Understanding the Role of Livestock Ownership on Anemia among Young Children in Ghana

by

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Dedication

This dissertation is dedicated to my mom, Marleen Van Steenberge. Thank you for all of the sacrifices you made so that I could pursue a career in science, and for supporting me every step of the way.

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List of Abbreviations

AAT	α-1-antitrypsin
aEPEC	atypical enteropathogenic *Escherichia coli*
AGP	α-1-acid glycoprotein
ASF	animal-source food
BRINDA	Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia
C. coli	*Campylobacter coli*
C. jejuni	*Campylobacter jejuni*
CI	confidence interval
CRP	C-reactive protein
Ct	cycle threshold
DDS	dietary diversity score
DHS	Demographic Health Survey
E. coli	*Escherichia coli*
EAEC	enteroaggregative *Escherichia coli*
EED	environmental enteric dysfunction
EIEC	enteroinvasive *Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
HAZ	length/height-for-age Z-score
Hb	hemoglobin
HRP2	histidine-rich protein 2
IQR	interquartile range
LMIC	low- and middle-income country
LSMS	Living Standards Measurement Study
LT-ETEC	heat-labile enterotoxin-producing *Escherichia coli*
MAL-ED	Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health
MDD	minimum dietary diversity indicator
MPO	myeloperoxidase
NEO	neopterin
OR	odds ratio
pLDH	*Plasmodium* lactate dehydrogenase
qPCR	quantitative polymerase chain reaction
RBP	retinol binding protein

RDT	rapid diagnostic test
SD	standard deviation
SF	serum ferritin
SSA	sub-Saharan Africa
STEC	Shiga toxin-producing *Escherichia coli*
ST-ETEC	heat-stable enterotoxin-producing *Escherichia coli*
sTfR	serum transferrin receptor
tEPEC	typical enteropathogenic *Escherichia coli*
TLU	tropical livestock unit score
V. cholerae	*Vibrio cholerae*
WASH	water, sanitation, and hygiene
WHO	World Health Organization
WHZ	weight-for-length/height Z-scores
YLD	years lived with disability

Abstract

Anemia affects two-thirds of children under the age of five in Ghana, and remains challenging to address given that micronutrient deficiencies and infectious diseases can cause anemia. Household livestock ownership may alleviate anemia among children by providing a source of micronutrient-rich animal-source foods (ASFs), yet could exacerbate anemia by exposing children to enteropathogens. This dissertation investigated linkages between household livestock ownership and child anemia in southern Ghana, assessing both dietary and infectious disease pathways. We conducted a cross-sectional study of children aged 6 to 59 months old in the Greater Accra Region, Ghana. Survey data were collected to examine household livestock ownership and to assess children's dietary diversity and consumption of ASFs. Blood and stool samples from children were analyzed for indicators of iron status and inflammation, anemia, malaria, helminth infections, and bacterial enteropathogen infections.

Nearly half of children were anemic, and anemia was associated with iron deficiency, vitamin A deficiency, malaria, and systemic inflammation. Overall, we found that children from households that owned cattle, along with poultry and goats or sheep, but not those from households that owned only poultry, had lower odds of anemia compared to children from non-livestock-owning households. However, there was no evidence that this association was mediated by ASF consumption, despite finding that children from poultry-owning households were more likely to consume chicken meat and those from cattle-owning households more likely to consume cow's milk. There was also no difference in symptoms of illness, including diarrhea, fever, or cough, nor of inflammation, among children from households with livestock, compared

to those without livestock. Overall, less than one-tenth of children presented with diarrhea, yet bacterial enteropathogens were detected in 87% of children's stools, and 55% of children were positive for at least one zoonotic enteropathogen. Of the zoonotic enteropathogens, only Shiga toxin-producing *Escherichia coli* (STEC) infection was associated with livestock-related risk factors, including household goat or sheep ownership, exposure to STEC-positive chicken feces, and fresh cow's milk consumption. However, *Campylobacter jejuni/coli* and atypical enteropathogenic *E. coli* infection were not associated with owning livestock, exposure to livestock feces, or ASF consumption. Nevertheless, *C. jejuni/coli* and aEPEC were detected in 45% and 39% of chickens' feces, respectively, and livestock fecal contamination in the home environment was common, even among non-livestock-owning households. Examining associations between pathogen infections and anemia, we found that zoonotic infections (*C. jejuni/coli*, aEPEC, STEC) were not independently associated with iron deficiency or anemia, though *C. jejuni/coli* infection was associated with elevated systemic inflammation. However, children positive for enteroinvasive *E. coli/Shigella* and enteroaggregative *E. coli* had higher odds of anemia.

