Do domestic animals contribute to bacterial contamination of infant transmission pathways? Formative evidence from Ethiopia

Sophie Budge, Paul Hutchings, Alison Parker, Sean Tyrrel, Tizita Tulu, Mesfin Gizaw and Camila Garbutt

ABSTRACT

Child stunting is associated with poor water, sanitation and hygiene (WASH), partly due to the effect of infection on intestinal nutrient absorption. WASH interventions, however, show little effect on growth. A hypothesis is that bacterial contamination of hands and floors from domestic animals and their faeces, and subsequent ingestion *via* infant hand-to-mouth behaviours, may explain this. This formative study used microbial testing and survey and observational data from 20 households in Ethiopia to characterise principle bacterial transmission pathways to infants, considering WASH facilities and practices, infant behaviours and animal exposure. Microbial swabbing showed the contamination of hands and floor surfaces from thermotolerant coliform bacteria. Animal husbandry practices, such as keeping animals inside, contributed significantly (all $p < 0.005$). There was no evidence that latrine facilities mitigated contamination across infant ($p = 0.76$) or maternal ($p = 0.86$) hands or floor surfaces ($p = 0.36$). This small study contributes to the evidence that animal faeces are an important source of domestic bacterial contamination. The results imply that interventions aiming to reduce pathogen transmission to infants should think beyond improving WASH and also consider the need to separate infants and animals in the home. Intervention studies will be required to determine whether this reduces infant infection and improves linear growth.

Key words | animal husbandry, contamination, malnutrition, sanitation, WASH

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ABBREVIATIONS

HAZ	height-for-age (*z*-score)
IYC	infants and young children
TTC	thermotolerant coliform
WASH	water, sanitation and hygiene
HEW	Health Extension Worker
CFU	colony-forming units

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INTRODUCTION

Stunting, water, sanitation and hygiene (WASH) and infection

Linear growth failure, or stunting, defined as a *z*-score (an age- and sex-normalised measure of child height in units of standard deviations) of less than −2, remains highly prevalent among low-income countries. Despite having fallen on a global level, stunting still affects around one in five children (UNICEF and WHO 2019) and remains a key public health issue, both in terms of infant morbidity and mortality and in loss to national economic productivity (The World

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Bank 2006; Martorell *et al.* 2010; Black *et al.* 2013; UNICEF and WHO 2019). Defining the precise causes of stunting remains elusive. While an inadequately diverse and nutrient-dense diet (Black *et al.* 2008) likely affects growth outcomes, supplementary and complementary feeding interventions may only improve stunting by a height-for-age *z*-score (HAZ) of around 0.7 (Dewey & Adu-Afarwuah 2008) – far from the average discrepancy of −2.0 in parts of sub-Saharan Africa (Victora *et al.* 2010). Diarrhoeal incidence, another factor correlated with undernutrition (Scrimshaw & Suskind 1976), also does not appear to explain a large proportion of the stunting burden: it is estimated that eliminating diarrhoea within the first 2 years of life would increase length by a HAZ score of only 0.13 (Richard *et al.* 2013). Given this marginal impact, it is apparent that other aetiological factors remain which have not yet been addressed to tackle early growth faltering.

Stunting is commonly observed in regions with poor water, sanitation and hygiene (WASH), supporting an underlying role of exposure to pathogenic bacteria and infection (Humphrey 2009; Ahmed *et al.* 2014). Certain pathogenic strains, which are widespread across low- and middle-income countries (Marquis *et al.* 1990; Lin *et al.* 2013; Ngure *et al.* 2013) and appear linked to stunted growth, include faecally associated *Campylobacter* (Harvey *et al.* 2003; Oberhelman *et al.* 2006; Headey & Hirvonen 2016), *Cryptosporidium* (Guerrant *et al.* 1999; Kotloff *et al.* 2013), *Giardia* (Kosek 2017) and pathogenic *Escherichia coli* (Steiner *et al.* 1998; Ngure *et al.* 2013). Although not well described, increased prevalence of these enteropathogens in healthy infants is associated with growth faltering (Checkley *et al.* 1997; Lee *et al.* 2013), even in the absence of other clinical insults like diarrhoea (Korpe & Petri 2012; Prendergast & Humphrey 2015; MAL-ED Network Investigators 2017). A quantitative framework which estimated the relative impact on stunting from different causes found infectious disease to be a significant contributory factor across global regions (Mosites *et al.* 2017). Preventing infection has, therefore, become an important focal point for WASH interventions addressing infant growth.

The five main faecal–oral routes of transmission are described in the 'F diagram' (fluids, fingers, fields, flies and food) – proposed some 60 years ago as an important map of causes of enteric infection (Wagner & Lanoix 1958). Fundamental to WASH research and programming, the diagram underpins WASH interventions to prevent infectious disease *via* both primary barriers (sanitation) and secondary barriers (water treatment and handwashing), which should block pathogen transmission. However, several recent, large trials which have assessed the effect of either an individual component or an individual and combined components of WASH across different study arms have demonstrated little impact on infant health, or a significant reduction in environmental faecal contamination (Clasen *et al.* 2014; Patil *et al.* 2015; Luby *et al.* 2018; Null *et al.* 2018; Humphrey *et al.* 2019). This remained the case even in combination with a nutrition programme (Clasen *et al.* 2014; Patil *et al.* 2015; Luby *et al.* 2018; Null *et al.* 2018; Humphrey *et al.* 2019). Following largely insignificant results across the different study designs and settings, researchers have concluded that such interventions, while necessary, were perhaps not sufficient in coverage to show noteworthy improvements in growth (Arnold *et al.* 2018). Perhaps, more importantly, these trials may have neglected to address, in particular, the main pathogen transmission pathways specific to infants and young children (IYC), having not considered the age-related behaviours which alter infection risks – as well as sources of faecal contamination other than human.

