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Ruminant-Related Risk Factors are Associated with Shiga Toxin–Producing *Escherichia coli* Infection in Children in Southern Ghana

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Abstract. Livestock can provide benefits to low-income households, yet may expose children to zoonotic enteropathogens that cause illness and negative long-term health outcomes. The aim of this cross-sectional study was to determine whether livestock-related risk factors, including animal ownership, exposure to animal feces, and consumption of animal-source foods, were associated with bacterial zoonotic enteropathogen infections in children 6–59 months old in Greater Accra, Ghana. Stool samples from 259 children and 156 household chickens were analyzed for atypical enteropathogenic *Escherichia coli* (aEPEC), *Campylobacter jejuni/coli* (*C. jejuni/coli*), *Salmonella*, and Shiga toxin–producing *Escherichia coli* (STEC) using quantitative polymerase chain reaction (qPCR). aEPEC, *C. jejuni/coli,* STEC, and *Salmonella* were detected in 45.6%, 11.6%, 4.3%, and 0.8% of children's stool samples, respectively. In adjusted logistic regression models, household ownership of goats or sheep was associated with STEC detection in children (odds ratio [95% confidence interval]: 4.30 [1.32, 14.08]), as were positive detection of STEC in chicken feces (7.85 [2.54, 24.30]) and frequent consumption of fresh cow's milk (3.03 [1.75, 5.24]). No livestock-related risk factors were associated with aEPEC or *C. jejuni/coli* infection in children. Our findings suggest that ruminant ownership in southern Ghana may expose children to STEC through household fecal contamination and foodborne routes. The lack of association between livestock risk factors and the more commonly detected pathogens, aEPEC and *C. jejuni/coli*, warrants further research, particularly to help explain how animal-keeping and sanitation practices affect transmission of fecal pathogens that were highly prevalent in chicken feces.

INTRODUCTION

Livestock keeping is common among poor households in low- and middle-income countries (LMICs), where animals support peoples' livelihoods by providing a source of income, savings, food, transport, and manure for fuel and fertilizer.[1,2] Production of small livestock, particularly chickens, has been incorporated into nutrition-sensitive interventions to promote consumption of nutrient-dense animal-source foods, increased income, and improved child health outcomes.[3] Yet domestic livestock are a major source of enteropathogenic organisms, such as non-typhoidal *Salmonella*, *Campylobacter*, and certain *Escherichia coli* (*E. coli*) pathotypes, that contribute to the global burden of childhood diarrhea.[4,5] Importantly, *Campylobacter* infection has been linked to linear growth faltering, even among asymptomatic children.[6] Recent large-scale household-level water, sanitation, and hygiene (WASH) efficacy trials were ineffective in preventing enteric infections in children[7] or eliminating *E. coli* contamination of the household environment,[8] prompting calls for greater attention to domestic animal fecal contamination as an important source of enteropathogen exposure.[9]

The classic F-diagram depicts fecal-oral transmission routes of enteric pathogens from human feces to new human hosts (via fluids, fields, flies, fingers, fomites [e.g., toys, cooking utensils], and food).[10] The transmission of zoonotic enteropathogens from animal feces occurs via similar pathways: animal feces can contaminate water sources, agricultural fields, household soil, food, flies, and fomites, and direct contact with animal feces or any of these sources can lead to ingestion of pathogens.[11] These transmission pathways are particularly relevant in LMIC settings where there is little physical separation between domestic livestock and humans and where manure is used for fuel, fertilizer, or housing materials.[11–14] Several studies have confirmed that domestic livestock and livestock feces in the household environment contribute to microbial contamination of household surfaces, soil, drinking water, food, and caregivers' hands in distinct settings in Southeast Asia, South America, and East and West Africa.[15–20] Young children may be particularly at risk of enteropathogen infection from environmental contamination given their frequent hand-to-mouth and exploratory behaviors.[21] Indeed, direct observational studies have shown that infants engage in geophagy and even consumption of chicken feces in normal day-to-day behavior.[22–26]

Zoonotic enteropathogens have distinct livestock reservoirs and transmission pathways. Data suggest that cattle and other ruminants are the primary hosts of Shiga toxin–producing *E. coli* (STEC),[4,27] which can be transmitted to humans via exposure to ruminant feces, direct contact with ruminants, and consumption of contaminated meat, milk, and water.[28] Atypical enteropathogenic *E. coli* (aEPEC) is present in the feces of many domestic animals including cattle, sheep, pigs, and chickens,[4,29,30] with evidence of transmission to children,[29] though the pathogenicity of animal aEPEC strains remains unclear.[4,27] Both non-typhoidal *Salmonella* and *Campylobacter* have many animal reservoirs (primarily poultry and cattle) and are transmitted through foodborne routes, mainly via consumption of contaminated poultry meat and dairy products.[4,31] *Campylobacter* can also be transmitted via direct contact with infected ruminants and poultry and by ingestion of contaminated water.[32]

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Although *Salmonella* can be spread by person-to-person fecal-oral transmission,[4,31] *Campylobacter* transmission is predominantly zoonotic.[31] Although much of this evidence is derived from studies conducted in European countries, the United States, Canada, Australia, and other high-income countries,[27,28,32] it provides insights into these pathogens' reservoirs and transmission pathways. Nevertheless, there is need to critically evaluate how such pathogen–host relationships may be different in other environmental and social contexts.