Overall, these studies demonstrate the complex relationships among livestock rearing, ASF consumption, enteropathogen infections, and anemia. Livestock ownership, particularly of cattle and small livestock, appears to provide non-diet mediated benefits that lower children's risk of anemia, yet also presents risks of infection through fecal exposure and foodborne routes. Furthermore, enteroinvasive bacterial pathogens contribute to anemia among young children. These complex relationships suggest that additional research is needed to understand and harness the potential benefits of livestock on children's health while mitigating risks of infectious disease. Finally, results presented in this dissertation highlight that reducing the anemia burden

among young children living in low-resource settings may require preventing enteropathogen exposures coming from both human and livestock sources.

CHAPTER 1 Introduction

Burden and pathophysiology of anemia in low- and middle-income countries (LMICs)

Anemia is a condition in which there is insufficient hemoglobin or red blood cells to meet the body's physiologic needs (1,2). Anemia is a significant public health concern, as children with anemia and iron deficiency are at greater risk of irreversible impairments in cognitive, motor, and socio-emotional development, of susceptibility to infection, and of mortality (3–5). Iron-deficiency anemia is the fourth leading cause of years lived with disability (YLD) globally and the leading cause in sub-Saharan Africa (6). Symptoms of anemia include fatigue, reduced concentration, pallor, dizziness, headache, and in young infants, poor feeding and irritability (7). Young children are especially vulnerable to developing anemia because of their physiologic needs for iron during early periods of rapid growth (1).

Anemia affects 42% of children under five years old globally, with this burden disproportionately higher among children in Africa (59%) and South-East Asia (51%) compared to other regions of the world (ranging from 9% to 35%) (8). In Ghana, the country of focus for this dissertation research, the most recent 2014 Ghana Demographic and Health Survey (DHS) reported that the prevalence of anemia was 66% among preschool age children (aged 6 to 59 months old), with regional prevalences ranging from 60% in the Greater Accra Region to 82% in the Northern Region (9). Although the prevalence of anemia among Ghanaian preschool age children has decreased by 14 percentage-points over the last two decades (8), it is still of severe public health significance (prevalence of 40% or higher) (10).

Anemia is caused by ineffective erythropoiesis (red blood cell production) or red blood cell loss/destruction, which can result from multiple factors including micronutrient deficiencies, infectious diseases and inflammation, or genetic hemoglobin disorders (1,2) (**Figure 1.1**). Hemoglobin and red blood cell production and function require several micronutrients including iron, folic acid, vitamin B12, and vitamin A, and inadequate intake or poor absorption of any of these nutrients can lead to nutritional anemias. Diets low in leafy greens, vitamin A-rich fruits and vegetables, and animal-source foods (which contain high concentrations of bioavailable heme iron, vitamin A, and vitamin B12) contribute to iron-deficiency and nutritional anemias (7,11). Additionally, malaria, soil-transmitted helminth infections, and other infectious and neglected tropical diseases are among the primary contributors to anemia in children under five globally (12). Soil-transmitted helminths, particularly hookworm (*Necator americanus* and *Ancylostoma duodenale*), cause gastrointestinal blood loss, while malaria (infection with *Plasmodium* spp. parasites) leads to destruction of red blood cells. These and other infections can also cause systemic inflammation which alters iron metabolism (13). Briefly, the release of proinflammatory cytokines (i.e., IL-6) upregulates hepcidin, the iron-regulatory hormone. Hepcidin induces iron sequestration in intestinal enterocytes, macrophages, and hepatocytes, and also blocks iron absorption, thereby reducing circulating iron stores and impairing red blood cell production and maintenance. Hemoglobinopathies, for example sickle cell disease, thalassemias, and glucose-6-phosphate dehydrogenase (G6PD) deficiency, are also important contributors to the burden of anemia among young children, particularly in Africa where malaria is endemic, and cause impaired red blood cell production and hemolytic anemia (2,12).