WASH interventions and infant-specific behaviours and risks

While WASH interventions, following the seminal F diagram, have traditionally focussed on improving latrine facilities, communal and household water supply and adult hygiene practices (ACF and UNICEF 2017), for IYC different needs and behaviours, create additional exposure to pathogens (Ngure *et al.* 2013; Morita *et al.* 2017). That is, developmental behaviours, such as crawling and the touching, mouthing and sucking of objects from frequent hand-to-mouth activity, mean that primary transmission pathways differ from adults and exposure risk is increased (ACF and UNICEF 2017; Kwong *et al.* 2019). This risk is higher across low-income countries where, while crawling and playing, IYC may come into contact with contaminated soil, faeces and enteric pathogens which are brought inside the home (Ngure *et al.* 2013; Reid *et al.* 2018). Faecal indicator bacteria and associated pathogens have been detected in soil (Pickering *et al.* 2012),

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and hand-to-mouth contact appears to result in significantly higher faecal intake in IYC than consumption of stored water (Mattioli *et al.* 2015). While direct ingestion of faeces is more rare, IYC frequently place soil in their mouth or put their hands in their mouths after touching soil (Ngure *et al.* 2013; Ercumen *et al.* 2017; Bauza *et al.* 2018; Kwong *et al.* 2019), meaning that hand recontamination is likely (Kwong *et al.* 2019). Both direct and indirect consumption are associated with diarrhoea, markers of infection and inflammation and growth failure in infants (Shivoga & Moturi 2009; George *et al.* 2015a, 2015b). While pathogenic bacteria of both human and animal origin can cause diarrhoea, even with improvements in sanitation these associations remain (Ercumen *et al.* 2017), suggesting that other external sources of faecal contamination are not illustrated in the original F diagram and considered in interventions.

In lower-income countries, domestic animals – usually livestock and poultry (WHO 2012; Ercumen *et al.* 2017) – are often not contained or separated from the household environment (Penakalapati *et al.* 2017), and animal faecal contamination appears widespread across the home (Boehm *et al.* 2016; Penakalapati *et al.* 2017). Recently, studies using molecular techniques have shown animal faecal markers in soil from the outside yard, household floor and infant hands (Boehm *et al.* 2016; Harris *et al.* 2016), and animal presence has been associated with higher levels of faecal contamination across multiple pathways, including soil (Ercumen *et al.* 2017) and contaminated food (Parvez *et al.* 2017). Both quantitative molecular techniques (Mattioli *et al.* 2015) and microbial source tracking (Parvez *et al.* 2017; Penakalapati *et al.* 2017) have shown a high burden of animal faecal contamination in living areas, particularly of hands and soil, which appears more prevalent than human contamination (Penakalapati *et al.* 2017). However, interventions underpinned by the F diagram have largely focussed on removing human faeces, with few objectives to remove animal faeces or reduce exposure. Such interventions that are limited in scope may miss animal contamination as a critical risk factor (also of importance given the differing pathogens present in animal faeces that cause enteric infection and present different risks to health) alongside not acknowledging the transmission pathways particular to IYC, like contaminated hands and soil. Considering the high prevalence of animal faeces and faecal contamination around the home (Ngure *et al.* 2013; Headey & Hirvonen 2016; Penakalapati *et al.* 2017), the behaviours which expose IYC to faeces and the associations with lower HAZ scores (Bukenya & Nwokolo 1991; George *et al.* 2015a, 2015b; Headey & Hirvonen 2016), animal faecal contamination of the domestic environment is an issue particularly significant to IYC in lower-income countries which may be stalling the progress of interventions to improve infant health. This may explain the failure of improved WASH infrastructure and facilities to reduce infectious disease and improve IYC health outcomes. Figure 1 illustrates the exposure pathways through which IYC are commonly and frequently exposed to, and ingest, pathogenic bacteria within the home (Budge *et al.* 2019). Where the dashed lines constitute the original 'F diagram', the figure illustrates how the original diagram does not consider specific infant behaviours (boxes in bold), nor the contribution of animal faeces to domestic contamination, but the integral relationship with those three components and microbial ingestion.

It follows that an improved understanding of the key sources of contamination and principal faecal–oral transmission pathways to IYC is necessary to understand the risk of infection to infants. Further evidence toward this may support and inform initiatives which are attempting to address sectoral integration to improve infant health such as the BabyWASH coalition (ACF and UNICEF 2017). In order to inform a larger trial intending to address pathogen transmission to infants, this small formative study sought (i) to quantify thermotolerant coliform (TTC) bacterial burden across infant-related environmental pathways (infant and caregiver hands, domestic floor surface) in rural Ethiopian households, (ii) to assess how the presence of animals within the household, household sanitation and key hygiene practices may affect levels of TTC contamination and (iii) to understand if and how these environmental transmission pathways within the home influence and impact one another through cross-contamination.