A growing body of evidence has linked household livestock ownership in LMICs to enteropathogen infections in young children, including in Ecuador,[29,30] Peru,[33] Egypt,[34] Ethiopia,[14] and Lao People's Democratic Republic.[35] Four of these studies found that exposure to household chickens and their feces was associated with *Campylobacter jejuni* infection in children,[14,29,33,34] suggesting that in low-resource settings, direct fecal exposure is an important transmission pathway for *Campylobacter* infection. The other two studies combined livestock species as an exposure, limiting interpretation of individual zoonotic transmission pathways.[30,35] In Ghana, where this study was conducted, household livestock ownership is common, yet research to date on zoonotic transmission from livestock has focused on infections in adult farmers.[36] The present study builds on prior work by assessing the infectious risks of domestic livestock exposure to children. In particular, we evaluated species-specific transmission risks for individual zoonotic pathogens, recognizing that aEPEC, STEC, *Campylobacter*, and *Salmonella* have distinct animal reservoirs and transmission pathways.

The aim of this study was to determine whether ownership of livestock, exposure to livestock feces in the home environment, and animal-source food consumption, were associated with bacterial enteropathogen infections in children 6–59 months old in Ghana. Given the ubiquity of free-roaming chickens in Ghanaian households, we hypothesized that poultry ownership and fecal contamination in the home environment, measured by household observation, would be associated with *Campylobacter* infections in young children.

MATERIALS AND METHODS

Study design. This cross-sectional study of preschool-age children was conducted in the Greater Accra Region, Ghana, from October to November 2018. To be eligible for inclusion in the study, children had to be 6–59 months old, and their primary caregiver, usually their mother, had to be at least 18 years old. This study analyzed a subsample of children from a larger study investigating livestock ownership and anemia.[37] Briefly, children were sampled in 18 communities within the Ga East and Shai Osudoku Districts of Greater Accra, with communities purposefully selected for variation in types of livestock being reared and community size. Ga East is a primarily urban district with about 6% of its population engaged in agriculture.[38] In contrast, three-quarters of the population of Shai Osudoku lives in rural areas, with 34% of its population is engaged in agricultural activities.[39]

From the main study sample of 484 children, 430 (88.8%) had a stool sample collected (Supplemental Figure 1). Of those with available stool samples, 265 (61.6%) were selected for fecal analysis in a two-step process based on financial constraints of conducting enteropathogen testing. First, all children with collected stool samples and who resided in chicken-owning households from which chicken stool was also collected were included ($N = 163$). Second, 102 of 267 (38.2%) additional children with collected stool samples and who were from non-chicken-owning households were included by random selection using a random number generator.

Survey data collection. Trained enumerators conducted interviews with the primary caregiver of each index child, most often the mother, using electronic tablets (Samsung Galaxy Tab A, Model Number SM-T285, Samsung Electronics Co., Suwon, South Korea) and the Qualtrics survey platform (Qualtrics, Provo, UT). Data were collected on household member demographic characteristics, household assets, the household's drinking water source, and sanitation and hygiene practices. Livestock ownership was assessed using a survey derived from the Living Standards Measurement Study (LSMS) Livestock Module.[40] Additional data were collected on livestock management practices, including whether livestock were free-roaming or confined during the day or night and where they were free-roaming or confined (e.g., in the yard, inside the house). Caregivers were also asked whether the index child consumed specific animal-source foods (e.g., fresh cow's milk, chicken meat) in the prior 3 months, and if yes, how frequently (ranging from less than once per month to two or more times per day). Caregivers were asked whether the child had experienced diarrhea ($\geq$ 3 loose stools in a 24-hour period) in the prior 7 days. During and after the interview, enumerators observed and recorded the household structure and cleanliness, and whether livestock entered the household living quarters, chickens were present around the household yard, and human and animal feces were present in the yard.

Stool sample collection and analysis. For children's stool sample collection, caregivers were provided with verbal instructions and a stool collection kit, which included a sterile fecal container (Sarstedt, Nümbrecht, Germany), a diaper or clean piece of paper, gloves, and a plastic bag to store the stool sample. Caregivers were instructed to collect their child's first morning stool, and to have the child defecate in either a diaper, for children under 24 months old, or on a clean piece of paper, for older children. Stool samples were collected each morning by the field team and stored in a cooler box with ice packs. If the child's stool sample was improperly collected or stored, caregivers were provided with a new stool collection kit and the field team followed up with the caregivers to collect a new stool sample in the following days.

One chicken stool sample per household was collected from households that reported owning chickens. After obtaining permission from a household member to collect a stool sample, a member of the field team set up a closed-top pen measuring 29-by-29-by-17 inches in the household yard (Supplemental Figure 2). The bottom of the pen was covered by a sheet of newspaper and a handful of corn feed was provided inside. The entrance of the pen was left open until one chicken from the household's flock entered the pen, upon which the field team member closed the entrance to keep the chicken inside the pen until it defecated. After the chicken was released from the pen, the field team member collected the stool sample into a sterile fecal container using the collection scoop attached to the fecal container

cap. If a chicken did not enter the pen, a fresh stool sample was taken from chickens where they were roaming, or if no chickens were near the household, a fecal sample was collected from where chickens had roosted the night before (e.g., the chicken coop). Chicken stool samples were stored in a small cooler with ice packs.