Globally, iron deficiency is the primary cause of anemia in young children (12). Yet, because of the multiple contributors to iron deficiency, from both nutritional and infectious

causes as described above, treating only nutritional iron deficiency, for example with iron supplements, is insufficient to reduce anemia and may even cause greater harm in regions endemic for malaria and other infectious diseases (14). Recent work has shown that there is substantial global variation in the fraction of anemia associated with iron deficiency by countries' infectious disease context (15,16). In Ghana, preschool age children experience several conditions that can contribute to anemia; analysis of the nationally-representative 2017 Ghana Micronutrient Survey found that 22% of preschool age children had iron deficiency, 21% had vitamin A deficiency, 46% had inflammation, 20% had malaria parasitemia, 13% had sickle cell trait, 1% had sickle cell disease, and 27% and 3% were heterozygous or homozygous, respectively, for α-thalassemia (17). Further analysis from this survey found that only 19% of anemia in these children was attributable to iron deficiency, while 8% was attributable to vitamin A deficiency, 15% to malaria parasitemia, 12% to inflammation, 9% to fever, and 6% to stunting (18). This fraction of anemia associated with iron deficiency is far lower than the World Health Organization's commonly cited estimate of 50%, and in line with more recent estimates of ~25% among preschool age children (16). In the Southern Belt of Ghana, which includes the Greater Accra Region (where this dissertation study was conducted), no associations between iron status and anemia were found, while malaria parasitemia, inflammation, and fever collectively contributed to three-quarters of the population attributable fraction (18). These findings highlight the importance of tackling infectious causes of anemia in this area of Ghana.

Prevention and control strategies targeting anemia have predominantly focused on addressing the *immediate* determinants of anemia (i.e., inadequate micronutrient intake or absorption and exposure to infectious diseases) (2). These strategies, termed nutrition-specific interventions, include dietary or micronutrient supplementation, improved complementary

feeding practices, food fortification, delayed cord clamping, and disease prevention and management (e.g., use of antimalarial drugs, distribution of insecticide-treated bednets, and deworming treatments) (1,19). Efforts to reduce anemia among preschool age children in Ghana have focused on such efforts, including malaria prevention (insecticide-treated bed net distribution and seasonal malaria chemoprevention in high-malaria regions), deworming coverage (38% coverage in 2014), vitamin A supplementation (65% coverage in 2014), vitamin A fortification of vegetable oils (95% coverage), and exclusive breastfeeding (52% among children in 2014) (20). National iron-folic acid supplementation and point-of-use micronutrient fortification for young children has not yet been implemented, though efforts to do so are underway (20). Research has found strong beneficial effects of iron supplementation, micronutrient powders, and insecticide-treated nets in reducing anemia (21). Yet these interventions require sustained implementation and coordination, and alone cannot reduce the anemia burden in LMICs (22). In conjunction with these efforts, the *intermediate* determinants of anemia must be addressed, including food insecurity, inadequate maternal and child care, limited access to health/nutrition services, inadequate health/nutrition knowledge, and inadequate access to water, sanitation, and hygiene (WASH) infrastructure.

One sector that may be particularly important to tackling food insecurity and unhygienic environments in LMICs is livestock rearing. Livestock are an integral component of the global food system; nearly one billion rural poor raise livestock for income, savings and insurance, food, transportation, and manure for fuel and fertilizer (23). Yet livestock are also major reservoirs of zoonotic diseases that cause human illness (23). Given that anemia may be caused by both micronutrient deficiencies and infectious diseases, livestock production has the potential to both reduce child anemia through improved diets, and increase anemia by exposing children to

infectious pathogens. These contradictory pathways are particularly important to understand, as livestock production projects are implemented as part of nutrition-sensitive interventions to reduce child anemia (22). Limited progress has been made in reducing anemia due its multifactorial etiology, and therefore understanding the role of livestock in potentially contributing to this burden is imperative.