MATERIALS AND METHODS

Study sites and sampling frame

Formative research is intended as an initial part of the process of a larger study design and can use both qualitative and

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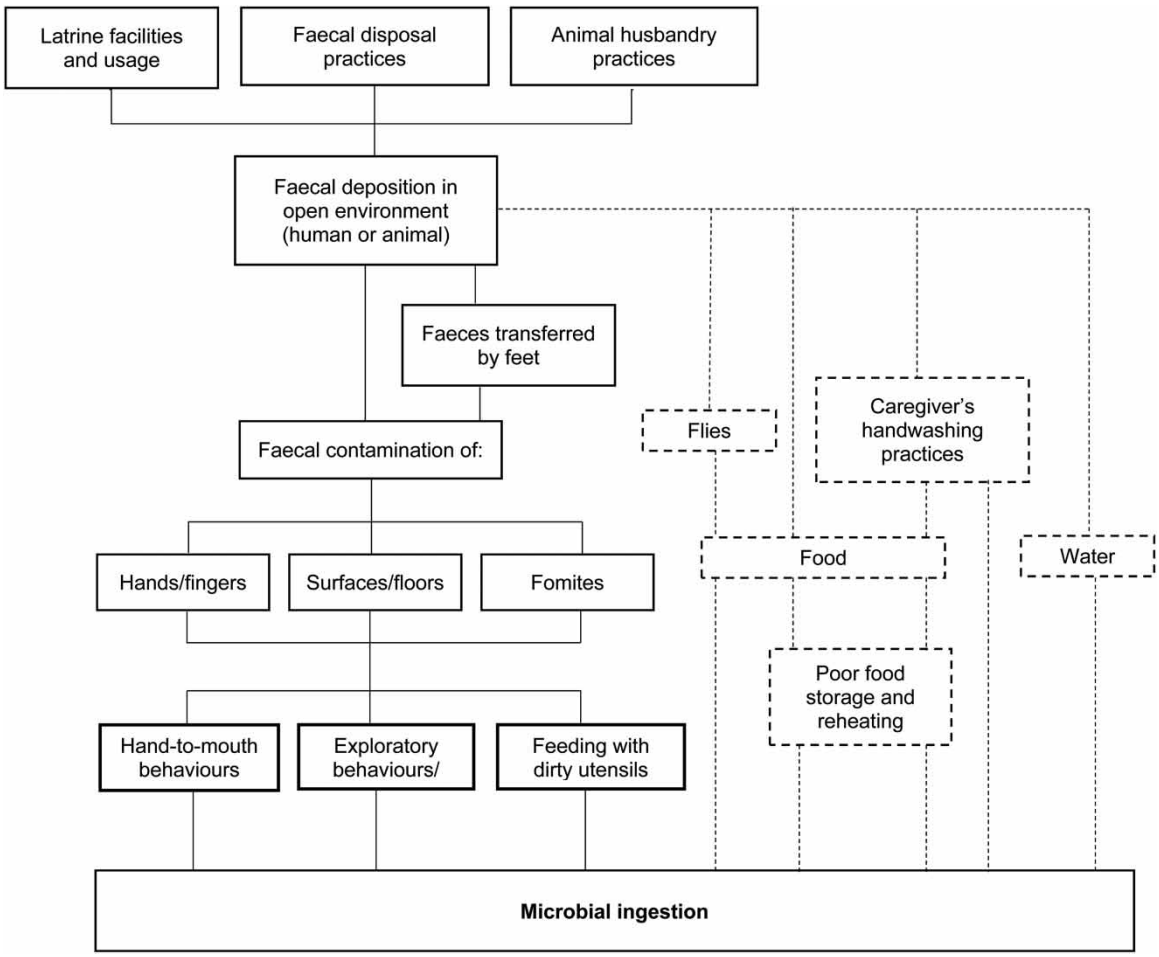

Figure 1 | Age-specific pathways and contributing sources by which infants are exposed to and ingest pathogenic bacteria within the home. Where the dashed lines constitute the original 'F diagram', the figure illustrates how the original diagram does not consider specific infant behaviours (boxes in bold), nor the contribution of animal faeces to domestic contamination, but the integral relationship with those three components and microbial ingestion. Adapted alongside the 'F diagram', as published by Wagner & Lanoix (1958).

quantitative methods to provide data for research teams to plan interventions or further data collection. While formative research is critical to designing and delivering interventions or programmes which are efficient and effective, it is early-phase data not powered to detect differences between groups (Gittelsohn *et al.* 2006). As such, this study must be interpreted in this context – it is intended to provide indicative evidence on a hypothesis but is not sufficiently powered to provide conclusive evidence in this area. This formative study was conducted in the Sidama zone, Southern Nations, Nationalities, and Peoples (SNNPR) region, Ethiopia as the geographical outreach area of the non-governmental organisation People In Need. The study took part in the month of May, which in the region is a mostly dry period with some afternoon rainfall. From the six woredas (districts) in the zone, 16 kebeles (neighbourhoods) were grouped into peri-urban ($n = 6$) or rural ($n = 10$). A simple random sampling method was used, whereby kebeles within the People In Need intervention area were listed, given a number and using a lottery method, eight kebeles were selected at random. From these eight kebeles, 20 households which included an infant aged 12–24 months old and not engaged in any other research with People In Need were selected. This involved communication with a Health Extension Worker (HEW) local to the kebele who was familiar with the ages of infants in the area. The final sample size was 20 infants ($n = 20$). Households were visited on one single occasion. A separate single location for piloting the study was chosen in the same manner and not included in the final sampling frame.