Child and chicken stool samples were transported to the Noguchi Memorial Institute for Medical Research (Accra, Ghana) every afternoon for processing. Each stool sample was manually homogenized while on ice and aliquoted into a sterile 2 mL cryovial (Corning, Corning, NY) using sterile techniques. Samples were stored at −80°C until DNA extraction. In separate batches for child and chicken stool samples, microbial nucleic acid was extracted using the QIAamp PowerFecal® DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For children's stool, approximately 250 mg of sample was used for extraction, while approximately 115 mg of sample was used for extraction from chicken stools. In the final extraction step, 100 μL of DNA was eluted into an Eppendorf™ DNA LoBind microcentrifuge tube (Eppendorf, Hamburg, Germany). An extraction blank that went through all DNA extraction steps but without any addition of stool sample was included in each extraction batch to control for laboratory contamination. Total DNA concentration and purity were measured using a NanoDrop™ 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA). DNA samples were then transported to the University of Michigan (Ann Arbor, MI) and stored at −80°C until enteropathogen analysis.

DNA samples were analyzed using probe-based quantitative polymerase chain reaction (qPCR) for the identification of zoonotic enteropathogens using the following gene targets: *cadF* for *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), *eae* and *bfpA* for atypical enteropathogenic *E. coli* (aEPEC), *ttr* for *Salmonella enterica*, and *stx1* and *stx2* for Shiga toxin–producing *E. coli* (STEC). Primer and probe sequences for each gene target were derived from Liu et al.[41] and validated for specificity using the National Institutes of Health (NIH) Nucleotide Basic Local Alignment Search Tool (blastn) Version 2.10 (Supplemental Table 1).[42] Child and chicken DNA samples and extraction blanks were diluted 1:10 and run single-plex in triplicate or quadruplicate on 384-well plates using the QuantStudio™ 5 System (Applied Biosystems™, Foster City, CA). Each amplification well contained 4.5 μL 1:10-diluted DNA, 5.0 μL TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Foster City, CA), and 0.5 μL of primer-probe mixture at a final concentration of 500:250 nM primer:probe. All probes were double-quencher probes with a 5′ 6-FAM™ fluorophore, internal ZEN™ quencher, and 3′ Iowa Black® Fluorescent Quencher (purchased from Integrated DNA Technologies, IDT). Each reaction plate also included a water control that replaced DNA with ddH_2O to control for reagent contamination. None of the extraction blanks or water controls amplified. Samples went through the following cycling conditions: 95°C for 10 minutes followed by 45 amplification cycles of 95°C for 15s and 60°C for 1 minute. For the *cadF* amplicon, annealing and extension was at 58°C for 1 minute.

Cycle threshold (Ct), the cycle number at which the PCR product can be detected above the background signal, was determined for each sample replicate using the Thermo Fisher Connect Design and Analysis Software Version 2.5 (Thermo Fisher Scientific, Carlsbad, CA). The Ct value is inversely associated with the amount of pathogen in the sample. The threshold line for each plate was determined by including a repeated positive child sample on each child and chicken sample plate to ensure comparability between plates for each gene target. Technical replicates for each sample were inspected if the SD between replicates was ≥ 0.5, and when warranted, outliers were manually removed. For each sample, the Ct value was calculated as the average of amplified replicates. Samples with $\leq 50\%$ amplification of replicates were classified as negative for that gene. A Ct cutoff of ≤ 35 was applied to define positive detection of a target gene for child samples. This cutoff was chosen as the lower bound at which incongruent amplification between replicates was observed, indicating the technical limits of the assay's precision. A higher cutoff ($Ct \leq 40$) was applied to chicken samples given that chicken samples had on average higher Ct values than child samples, possibly due to lower initial sample input and lower purity of the samples (child A260/280 mean $\pm$ SD: 1.8 ± 0.1, chicken: 1.7 ± 0.4). Positivity for each enteropathogen was defined according to the presence and absence of target genes as follows: aEPEC (*eae* without either *bfpA*, *stx1*, or *stx2*), *C. jejuni/coli* (*cadF*), STEC (*eae* with either *stx1* or *stx2*, or both, and without *bfpA*), and *Salmonella* (*ttr*).

Statistical analysis. Descriptive analyses were conducted of child- and household-level characteristics, and of livestock management, livestock observations, and household hygiene behaviors. Means and SDs were calculated for normally distributed continuous variables, whereas medians and interquartile ranges (IQR) were used for non-normally distributed variables. The prevalence of zoonotic enteropathogen detection was assessed in children and household chickens. We also determined the number of pairs of children and chickens that had detection of the same pathogen within a household.