The role of livestock production in LMICs and its potential to impact child anemia

The dietary benefits of livestock production

Animal-source foods (ASFs), which include meat, organ meats, fish and seafood, dairy, and eggs, are a nutrient-dense source of food. Livestock-derived ASFs contain many of the micronutrients essential for preventing anemia – meat and eggs are high in vitamin A and vitamin B12 and red and organ meats have high concentrations of heme iron (24,25). Importantly, ASFs contain these nutrients at higher concentrations and in more bioavailable forms compared to plant-based foods. In LMICs, diets are predominately plant-based and low in ASFs, and modest increases in ASF intake could reduce micronutrient inadequacies, particularly among young children (26,27). Analysis of the 2014 Ghana DHS found that among children 6-59 months old, 40% had consumed fish in the prior 24 hours, 11% had consumed meat, and 17% had consumed eggs (28), suggesting generally low levels of livestock-derived ASF consumption in Ghana. In a study of farming and non-farming households across Ghana's three agroecological zones, >99% of children 2 to 5 years old met nutrient adequacy requirements for iron, but only 52% met requirements for vitamin A adequacy and 16% met requirements for vitamin B12 adequacy (29). Lower mean micronutrient density adequacy in these children was associated with severe household food insecurity, with moderately and severely food insecure households being less likely to consume poultry, eggs, dairy, and red meat, but not fish (29).

ASF consumption has the potential to fulfill a gap in the dietary needs of vulnerable populations including young children. Meeting nutritional needs with ASFs is important to consider, even amidst calls to reduce global ASF consumption among adults and high-income populations in order to reduce environmental impacts (30). Supporting livestock production in LMICs could reduce anemia by increasing access to ASFs and improving the nutritional quality of children's diets, which is often part of the proposed impact pathway of nutrition-sensitive livestock interventions. This comes with caveats, however. Although observational studies have linked vegetarian diets to lower iron stores (31), randomized controlled trials in LMICs have not found that providing ASFs to children <5 years old improves hemoglobin concentrations (32). Furthermore, livestock ownership does not necessarily translate to higher ASF consumption. Evidence is mixed regarding whether livestock ownership is associated with higher ASF consumption among children, and this may depend on the livestock species, whether livestock are male- or female-owned, and access to markets (28,33–41). Qualitative investigations conducted in Ghana have highlighted that households preferentially use livestock for savings, allocate poultry eggs for reproduction, and only rarely slaughter poultry for household consumption (42,43). Thus, the use of livestock for purposes other than consumption by the owner's children may limit whether livestock production can effectively improve dietary quality and nutrition outcomes.

Livestock and infectious disease risks

Livestock are often infected by diverse microbial pathogens, including helminths, protozoa, viruses, and bacteria, that can cause illness in humans (i.e., zoonotic diseases) (44), and exposure to these pathogens may increase children's risk of anemia. Soil-transmitted helminths, for example, cause anemia via gastrointestinal blood loss, as described earlier. Enteric pathogens

(microorganisms infecting the intestinal tract) may contribute to anemia via two intertwined processes, though these mechanisms are not yet fully elucidated. First, continual exposure to enteric pathogens is thought to lead to environmental enteric dysfunction (EED), a subclinical disease of the small intestine characterized by intestinal and systemic inflammation, blunted microvilli, intestinal permeability, and nutrient malabsorption (45,46). It is hypothesized that EED underlies, at least in part, growth stunting in children in LMICs (47). EED may also contribute to iron deficiency and anemia, both by limiting absorption of micronutrients essential to hematopoiesis and by causing inflammation (14). Recent studies have indeed found negative associations between EED biomarkers and iron status and hemoglobin in children (48–50). Second, enteropathogen infections may induce a systemic inflammatory response that prompts the body to upregulate hepcidin, sequester iron, and reduce iron absorption in an effort to starve the invading pathogen of iron (51). This systemic inflammation likely occurs from acute responses to infection, but chronic systemic inflammation via EED may also lead to persistent upregulation of hepcidin, and hence anemia that develops not due to true iron deficiency, but functional iron deficiency (7). However, population-based research on associations between enteric pathogens and anemia is sparse. One study has found that higher rates of asymptomatic enteropathogen infections in children during their first two years of life are associated with lower hemoglobin concentrations (52). Also, there is some evidence suggesting that poor water and sanitation, which may proxy exposure to enteropathogens, is associated with child anemia (53,54).