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Survey and infant observation period

A survey was designed to assess sanitation facilities, handwashing practices, animal presence, livestock husbandry and diarrhoea prevalence and duration of diarrhoeal episodes (where to avoid reporting bias, researchers asked caregivers for the frequency of loose or watery stools over the past 7 days and subsequently applied World Health Organization criteria) (Baqui *et al.* 1991). This was administered alongside a 1-hour observation period which noted infant hand-to-mouth behaviours and general sanitary conditions. During this, a pre-tested semi-structured survey tool was used to record every object that was either mouthed or touched by the infant, where mouthing was defined as an infant putting fomites or fingers into their mouths whether swallowed or not. This semi-structured tool was calibrated to capture some of the underemphasised key pathogen transmission pathways in infants. As illustrated in Figure 1, this is also well described by the 'modified F diagram' published by the Water, Sanitation and Hygiene Partnerships and Learning for Sustainability (WASHPaLS) consortium (Ngure *et al.* 2017), which illustrates additional key pathways specific to infants – namely geophagy and direct faecal ingestion. After entering into the home, primary introductions with the caregiver and the consent process, a fieldworker conducted the survey with the help of the local HEW. Concurrently, the primary researcher conducted the 1-h observation period of the infant. This included mouthing and exploratory behaviours. Other observations included infant interaction with others, open defecation practices, general sanitation and hygiene, animal presence and husbandry. Observations of animal faeces and other hygiene markers were also visually assessed by the researcher, including cleanliness of caregiver and infant hands, which were visually inspected for visible dirt on the palms and underneath nails. General infant cleanliness was also noted by observing visible dirtiness of infants' clothing and skin.

Microbiological analysis

Microbial samples were collected during the same visit as the survey in order to minimise social desirability bias and changes in behaviour. Nineteen of 20 households were sampled due to one baby asleep in the caregivers' arms during the observation period. Following the survey and observation period, swabs were taken of infant and caregiver hands and a weighed sample of floor material (soil, dirt) within crawling reach of the infant was also collected from 18 households. For each sample approximately 1.0 g (weighed to the nearest 0.01) was collected using a sterile scoop and put into Whirl-Pak® 710 mL bags containing a buffer solution of 100 mL of bottled water with Ringer's solution ¼ strength tablets and 0.1% v/v Tween® 20. It should be re-emphasised that this methodology could not distinguish between TTC of human or animal origin. Faeces were observed within the homes but were not sampled. Hand swabs used a similar methodology as described by Ngure *et al.* (2013) who found replicable results and is described briefly as follows. Hand swabs were collected using commercially available environmental sponge sampling kits (Whirl-Pak® Speci-Sponge® Environmental Surface Sampling Bags, Sigma-Aldrich, UK) which were pre-moistened with the same buffer solution previously described. Sponges swabbed both sides of both the caregivers' and infants' hands (palm, back of hand and in-between fingers). After swabbing, sponges were returned aseptically to the bag. Bags were sealed and transported in a cool-box for microbiological analysis within 6 h. All samples were analysed for TTC counts with a DelAgua single incubator using the water filtration method (DelAgua Water Testing Ltd 2015). Membrane Lauryl Sulphate Broth was pipetted on 0.45 μm 25 mm gridded cellulose nitrate membranes (DelAgua, UK) to grow TTC. Samples were incubated overnight for 16–18 h at 44 °C. To control for potential contamination in field laboratory conditions, a blank sample was incubated with every other set of samples. At the end of data collection, only one blank sample was found to be contaminated during sample processing.

Data analysis

Means for bacteria counts from swabs were calculated as TTC colony-forming units (CFU) per hand (TTC CFU/hand). Bacterial populations from the solid samples were calculated as TTC colony-forming units per dry gram (TTC CFU/dry g). Anonymised survey data were entered into a tablet using KoBoToolbox (Harvard Humanitarian Initiative, Massachusetts, USA) into preconfigured fields. Data

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were downloaded into Microsoft Excel and coded for descriptive analysis and then transferred and further analysed using SPSS statistical software version 22.0 (IBM, New York, USA). Boxplots showed associations between infant and caregiver hand TTC CFU count, floor surface sample CFU count and associated variables. An unpaired *t*-test tested the difference in sample means of TTC count between vectors for statistical significance.

Ethics

Infants and their caregiver were visited in the home between the hours of 10:00–12:00 pm and 14:00–16:00 when the infant was most likely to be awake and playing. Households were visited unannounced to avoid researcher bias. However, at the start of the household visit, free and informed consent of the participants was obtained. To do this, the study was introduced by the field team and the HEW, and an informed consent statement was read to the caregiver in their first language of Amharic or Sidamigna (Sidamo). Fieldworkers tested the caregivers' understanding of consent by asking them questions regarding the study or the consent process, and explained data were anonymised. The survey was written in English, translated to Amharic by local fieldworkers and verbally translated into Sidamo by a local HEW. The study protocol was approved by an institutional Committee for the Protection of Human Participants (Cranfield University Research Ethics Committee; CURES/4955/2018).