The primary outcome variables were positive detection ($Ct \leq 35$) of *C. jejuni/coli*, aEPEC, and STEC in children. Because *Salmonella* was detected in $< 1\%$ of children, it was not analyzed further. Livestock-related risk factors were examined to explore hypothesized associations between exposure to livestock through ownership of distinct livestock species (i.e., cattle, goats or sheep, and poultry), through exposure to livestock feces in the home environment (i.e., observation of livestock feces in the household yard and pathogen detection in household chicken feces), and through frequent consumption of a high-risk animal-source foods (i.e., fresh cow's milk, chicken meat). Frequent consumption of fresh cow's milk and poultry meat was defined as a child having consumed the animal-source food one or more times per week, on average, during the prior 3 months. Pig ownership was not examined as a predictor as ownership was rare ($< 3\%$).

Potential confounding variables were selected *a priori* based on the literature,[30,35] and included child sex and age, caregiver highest attained education, and the household's size, asset-based wealth quintile, drinking water source, and latrine facility. The child's date of birth was verified by enumerators using their clinical Child Health Records. An improved drinking water source, defined according to the WHO and the United Nations Children's Fund (UNICEF) Joint Monitoring Program for Water Supply and Sanitation (JMP)

guidelines, includes any of the following: piped water, public pipe/standpipe, tube well or borehole, protected dug well, protected spring, bottled water, sachet (packaged) water, cart with tank, and rainwater.[43] We created a household asset-based wealth index score using principal components analysis (PCA) of 26 assets, excluding land holdings, livestock, and WASH indicators.[44] Asset scores from the first component were categorized into quintiles and used to characterize household wealth from lowest to highest. Household size was not included in the final adjusted models as it was not associated with any of the outcomes in bivariate analyses and inclusion of this variable in the model did not meaningfully change effect estimates. Also, asset-based wealth quintile was not included in the final adjusted models due to the low number of observations in certain wealth quintiles for *C. jejuni/coli* and STEC infection. Furthermore, inclusion of asset-based wealth quintile did not meaningfully change results from the final adjusted models.

Unadjusted and adjusted logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between the livestock-related exposures and child enteropathogen infection, with robust standard errors clustered at the community level. Adjusted models included the covariates described earlier, as well as a fixed effect for district. Adjusted models examining cow's milk consumption as the main predictor also included cattle and goat or sheep ownership as covariates. We also examined associations between enteropathogen detection and caregiver-reported diarrhea, adjusting for child age and sex, household open defecation, maternal education, and district.

Data cleaning and statistical analysis were conducted using Stata SE version 14.2 (StataCorp, College Station, TX). Statistically significant associations are reported at the $P < 0.05$ level.

Ethical considerations. This study was approved by the University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board (protocol no. HUM00145171), the University of Michigan Institutional Animal Care and Use Committee (protocol no. 00008493), and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18). Informed written consent was provided by the index child's caregiver for their child's participation in the study and for their participation in the interview with a signature, or with a thumbprint and with a witness' signature. Households were given a small, nonmonetary gift as compensation for their participation in the study. Each study field team member was trained in animal ethics prior to the start of the study.

RESULTS

Child and household characteristics. Overall, of the 265 children selected in the subsample, 259 were included in the analysis after excluding children from poultry farms ($N = 2$) and those with missing data on age ($N = 1$) and caregiver education ($N = 3$). Children were, on average, 27.5 months old (range 6.2–57.9 months), and approximately half (48.3%) were female (Table 1). Most households reported using an improved water source for drinking water, primarily a public tap/standpipe (45.2%) or packaged (sachet) water (44.0%), whereas seven households (2.7%) used an unimproved drinking water source, mainly surface water. Almost one-third (30.5%) of households practiced open defecation, whereas 59.1% used a pit latrine and 10.4% used a flush toilet. One-quarter of primary caregivers (mothers) had no formal education or up to nursery education, whereas over half had completed junior education or higher.

Household livestock ownership and management characteristics. Among this purposefully selected sample of chicken- and non-chicken owning households, a total of 61% of households owned livestock (Table 1). Of households that owned livestock, 62.9% owned only chickens and other poultry (guinea fowl, turkeys, and ducks) while the others owned poultry as well as small ruminants, pigs, or cattle. Poultry-owning households reared a median of 12 poultry (IQR: 6, 25), and goat, sheep, and cattle-owning households reared a median of five goats (IQR: 2, 10), 10 sheep (IQR: 5, 18), and 70 cattle (IQR: 42, 100), respectively. Cattle were reared solely in the Shai Osudoku district. Small ruminants and poultry were reared in both districts, though the number of sheep and goats per household was higher in Shai Osudoku (median: 7, IQR: 2, 16) compared with Ga East (median: 4, IQR: 2.5, 6).

Chickens were almost entirely free-roaming in the yard or compound of most households during the day, while about two-thirds of goats and sheep were free-roaming (Supplemental Table 2). About two-thirds of households confined chickens in a coop at night, and only four households reported keeping chickens inside the household dwelling at night. Fifty-four percent of households confined goats in a pen during the night and 66.7% of households penned their sheep at night. Most cattle-owning households moved cattle out of the yard or compound during the day, but kept them confined in fenced corrals at night.

Livestock and livestock feces were observed in household yards of both livestock- and non-livestock-owning households among this purposefully selected sample. Chickens were observed free-roaming in the yards of 97.5% of livestock-owning households and animal feces were observed in the yards of 93.7% of these households. Among non-livestock owning households, chickens were observed in 76.3% of households' yards and animal feces were observed in 67.4% of yards. Enumerators also observed chickens entering people's living quarters in 59.0% of livestock-owning households and 36.1% of non-livestock owning households.