Exposure to bacterial enteropathogens in livestock feces may be more common in LMICs, where sanitation is often poor and animals free-roam within household areas that are also used for cooking, sleeping, and other day-to-day activities. Transmission of pathogens from

livestock feces to a human host can occur through contamination of fluids (e.g., water), fields/soil, fingers, flies, fomites (e.g., cooking utensils) and food (55). Microbial contamination from livestock feces has been identified in household yards, on household surfaces, in drinking water and food, and on caregivers' hands in distinct LMICs, including in Ghana (56–59). The risk of exposure to contamination from livestock feces may be especially high for young children who crawl and play in household yards and engage in frequent hand-to-mouth contact (60,61). A multi-site, cohort study conducted in Africa, Asia, and South America in the last ten years confirmed that burden of enteropathogen infection in children under two years old is substantial, with at least one enteric pathogen being detected in 77% of diarrheal stool samples and 65% of non-diarrheal stool samples (62). Though improving WASH infrastructure decreases transmission of these pathogens, reducing children's exposure to animal feces is also fundamental to decreasing the enteropathogen burden in children living in low-resource settings (63). Studies have shown that livestock ownership is associated with higher odds of diarrhea (64), elevated EED (65), and enteric pathogen infections in children (66–71). Thus, nutrition-sensitive interventions that seek to *promote* livestock may inadvertently expose children to a greater burden of infectious pathogens that can increase, rather than decrease, children's risk of anemia. However, the extent to which livestock production may impact anemia is poorly understood.

A systematic review of the literature assessing associations between livestock ownership and child anemia

In order to understand the current evidence on the potentially contradictory dietary and infectious disease pathways linking livestock production and anemia in children, I conducted a systematic literature review (72). This review sought to understand linkages between livestock

ownership and anemia among children and women in LMICs, assessing evidence from observational studies and livestock-based interventions.

The study methods are described in detail in Lambrecht et al. (2019) (72). Briefly, I conducted a database search of peer-reviewed and grey literature published through September 1, 2017. Studies that assessed the relationship between animal husbandry and capture (e.g., livestock rearing, fishing) and anemia (i.e., hemoglobin, anemia) in children and women, and that were conducted in a LMIC were included, yielding 23 studies. Fourteen of these studies included children aged 6 to 59 months, the focus age group of this dissertation. Half of the identified studies were nutrition-sensitive interventions, primarily conducted in South-East Asia, while the other half were observational studies conducted in sub-Saharan Africa and South America.

Three studies examined the influence of pastoralist livelihoods on children's anemia status. Of these, two found lower anemia among children from communities practicing nomadic pastoralist livelihoods compared to those in sedentary pastoral or agricultural communities in Mali (73) and Kenya (74), while one study found higher anemia among children in mobile pastoralist groups in Chad (75). There were no differences in morbidity outcomes (diarrhea, fever, respiratory illness, intestinal parasites) between comparison groups (74,75), and Kenyan pastoralist children consumed more milk but not meat than children from sedentary livelihoods (74).

Among observational studies, associations between livestock ownership and anemia were mixed, though poultry ownership appeared to be particularly important. One study found that poultry ownership was negatively associated with anemia (76), while two studies found that poultry ownership was positively associated with anemia (28,77). In particular, Jones et al.

(2018) conducted an analysis of the 2014 Ghana DHS, finding that household livestock ownership was associated with 1.5 times higher odds (95% Confidence Interval: 1.2, 2.2) of anemia in children 6 to 59 months old. In analysis by livestock species, only chicken ownership, but not ownership of other types of livestock, was associated with higher odds of anemia in children. Furthermore, household livestock ownership was not associated with children's consumption of ASF in the prior 24 hours. Two observational studies published after the literature review search was completed, also examined associations between livestock ownership anemia. A cross-sectional study of 300 households in southern Ghana found that overall household livestock ownership was associated with 0.32 times lower odds of anemia in children 2 to 5 years old (95 CI: 0.12, 0.81), but that children from households owning a greater number of sheep and goats had 10% higher odds of anemia (95% CI: 1.03, 1.17) (78). An analysis of 28 countries' DHS surveys in sub-Saharan Africa found that ownership of cattle, sheep, or goats, but not chickens, was associated with lower hemoglobin concentrations in children 6 to 59 months old, though the coefficients were quite small (-0.004 to -0.005 g/dL in hemoglobin concentration for one additional unit of livestock owned) (79). Chicken ownership was associated with children's consumption of eggs and meat, but consumption of these foods was not associated with hemoglobin concentration (79).