RESULTS

Survey results

The WASH survey asked questions regarding infant characteristics, diarrhoea prevalence and episode duration (as described), latrine ownership and use, handwashing and animal husbandry practices. Briefly, most houses had a pit latrine either with a slab (40%) or without (30%). The remainder (20%) had no toilet at all (assumed to openly defecate) or used the toilet of a neighbour (10%). Only five (25%) households had a specific place to wash their hands; of those, all households had water available, but only three (15%) had soap (in two instances visual inspection indicated the soap was likely not used). Seventy per cent of households raised animals of some kind, with the most common chickens (93%) and cattle (71%). When asked where animals lived during the day, only one household reported that their animals lived outside enclosed in an area, with the rest kept outside either unenclosed or living inside with the family, suggesting that animals were mostly uncontained. One hundred per cent of households reported that during the night animals lived inside with the family. Regarding diarrhoea prevalence, three infants (15%) were reported to have experienced three or more loose stools within a 24-h period; across these infants, the reported mean duration of a diarrhoeal episode was 3.3 days. Table 1 illustrates these findings along with general hygiene characteristics of the infant's environment.

Infant observation period results

Nineteen infant–caregiver pairs were observed for 20 h during the infant observation period. Infants were frequently observed to mouth their own hands (a mean of 31 times over 1 h), or to mouth those of their caregiver (mean of 21 times over 1 h), which in the majority of instances were both visibly dirty (90% and 86%, respectively). Throughout the observation, infants would typically have nothing to play with other than a plastic bottle, which may explain why infants were observed to frequently suck their hands or those of their caregiver. Animal faeces were directly ingested by two infants, and the floor surface material was also picked up and directly entered into the infants' mouths on seven occasions.

Table 1 details hygiene characteristics as noted during the observation. In 50% of households, there were faeces visible on the floor (usually from chickens as the predominant livestock) and almost half of all infants were visibly dirty (often naked from the waist down and dirty). On four occasions, the infant crawled near-visible pools of urine and/or faeces. Animals, most commonly cattle or chickens, were often in the house during the observation and were rarely separated from the living area other than by a rudimentary wooden beam. Thus, animals tended to occupy the same space as the infant and were frequently around them at play.

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Table 1 | General characteristics of the home environment and specific hygiene characteristics as reported from survey data and as noted during the infant observation period

	n	%
Characteristic (survey)		
Rural	14	70
Infant male sex	14	70
Diarrhoea within the last 7 days	3	15
Mud house	15	75
Mud floor	18	90
Open defaecation	4	20
Share neighbour's toilet	2	10
Pit latrine without slab	6	30
Pit latrine with slab	8	40
Specific handwashing station	5	25
Water available	5	25
Soap available	3	15
Raises animals	14	70
Hygiene characteristics (observed)		
Flies on baby	12	60
Mud house	15	75
Mud floor	18	90
Animal faeces visible on the floor	10	50
Baby visibly dirty	9	45
Baby crawled near urine/faeces	4	20
Any animal in the house	12	60
Cattle in the house	5	25
Goats or sheep in the house	2	10
Chickens in the house	10	50

Microbiological data

Samples from the inside floor surface showed the highest bacterial count with a mean TTC CFU/dry g of 76.5 (1.88 $\log_{10}$). The higher count in the floor sample may reflect the typical presence of animals inside as well as the common occurrence of animal faeces. High counts in the domestic floor sample are significant, given the sample was collected within crawling reach of the infant and was observed to enter the infants' mouths and contaminate their hands. Infant hand contamination showed a slightly higher mean count than those of their caregiver (mean TTC CFU/hand 33.3 (1.52 $\log_{10}$) versus 23.6 (1.37 $\log_{10}$), respectively) ($p < 0.005$, data not shown). This is unsurprising in a context where infants were frequently crawling on the floor and touching and mouthing objects, which were usually visibly dirty (Table 1). The relationship between key vectors and transmission pathways, as measured by microbial testing, is presented in Figure 2. The *p*-values presented are from a *t*-test that assessed any statistically significant differences between TTC CFU counts on hands and between floor surface samples and key transmission pathways. The data, illustrated by the striking differences in the box plot figures, indicate that levels of TTC were much greater in households than raised livestock and where livestock were kept indoors. Infants and caregivers who lived in a house which raised animals showed a significantly higher hand TTC CFU count than those who did not (Figure 2, graphs 3A [$p < 0.005$] and 3B [$p < 0.005$]), and the floor surface TTC CFU count was also higher in these households (Figure 2, graph 3C, $p = 0.006$). Similarly, graphs 4A, 4B and 4C show CFU animals living inside during the day were significantly related with an increased CFU count on both infant and caregiver hands and in floor surface samples. In contrast, from the data, it appears that owning a handwashing facility did not reduce TTC CFU count on hands (Figure 2, graphs 1A [$p = 0.57$] and 1B [$p = 0.38$]); nor for floor surface (graph 1C [$p = 0.68$]). A similar observation can be made for whether the household owned a latrine (Yes) or openly defecated (No) (graphs 2A, 2B and 2C), where owning a latrine was not related to a reduced hand CFU count for infant or caregiver hand ($p = 0.76$ and 0.86, respectively), nor for floor surface samples ($p = 0.36$).