Household hygiene characteristics. In observational spot-check surveys, enumerators observed rubbish in the yards of 43.6% of households, gray (waste) water pooled in 11.8% of yards, and human feces lying in 5.1% of yards (Supplemental Table 2). Half of caregivers reported that their child spent the majority of the day in the household yard, while 25.1% of children spent the day at daycare or school and 17.4% traveled with their mothers to another household, to an agricultural field, or to another location during the day. Fifty-seven percent of households also cooked outside in the household yard, whereas others reported cooking inside the house or another building.

Enteropathogen detection in stools of children and chickens. Zoonotic enteropathogens were detected in over half (55.2%) of children's stool samples (Table 2). In particular, aEPEC was detected in 45.6% of stool samples, *C. jejuni/coli* in 11.6%, and STEC in 4.3%. *Salmonella* was

TABLE 1
Child and household-level characteristics of study sample in Greater Accra Region Ghana, October and November 2018 ($N = 259$)

Characteristics	Value
Child-level characteristics	
Female; *n* (%)	125 (48.3%)
Age, months; mean (SD) [range]	27.5 (13.9) [6.2–57.9]
Caregiver-reported diarrhea in past 7 days; *n* (%)	16 (6.2%)
Fresh cow's milk consumption ≥ 1 time/week; *n* (%)	19 (7.3%)
Chicken meat consumption ≥ 1 time/week; *n* (%)	123 (47.5%)
Household-level characteristics	
Number of household members; mean (SD) [range]	5.2 (2.1) [2–17]
Number of children < 5 years; mean (SD) [range]	1.3 (0.6) [1–4]
Female-headed household; *n* (%)	55 (21.2%)
Caregiver education level; *n* (%)	
None or nursery	65 (25.1%)
Primary	54 (20.9%)
Junior	106 (40.9%)
Senior or higher	34 (13.1%)
Drinking water source; *n* (%)	
Piped water (into dwelling)	3 (1.2%)
Piped water (into yard/compound)	10 (3.9%)
Public tap/standpipe	117 (45.2%)
Tube well or borehole	3 (1.2%)
Unprotected dug well	1 (0.4%)
Protected spring	1 (0.4%)
Rain water	4 (1.5%)
Surface water	6 (2.3%)
Sachet water	114 (44.0%)
Improved drinking water source; *n* (%)	252 (97.3%)
Latrine facility	
Flush or pour flush	27 (10.4%)
Pit latrine	153 (59.1%)
No toilet/open defecation	79 (30.5%)
Access to electricity; *n* (%)	225 (86.9%)
District; *n* (%)	
Ga East	87 (33.6%)
Shai Osudoku	172 (66.4%)
Livestock ownership	
Household owns any livestock; *n* (%)	158 (61.0%)
Household owns cattle; *n* (%)	13 (5.0%)
Household owns goats; *n* (%)	39 (15.1%)
Household owns sheep; *n* (%)	15 (5.8%)
Household owns pigs; *n* (%)	7 (2.7%)
Household owns chickens; *n* (%)	157 (60.6%)
Household owns turkeys, guinea fowl, or ducks; *n* (%)	21 (8.1%)
Total number of livestock, among owners; median (IQR) [range]	16 (8, 30) [1–212]
Number of cattle, among owners; median (IQR) [range]	70 (42, 100) [8–120]
Number of goats, among owners; median (IQR) [range]	5 (2, 10) [1–60]
Number of sheep, among owners; median (IQR) [range]	10 (5, 18) [2–45]
Number of poultry, among owners; median (IQR) [range]	12 (6, 25) [1–85]

IQR = interquartile range.

TABLE 2
Prevalence of enteropathogens in children 6–59 months old and household chickens, and the number of children and chicken pairs from the same household with a shared enteropathogen detection in Greater Accra, Ghana (October and November 2018)*

Pathogen	Children ($N = 259$)	Chickens ($N = 156$)	Child and chicken pairs†
aEPEC	118 (45.6%)	60 (38.5%)	33/156
STEC	11 (4.3%)	18 (11.5%)	3/156
C. jejuni/C. coli	30 (11.6%)	70 (44.9%)	7/156
Salmonella	2 (0.8%)	6 (3.9%)	0/156

aEPEC = atypical enteropathogenic *Escherichia coli*; *C. jejuni/coli* = *Campylobacter jejuni* or *Campylobacter coli*; STEC = Shiga toxin–producing *E. coli*.

* Values are *n* (%). Ct cutoff for enteropathogen detection in children's stool is Ct ≤ 35 and in chickens' stool is Ct ≤ 40.