All of the studies examining livestock production interventions were multi-component, integrating poultry production with improved home vegetable gardening and health and nutrition education. Of the six studies that included a comparison group, only two found significant improvements in anemia outcomes among children that received the integrated animal production intervention compared to controls (80–85). All of these interventions were evaluations of Helen Keller International (HKI) Homestead Food Production (HFP) programs,

which seek to improve child nutrition outcomes by (1) increasing access to micronutrient rich foods via increased poultry, vegetable, and fruit production, (2) increasing income through surplus production, and (3) eliciting improved caregiver nutrition knowledge and behaviors via health and nutrition education (86). Among the interventions that evaluated ASF consumption, egg production and consumption were increased (83–85). However, qualitative evaluation of one of these HKI programs in Cambodia found that households prioritized chicken eggs for reproduction and subsequent income, and not for household consumption (86). Though income from sales could be used for the purchase of ASFs, the limited impact on the poultry production-consumption pathway may explain why these interventions did not see improvements in anemia.

As has been noted in other literature reviews, these interventions may also have been unable to achieve meaningful changes in nutritional status given their timeline and scope and because they were statistically underpowered to do so (87–89). For the purposes of this review, it was also not possible to independently assess effects due to the livestock production component versus effects from other components, such as improved knowledge of young child feeding practices and access to a greater diversity of foods from home gardening. Of note, a chicken production intervention conducted in Ethiopia published after this systematic review search compared two intervention groups that did allow assessment of the independent effect of chicken production (90). Two intervention groups were examined in that study, one which received genetically improved chickens and chicken husbandry education, and the other, which received the chicken intervention in addition to home gardening and nutrition-sensitive behavior change interventions. Both chicken intervention groups increased egg production, children's egg consumption, and income from chickens compared to the control group, though the effect was higher in the multi-component intervention group. However, no differences in child anemia were

observed compared to the control group at endline, though it is important to note that the study was not designed to detect differences in anemia.

Overall, this systematic review yielded mixed evidence on the relationship between livestock production and anemia in children. While there were some benefits of poultry production in intervention studies, observational studies noted predominantly negative associations with livestock ownership, particularly of chickens. In one of these studies (28), conducted by our research group, we hypothesized that the positive association between chicken ownership and anemia may be due to enteropathogen exposure from chickens, but were unable to assess this pathway. Unfortunately, none of the studies that were reviewed measured enteropathogen infections, and only one study measured inflammatory biomarkers, limiting our understanding of how livestock may affect anemia through infectious disease pathways. Furthermore, even intervention studies that included nutrition education components which emphasized the use of own-produced eggs to improve child diets had difficulty achieving this impact due to preferential allocation of eggs and chickens for income. In conclusion, research is needed that jointly assesses the potential dietary benefits of livestock ownership against the potential drawbacks due to infectious diseases.

Dissertation aims

The overarching objective of this dissertation is, therefore, to identify and characterize linkages between household livestock ownership and anemia in young children aged 6 to 59 months, assessing both dietary and infectious disease pathways (see conceptual framework in Figure 1.1). The findings from this research are intended to improve our understanding of the benefits and risks of household livestock ownership on the health of young children, and the role that livestock production and infectious disease exposure may have in the burden of anemia. I

hypothesized that livestock ownership would be associated with higher likelihood of anemia in children, and that this association would be mediated by enteropathogenic infection from exposure to zoonotic bacteria in livestock feces, primarily that of chickens. To test these hypotheses, I conducted a cross-sectional study in the Greater Accra Region, Ghana of children living in livestock- and non-livestock owning households, and addressed the three aims described below.