Study limitations

This study presents some limitations. Firstly, the small sample size in this study of 20 infants/households would not have comprehensively captured variability in TTC contamination across pathways, which likely varies considerably. As is noted elsewhere (Navab-Daneshmand *et al.* 2018), high variability in contamination across pathways and vectors requires a large sample size to provide good statistical power. However, the results are emphasised as formative evidence and do support the primary hypothesis regarding the diversity of contamination across pathways and animals as a contributor to TTC contamination.

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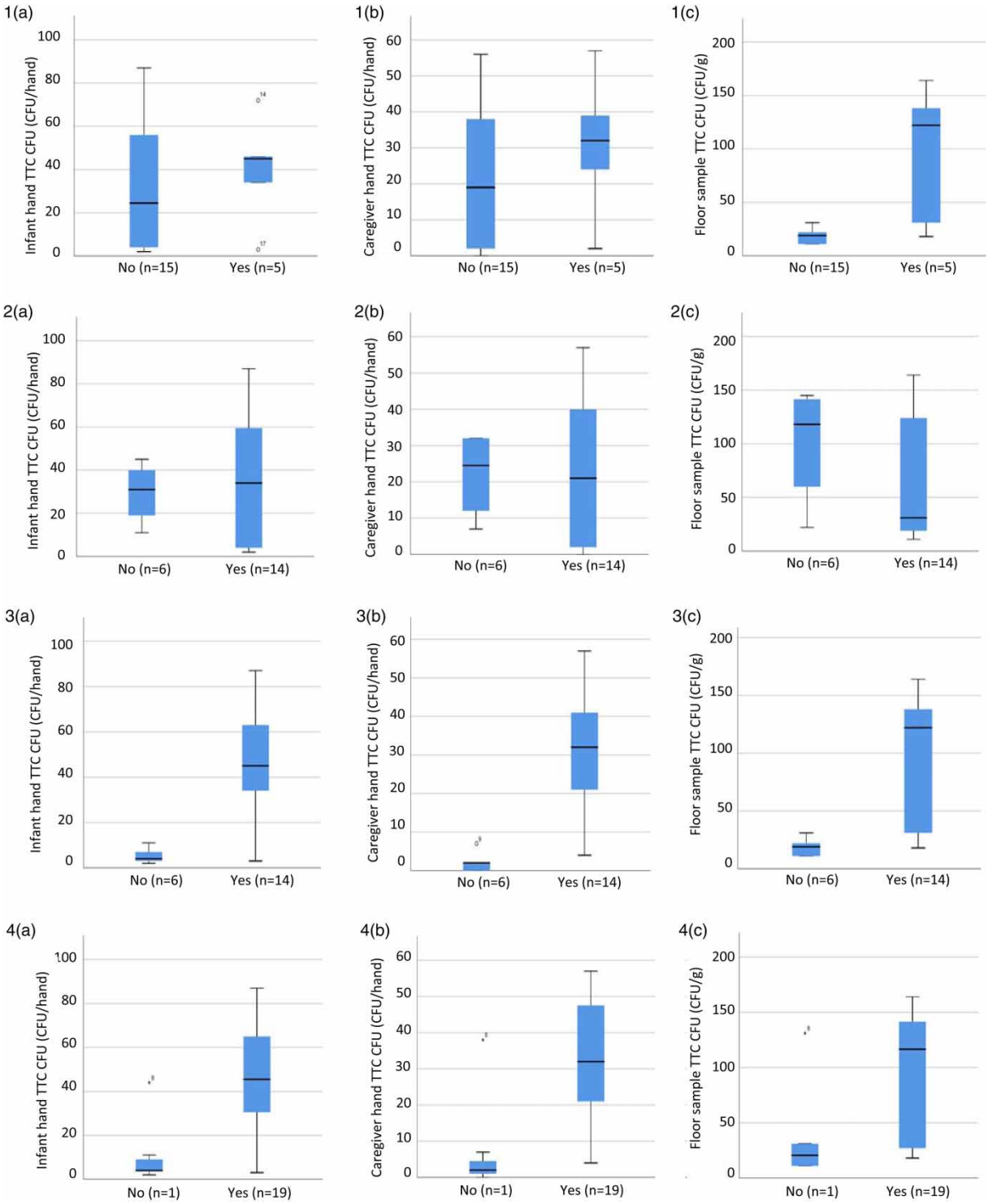

Figure 2 | Association with an increase in infant and caregiver hand and floor surface sample TTC CFU count with specific hygiene characteristics. From top left: (1) Relationship between if the household had a specific handwashing facility ($n = 5$) with increased TTC CFU count for: (1A) infant hands ($p = 0.57$), (1B) caregiver hands ($p = 0.38$) and (1C) floor surface sample ($p = 0.68$); (2) Relationship between if the household owned a latrine (Yes) ($n = 16$) or openly defecated (No) ($n = 4$) with increased TTC CFU count for: (2A) infant hands ($p = 0.76$), (2B) caregiver hands ($p = 0.86$) and (2C) floor surface sample ($p = 0.36$); (3) Relationship between if the family raised animals ($n = 14$) with increased TTC CFU count for: (3A) infant hands ($p < 0.005$), (3B) caregiver hands ($p < 0.00$) and (3C) floor surface sample ($p = 0.006$); and (4) Relationship between if animals lived inside during the day ($n = 13$) and increased TTC CFU count for: (4A) infant hands ($p < 0.005$), (4B) caregiver hands ($p < 0.005$) and (4C) floor surface sample ($p = 0.04$).