† Value indicates the total number of pairs in which a child and chicken from the same household were both positive for a pathogen (e.g., in 33 households out of the 156 chicken-owning households, children and chickens both tested positive for aEPEC).

detected in only two children's stool samples. Children's diarrheal prevalence in the preceding 7 days was low (6.2%). Enteric infections were predominantly asymptomatic, with no diarrhea reported among children with *C. jejuni/coli* infection and 4.2% of children positive for aEPEC having had diarrhea in the prior 7 days. However, STEC infection was associated with higher odds of diarrhea (OR: 10.37, 95% CI: 4.04, 26.65; P value < 0.001). Children positive for *C. jejuni/coli* were on average younger (≈22 months old) than those negative for *C. jejuni/coli* (≈28 months old). Conversely, children positive for STEC were on average 36 months old, compared with about 27 months for children negative for STEC. STEC was detected only among children living the Shai Osudoku district, while aEPEC and *C. jejuni/coli* were

detected in both districts. There was also a higher prevalence of STEC detection in children from households that practiced open defecation (10.1%) compared with those using a pit latrine or flush toilet (1.7%) (unadjusted OR: 6.65, 95% CI: 2.31, 19.13; P value < 0.001).

Chicken stool samples were analyzed from all but one of the 157 households that reported owning chickens. Seventy-one percent of chickens carried at least one enteric pathogen (Table 2). *Campylobacter jejuni/coli* was most prevalent, detected in 44.9% of chicken stool samples. aEPEC was detected in 38.5% of chicken stool samples, whereas STEC was detected in 11.5% and *Salmonella* was detected in 3.9%. The number of instances in which children and chicken pairs from a household carried the same pathogen in their stool samples are shown in Table 2. Household cattle ownership was positively associated with STEC detection in chicken stool (OR: 3.85, 95% CI: 1.48, 10.05; $P = 0.006$), after controlling for open defecation and district, but not with detection of other enteropathogens.

Associations between livestock-related risk factors and enteropathogen infection in children. Several livestock-related risk factors, including exposure to livestock through ownership, exposure to livestock feces, and consumption of animal-source foods, were examined for associations with enteropathogen infection in children (Table 3). None of the livestock-related risk factors were associated with *C. jejuni/coli* nor aEPEC infection in children. However, in adjusted analyses, ownership of goats or sheep was associated with 4.30 times higher odds of STEC infection in children (95% CI: 1.32, 14.08; P value: 0.016). Ownership of poultry with other livestock (cattle, goats, sheep, or pigs) was also associated with higher odds of STEC infection, while ownership of only poultry was not.

Cattle ownership was also associated with higher odds of STEC detection in unadjusted analyses (OR: 4.79, 95% CI: 1.20, 19.17; P value $= 0.027$), but not after controlling for household drinking water source, household open defecation, caregiver education, and child age and sex. However, consumption of fresh cow's milk at least once per week was associated with 8.88 times higher odds of STEC detection in children (95% CI: 3.72, 21.17; P value < 0.001). After adjusting for child- and household-level covariates as well as ruminant ownership, this association was attenuated (OR: 3.03, 95% CI: 1.75, 5.24); $P < 0.001$), but still highly significant. Goat or sheep ownership remained significantly associated with higher odds of STEC detection independent of cow's milk consumption (OR: 3.71, 95% CI: 1.26, 10.89; P value: 0.017), while the association between STEC and cattle ownership was attenuated (OR: 1.34, 95% CI: 0.15, 11.62) (Supplemental Table 3). Among the 19 children who consumed fresh cow's milk at least weekly, four were STEC positive. Of these, one child consumed milk twice per week, one child consumed milk 3–4 times per week, and two children consumed milk once or more per day. In two of these

TABLE 3
Unadjusted and adjusted OR and 95% CI for the associations between livestock-related risk factors and zoonotic enteropathogen infection in children 6–59 months old in Greater Accra, Ghana ($N = 259$)†

	C. jejuni/coli		aEPEC		STEC	
Risk factors	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Presence of livestock						
Cattle ownership	–	–	0.51 (0.21, 1.26)	0.63 (0.23, 1.72)	4.79* (1.20, 19.17)	4.32 (0.74, 25.10)
Goats or sheep ownership	1.32 (0.54, 3.22)	1.59 (0.59, 4.29)	0.92 (0.50, 1.71)	0.89 (0.45, 1.76)	5.56** (1.64, 18.92)	4.30* (1.32, 14.08)
Poultry ownership, with other livestock	1.37 (0.64, 2.95)	1.58 (0.67, 3.73)	1.05 (0.59, 1.85)	1.00 (0.54, 1.84)	7.11** (1.76, 28.65)	6.31* (1.27, 31.22)
Poultry ownership only	0.65 (0.34, 1.25)	0.63 (0.31, 1.26)	1.34 (0.79, 2.27)	1.37 (0.81, 2.32)	0.58 (0.12, 2.84)	0.57 (0.09, 3.38)
Presence of animal feces						
Pathogen detected in chicken stool ($N = 156$)	0.84 (0.31, 2.27)	0.73 (0.27, 1.96)	1.51 (0.92, 2.48)	1.36 (0.76, 2.41)	3.74* (1.02, 13.76)	7.85*** (2.54, 24.30)
Animal feces observed around household ($N = 256$)	0.49 (0.16, 1.52)	0.51 (0.16, 1.65)	0.65 (0.33, 1.26)	0.71 (0.34, 1.50)	–	–
Livestock-derived food consumption						
Fresh cow's milk $\geq$ 1 time/week‡	0.89 (0.38, 2.06)	1.10 (0.41, 2.94)	0.53 (0.17, 1.64)	0.55 (0.17, 1.80)	8.88*** (3.72, 21.17)	3.03*** (1.75, 5.24)
Chicken meat $\geq$ 1 time/week	0.83 (0.36, 1.88)	1.03 (0.40, 2.66)	1.06 (0.66, 1.70)	1.17 (0.67, 2.05)	1.34 (0.83, 2.18)	1.60 (0.84, 3.03)