The first aim of this dissertation was to assess the overall relationship between household livestock ownership and anemia in young children and examine whether this relationship was mediated by child ASF consumption or by child morbidity and inflammation. Household livestock ownership was categorized into typologies based on the types and number of livestock owned. We assessed the overall relationship between livestock ownership typologies and child iron deficiency and anemia, and the relationship between livestock ownership typologies and children's consumption of ASF in the past three months, symptoms of illness (diarrhea, fever, cough or cold), and elevated systemic inflammation (measured via C-reactive protein and α-1-acid glycoprotein). Formal mediation analysis examining dietary and morbidity indicators was conducted for factors associated with livestock ownership. Whether households used livestock for food and income was also assessed. We hypothesized that livestock ownership, particularly among households that owned chickens, would be positively associated with child anemia, mediated by children's symptoms of morbidity and elevated inflammation. We further hypothesized that ASF consumption from livestock would not substantially contribute to children's diets. Results from this aim, presented in Chapter 2, add to our current understanding by (1) investigating the potential benefits and drawbacks of household livestock ownership simultaneously in one sample, and (2) using biomarkers of inflammation and iron status that

allow for a more precise understanding of children's nutrient and inflammatory state (in comparison to proxies such as dietary intake and symptoms of illness).

The second aim of this dissertation was to determine whether livestock-related risk factors are associated with bacterial enteropathogen infections in children. Four zoonotic enteropathogens [atypical enteropathogenic *Escherichia coli* (*E. coli*), Shiga-toxin producing *E. coli*, *Campylobacter jejuni/coli*, and *Salmonella enterica*] were measured in children's stool samples using quantitative polymerase chain reaction (qPCR). Several livestock-related risk factors were examined for associations with each enteric pathogen, including cattle, goat, sheep, and poultry ownership, exposure to livestock feces in the home environment (measured via observation of livestock feces in the household yard), enteropathogen detection in chicken feces, and at least weekly consumption of high-risk animal-source foods (i.e., poultry meat and fresh milk). 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. The findings of this aim, presented in Chapter 3, enhance our current knowledge by assessing species-specific transmission pathways (i.e., fecal and food-borne) for individual zoonotic pathogens.

The third aim of this dissertation was to assess associations between children's bacterial enteropathogen infections and their inflammation, iron deficiency, and anemia status. A sub-aim was to examine whether children's diet quality, proxied by dietary diversity, modified the associations between enteropathogen infections and anemia. Children's stool samples were tested for the presence of multiple enteropathogens including *Campylobacter jejuni/coli*, enteroaggregative *E. coli*, atypical enteropathogenic *E. coli*, typical enteropathogenic *E. coli*, heat-stable enterotoxin-producing *E. coli*, heat-labile enterotoxin-producing *E. coli*, Shiga toxin-

producing *E. coli*, enteroinvasive *E. coli*/*Shigella* species, *Salmonella enterica*, and *Vibrio cholerae*. We hypothesized that enteropathogen infections of children would be associated with higher odds of systemic inflammation, iron deficiency, and anemia, and that associations with anemia would be attenuated in children with higher dietary diversity. This aim, presented in Chapter 4, represents the first study to assess individual effects of enteric pathogens on anemia, and expands our understanding of potential intermediary pathways (inflammation and iron deficiency) by which enteric pathogens may contribute to anemia in young children.

Limited progress has been made in reducing anemia among young children in Ghana, emphasizing the need to address underlying determinants of anemia. Small-scale livestock production is an appealing nutrition-sensitive strategy to address anemia because it provides families with food and income, yet the risks of increased exposure to pathogens may be substantial. There is limited understanding of whether exposure to pathogens from livestock may increase children's risk of anemia, and little research on the role of bacterial enteropathogens in the burden of child anemia. By using biomarkers of iron status and inflammation, and through molecular detection of enteric pathogens in both humans and chickens, this work will characterize nutritional and infectious disease determinants of anemia and enteropathogen infection in young children in southern Ghana. Results from this study can help inform livestock production and management interventions by clarifying benefits and risks from livestock and the pathways underlying these associations.