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Secondly and relatedly, it was not possible to determine the origin of TTC bacteria. As such, it is possible that the bacteria detected in animal-rearing households were of human origin. However, given the lack of human faeces observed within homes versus the high prevalence of animal faeces, and the correlation between animal-rearing households and TTC counts across different measures, we have confidence in supporting the theory describing a link between animal practices and environmental contamination. This is also backed up by broader studies (Ngure *et al.* 2014; Schriewer *et al.* 2015; Ercumen *et al.* 2017). Thirdly, the presence of TTCs indicates the presence of faecal contamination but cannot directly quantify the burden of pathogens that cause enteric infection. Fourthly, due to a lack of facilities, soil moisture content was not measured. This limits the ability to compare results between soil samples of different moisture content, as well as across studies, and should be a methodological consideration in further research. Lastly, this study only provides part of the picture of total infection risks to IYC. While hands and floors are key transmission pathways (Davis *et al.* 2018), contaminated food (Parvez *et al.* 2017) and water (Barnes *et al.* 2018) also constitute important pathways, of which we did not measure contamination. A broader study that seeks to measure each of these and assesses additive effects would be a productive route for extending this research.

DISCUSSION

This formative study found faecal contamination common across different transmission pathways in rural Ethiopian households with high sanitation access, contributing to a growing evidence base that improved sanitation access alone is not enough to improve overall environmental hygiene (Ercumen *et al.* 2017; Barnes *et al.* 2018). The contamination of caregiver and infant hands and domestic floor surface samples with TTCs suggests that IYC are frequently exposed to faecal pathogens through transmission pathways which are intrinsically linked. Through normal exploratory and hand-to-mouth behaviours, frequent contact with dirty floors meant that infant hands themselves became vectors for the transmission of faecal microbes, corroborating research found in similar settings (Ngure *et al.* 2013; Ercumen *et al.* 2017; Bauza *et al.* 2018; Reid *et al.* 2018). In this study, 35% of infants directly ingested soil over the 1-h period. Only a few other studies have sought to correlate high levels of hand-to-mouth behaviours and direct and indirect floor surface material ingestion. In rural Bangladesh, 25% of children aged 3–18 months old directly ingested soil during a 5-h observation (Kwong *et al.* 2016); in another study, in rural Ghana, 28% of children aged 6–36 months old reportedly ingested soil a median of 14 times in the past week (Bauza *et al.* 2018). Ingestion of floor surface materials by IYC is of concern giving the growing number of studies linking ingestion with negative health outcomes, such as diarrhoea (Yeager *et al.* 1991; Shivoga & Moturi 2009), and both enteric dysfunction from infection and linear growth failure (George *et al.* 2015a, 2015b).

Regarding the reliability of the testing method, the CFU counts captured in this study are a similar magnitude to others with similar methodologies, including a recent study in urban Harare which reported a mean 1.62 $\log_{10}$CFU/g in soil (per dry gram) and a mean 1.52 $\log_{10}$CFU/hand before handwashing (Navab-Daneshmand *et al.* 2018). An earlier study in a Tanzanian community with improved, non-networked water supplies found a mean *E. coli* count of 3.1 $\log_{10}$CFU, but over two hands (Pickering *et al.* 2010). Although in many settings the original source of contamination is not clear, strong evidence supports a relationship between domestic animal ownership and residual contamination from faeces (Marquis *et al.* 1990; Reid *et al.* 2018) and animal presence is associated with high levels of faecal contamination across multiple pathways (Ercumen *et al.* 2017; Parvez *et al.* 2017). These results suggest that faecal contamination of different transmission pathways is related, with the presence of animal faeces as the common contamination factor – supported by this study by the strong difference in the CFU count in households with animals (Figure 2, graphs 3–4). Due to the small number of sampled households, it was not possible to determine differences in bacterial contamination across floor types, which may point to a wider need for improved housing. However, other research has found no association with floor material (mud or concrete), the amount and frequency of infant soil ingestion and diarrhoeal episodes (Bauza *et al.* 2018), suggesting an independent contamination factor. In this study, where animals (particularly poultry) were present in most

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households, their presence likely led to floor contamination regardless of flooring type. In another study in Zimbabwe, floor surface contamination could not be explained by household-level WASH factors, but households with animals showed significantly higher concentrations of *E. coli* (Navab-Daneshmand *et al.* 2018). In this study, poultry was likely a key factor in contamination levels due to their common presence in the home – of concern given demonstrated associations between poultry faeces, diarrhoea (Yeager *et al.* 1991; Zambrano *et al.* 2014) and poor growth (Headey *et al.* 2017).