aEPEC = atypical enteropathogenic *Escherichia coli*; *C. jejuni/coli* = *Campylobacter jejuni* or *Campylobacter coli*; CI = confidence intervals; OR = odds ratios; STEC = Shiga toxin–producing *E. coli*.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Values are OR (95% CI) using logistic regression models. Adjusted logistic regression models control for child sex, child age (months), household uses improved drinking water source (aEPEC and STEC only), household practices open defecation, caregiver highest attained education, and district (*C. jejuni/coli* and aEPEC only). Dash (–) indicates that the logistic model could not run due to complete separation. Robust standard errors are clustered at community level in unadjusted and adjusted models.

‡ Adjusted for covariates above and cattle and goat or sheep ownership in aEPEC and STEC models.

cases, caregivers reported that children consumed milk boiled, whereas two said children consumed milk raw. Among all children consuming cow's milk, approximately half of these children (52.6%) drank cow's milk raw, whereas the other half (47.4%) consumed milk boiled.

DISCUSSION

This cross-sectional study examined associations between livestock-related risk factors and zoonotic enteropathogen infections in children 6–59 months old in Greater Accra, Ghana. Fifty-five percent of children had one or more zoonotic enteropathogens in their stool, yet only STEC, detected in 4% of children, was associated with recent diarrhea. STEC infection in children was associated with owning ruminants and with consumption of fresh cow's milk. For aEPEC or *C. jejuni/coli* infection, however, there were no associations with livestock ownership, exposure to livestock feces, or consuming animal-source foods. Nevertheless, half of household chickens were reservoirs of *C. jejuni/coli* and about one-third carried aEPEC in their feces. Fecal contamination from livestock in household yards was widespread, and animal feces and free-roaming livestock were present even in the yards of households that owned no livestock.

These findings suggest that ruminant exposure may contribute to STEC infection in Ghanaian children, particularly in the Shai Osudoku district. According to the 2010 Ghana census survey, there were one-tenth as many cattle reared in Ga East (≈2,600)[38] compared with Shai Osudoku (≈28,300),[39] which supports our observations that cattle rearing is a predominant livestock activity where STEC was detected in children. However, in this study, sheep and goat ownership, but not cattle ownership, was associated with higher odds of STEC infection in children. It is possible that the lack of association with cattle ownership is due to the small number of cattle-owning households sampled (13/259). Since most household cattle were not free-roaming in the yard and confined at night, it is also possible that children had less direct contact with cattle and their feces than with small ruminants. Small ruminants are important reservoirs of STEC in addition to cattle,[45,46] and contact with goats and sheep at petting zoos and farms has been documented as a source of STEC outbreaks in children in North America.[47] Given that over two-thirds of households allowed goats and sheep to free-roam around the household yard during the day, ruminant fecal contamination in the home environment is a likely exposure route for children. Nevertheless, because we did not test cattle, goat, or sheep feces for enteropathogens, we could not determine whether ruminant feces were sources of STEC in the study communities. Secondary transmission of STEC can also result from asymptomatic adults and children shedding STEC in their feces.[46] In our study, open defecation was associated with STEC detection in children, suggesting that person-to-person transmission may also be important in this region of Ghana.

Although chickens are not considered to be a major zoonotic reservoir of STEC,[46,48] STEC was detected in 12% of chicken fecal samples in our study (78% of these were detected in Shai Osudoku and 22% in Ga East). In comparison, STEC was identified in only 1–2% of chicken fecal samples in Ecuador households[29,30] and 6% of Burkina Faso local markets.[49] Although chickens are not natural reservoir hosts of STEC (i.e., they are not able to maintain STEC colonization in the absence of reexposure),[46] they are potential spillover hosts that harbor and shed STEC in their feces.[45,48] As with STEC spillover to wild animals living near livestock operations,[48] positive chicken feces may result from chickens residing in environments with high-densities of ruminant fecal contamination. Indeed, we found that household cattle ownership, but not goat or sheep ownership, was positively associated with STEC detection in chicken feces. We identified associations between STEC infection in children and STEC detection in chicken feces independent of cattle ownership, which may suggest that chicken fecal contamination is a source of STEC exposure in cattle-rearing communities.

Contaminated food products, particularly poultry meat and unpasteurized dairy, are a major source of STEC, *C. jejuni* and *C. coli*, and *Salmonella* exposure.[32,50,51] In our study, we did not find associations between poultry meat consumption and infection, nor between cow's milk consumption and *C. jejuni/coli* or aEPEC infections. However, children who consumed fresh cow's milk at least once per week were more likely to have STEC infection than children who did not consume fresh milk or consumed it infrequently. Four cases of STEC were associated with consumption of fresh cow's milk, in which two children consumed milk raw and two consumed it boiled. Interestingly, the milk was not directly sourced from own-produced cattle; rather, caregivers reported purchasing the cow's milk or receiving the milk as a gift. Consumption of unpasteurized milk and dairy products in high-income countries has been linked to STEC outbreaks, mainly of STEC O157:H7,[27,52] and was identified as a risk factor for STEC infection in children under 3 years old in Germany.[53] In Accra, Ghana, fecal coliforms and *E. coli* have been identified in both boiled and raw milk and milk products sold by dairy vendors.[54–56] These investigations, along with our findings, suggest that informal sharing and selling of fresh cow's milk, even if milk is boiled, may be a risk factor for STEC infection in children.

