household hygiene like a refrigerator. Such households are typically not recruited into diarrhoea disease studies unless they happen to live in a poorer neighbourhood but presumably, these living conditions prevent their infants from experiencing the high baseline level of enteric infections and diarrhoea as observed here.

In summary, our results point to a need for interventions that limit the presence of animals in households, increase handwashing after handling animals and using the latrine, and before eating, and promote safe management of milk-based infant foods to reduce the high population prevalence of enteric disease in <6-month infants. Interventions must target commercial packaged cow milk as one of the most common supplemental foods, including packaged milk products that are rapidly growing in popularity in urban populations.

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Contributors KKB and OC conceived of this study concept. JAM, OC, KKB, JDA and RD designed the parent study. SS and KT collected data. JAM and KKB supervised data collection. JAM and DS performed the analysis and drafted the manuscript. AM provided additional statistical review. All authors reviewed and submitted comments on the final manuscript. KKB is responsible as guarantor for the finished work, including the conduct of the study, access to the data, and the decision to publish.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. This study includes enrolment data from a clinical trial. De-identified human data will be made available upon request once the primary results of the trial are published.

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