In this study, even in households with a latrine (improved or other) contamination was still common, suggesting that even with sufficient sanitation infrastructure the presence of animals within the home may propagate contamination. In one study in Bangladesh, while households with fewer contaminated toys and objects were those with high latrine coverage and WASH infrastructure (Torondel *et al.* 2015), the absence of animals was highlighted as a possible noteworthy factor to low levels of contamination (Penakalapati *et al.* 2017). In this study, it is possible that latrines were not being used and open defecation was practised, although the likelihood of latrine use, determined via spot check if the path was trodden and if faeces were present, suggested they were. Owning a specific handwashing facility was not common here, but even where facilities existed contamination remained. This may be due to poor handwashing practices (soap was observed to be unused on three occasions); however, it is possible that even where good hygiene behaviours exist, if the environment is continually contaminated by an external source contamination, transmission and infection will not decrease (Curtis *et al.* 2000; Langford *et al.* 2011). Studies have shown that even after handwashing, *E. coli* count on hands, possibly from human and animal contamination of dirt and sand (Pickering *et al.* 2012; Lupindu *et al.* 2015), can increase 2–3 $\log_{10}$CFU within minutes of resuming normal activity (Pickering *et al.* 2011; Ram *et al.* 2011; Devamani *et al.* 2014). Furthermore, Barnes *et al.* (2018) found that despite high coverage of improved water sources, two-thirds of households in Kenya had drinking water contaminated with *E. coli* at point-of-use, which was significantly correlated with animal ownership and the presence of animal waste in the home. It is worth bearing in mind that poor water quality within the home influences not only personal and domestic hygiene but also the safety of food, propagating whole environment contamination and further reducing the capacity for domestic and personal hygiene and food safety. Parvez *et al.* (2017) found an increase in *E. coli* count in complementary foods in houses where mothers transferred food and fed infants with their hands, along with animal presence in the compound. Ercumen *et al.* (2017) found significantly higher levels of *E. coli* in food in compounds where animals lived – primarily increased by the presence of poultry. It, therefore, stands to reason that if other external sources of contamination from animals are not considered, all pathways will remain contaminated and bacterial transmission to infants may not be reduced – regardless of improvements in sanitation.

Notwithstanding this study does not suggest that animal husbandry should be restricted, as it remains critical for socio-economic development – especially in lower-income countries. Indeed, studies have found both non-significant and protective effects from domestic animal husbandry, for example, through the nutritional benefit of consuming animal products. A cross-sectional study in Nigeria found a significant protective effect against diarrhoea linked with animal exposure (rate ratio 0.8, 95% CI: 0.7–0.9), although confounding by other factors was suspected (Huttly *et al.* 1987). An analysis of cross-sectional datasets from Ethiopia, Kenya and Uganda found a negligible beneficial effect of household livestock ownership on child stunting prevalence (Mosites *et al.* 2015). In another study in Kenya, greater household livestock ownership at the baseline was not related to the baseline infant HAZ score (Mosites *et al.* 2016). What seems apparent is that while livestock ownership may provide benefits in terms of nutrition and economic development, these benefits must be utilised and capitalised on and at the same time are not without risk. The ways in which households and their livestock interact and share space vary from setting to setting and in the absence of integrated WASH, nutrition and agriculture programmes, it is possible that the close proximity of livestock may be of detriment to the health of IYC. This research advocates that further research within the WASH field should consider animal husbandry practices and work more closely (i) with the agricultural sector to better understand how exposure and transmission risks differ across settings and (ii) with nutrition experts to understand how

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risk might be mitigated. This may be especially pertinent for certain animals. In this study, in corroboration with findings in Bangladesh (Ercumen *et al.* 2017), Zimbabwe (Ngure *et al.* 2013), Peru (Marquis *et al.* 1990) and Zambia (Reid *et al.* 2018), poultry was the most common animal found within the home; poultry faeces are often found inside near the playing infant and are frequently ingested along with soil (Yeager *et al.* 1991; Ngure *et al.* 2013; Reid *et al.* 2018). Due to their mobility and the difficulty with which their faeces are noticed, small animals like poultry may pose a greater risk to infants.

CONCLUSION

Although the evidence presented here is of a small sample, results support the growing body of evidence which suggests that WASH interventions must address animal faecal contamination across the domestic environment: a 'total environmental hygiene' approach, which fully addresses multiple sources of contamination. Increased attention should be placed not only on WASH infrastructure and quality but also on addressing barriers from widespread faecal contamination to overall improved hygiene on a much wider level. Similarly to another recent study (Kwong *et al.* 2019), this study did not observe human faeces within the home and suggests that faecal contamination from animals may be a primary limitation in WASH interventions, which tend to focus on that of humans. Further interventions, which aim to improve infant growth by addressing contamination, are likely to benefit from considering certain common animal husbandry practices, such as keeping animals indoors during the day and night, and the need to separate IYC from animals. If not considered, in this setting and other similar settings, it is possible that WASH interventions may not interrupt faecal–oral transmission of bacteria and pathogens to IYC. Similarly, if interventions targeted towards IYC are predicated on health grounds, effects may be limited when animals share household space. While new, more targeted programmes such as the 'Baby WASH' initiative may reduce infant zoonotic transmission and diarrhoea, large-scale interventions must focus on controlling animal faecal pathogen transmission and limiting infant exposure. These findings alongside similar, larger studies may aid policymakers to better understand the contribution of specific risk factors and transmission pathways within the home and in the allocation of resources to infant-focussed WASH interventions that aim to improve growth.

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