No associations between livestock ownership and *C. jejuni/coli* infection in children were found in our study, despite detecting *C. jejuni/coli* in almost half the sampled chicken feces. Chickens are known to be a primary source of *Campylobacter* infections in humans,[32] and many other epidemiologic studies have found associations between chicken ownership and *C. jejuni* infection in children.[5,14,29,30,34] It is therefore surprising that results from our study did not corroborate this relationship. The extent of interaction between children and animals may have modified associations between ownership and enteropathogen infection. For example, Lowenstein et al.[30] did not find an association between the presence of animals in the home and enteropathogen detection in children under 5 years old in Ecuador, yet among livestock owners, children who regularly interacted with animals were at higher risk of enteropathogen carriage. Similarly, Budge et al.[14] found that keeping animals indoors at night was associated with higher odds of *Campylobacter* infection in Ethiopian infants. Also in Ethiopia, Headey and Hirvonen[12] found positive associations between poultry ownership and height-for-age Z-scores (HAZ) in children under 5 years old, yet negative associations with HAZ when chickens were kept in the household dwelling at night. In our study, only four households reported keeping poultry indoors at night, which

precluded us from investigating differences in infection by this type of livestock management. Person-to-person *Campylobacter* transmission is possible, but rare,[31] and we did not find an association between open defecation and *C. jejuni/coli* infection in children.

For aEPEC infections in children, no associations with livestock-related risk factors were found, despite a high infection prevalence in household chicken feces. aEPEC has been detected in household chickens in Ecuador,[29,30] though at lower prevalences than detected in our sample (7% and 9% versus 39%). There is some evidence of aEPEC transmission between animals and humans,[29,57] but it is unclear whether animal feces are the source of this aEPEC.[4] In our study, open defecation was associated with aEPEC in children, suggesting that chickens may have acquired aEPEC from human shedding, or that human-to-human transmission plays a more important role in childhood infections than zoonotic transmission. Further research on the pathogenicity and transmission of aEPEC from animal versus human reservoirs is warranted.

This study has several important limitations. First, because the design is cross-sectional, we cannot infer causality between the examined livestock risk factors and child infection. We did not test ruminant fecal samples for enteropathogens and therefore cannot confirm whether ruminants are a source of STEC exposure in this setting, despite the biologic plausibility. This limits our confidence in attributing STEC infection in children to these animals. Future investigations should test ruminant feces and milk, as well as other potential environmental sources, such as drinking water and household soil, to better evaluate transmission routes in this setting. The use of microbial source tracking methods and genomic sequencing would also provide stronger evidence of animal-to-human transmission pathways.[58] Nevertheless, our testing of household chicken feces demonstrated certain zoonotic enteropathogens to which these children may be exposed. Last, we purposefully selected communities that reared livestock, so the prevalence of zoonotic enteropathogens in children in our sample may overestimate that of other children in the region.

In conclusion, our study found that aEPEC and *Campylobacter* are prevalent in both children and chickens in Ghanaian households, but there is no clear evidence of an association between the two. Conversely, though STEC prevalence was less than 5% in children, we identified several ruminant-related risk factors that may expose children to STEC. Although STEC infection related to ruminant exposure and consumption of contaminated food products has been extensively documented in many high-income countries, it remains understudied on the African continent.[59,60] Despite its sporadic incidence, STEC can cause mild to more severe bloody diarrhea in children, and possibly hemolytic uremic syndrome (HUS), yet surveillance in sub-Saharan Africa is limited.[27] Given the importance of ruminants to the livelihoods of many low-income households in Ghana and other African countries, further research on mitigating exposure to livestock reservoirs of zoonotic pathogenic bacteria is warranted. Several studies have documented ruminant contamination in household environments and drinking water,[16,18,20] thus improved handwashing facilities and behaviors in addition to point-of-use water treatment are critical to reducing children's exposure to ruminant zoonotic pathogens. Furthermore, given the risk of STEC infection from consuming raw milk, efforts should be made to ensure that fresh milk, whether sold formally or informally, meets sanitary standards. Finally, our study emphasizes the need for livestock management interventions that are context- and species-specific. Although we did not find associations between livestock ownership, fecal exposure, or animal-source food consumption and *Campylobacter* infection in children, 12% of children had *C. jejuni/coli* detected in their stools. Since *Campylobacter* infection is a significant contributor to diarrhea and growth stunting in young children in LMICs,[6,61] further longitudinal studies are needed to understand predominant exposure sources to prevent transmission. Exposure routes that are important in some contexts (e.g., chickens housed indoors) may not be as relevant in other locations depending on the animal-rearing practices of households in that community. Interventions that seek to promote livestock production may therefore need to examine predominant sources of pathogen exposure to determine which animal management and hygiene interventions to prioritize.

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