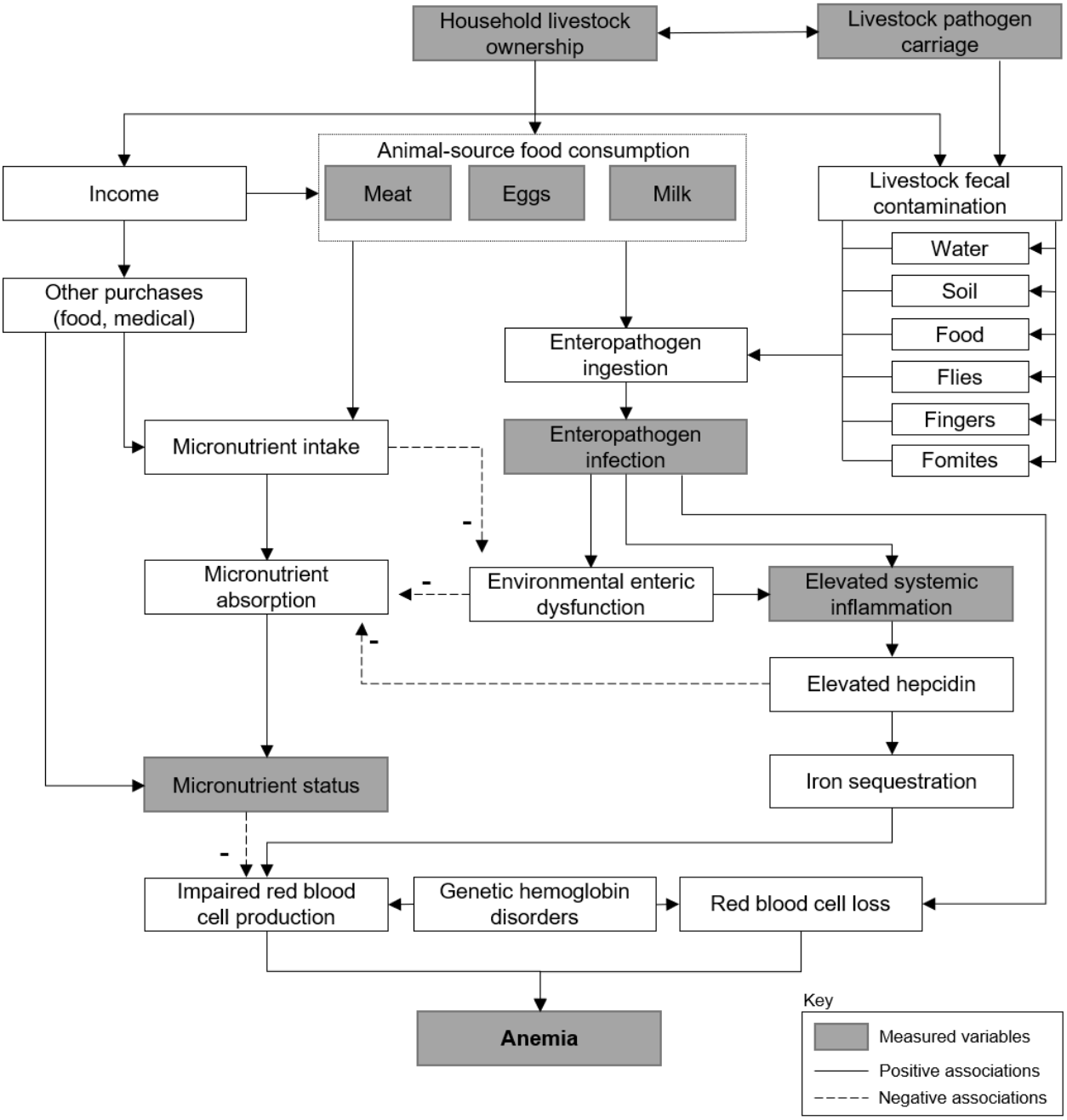

Figure 1.1 Conceptual framework of the hypothesized pathways between household livestock ownership and anemia risk among young children. F-diagram linking livestock fecal contamination with enteropathogen ingestion adapted from previous interpretations (55,66) of the original F-diagram by Wagner and Lanoix (1958) (91). Linkages between micronutrient deficiencies, inflammation, infections, and anemia adapted from Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia conceptual framework (92).

References

1. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. Lancet. 2011;378:2123–35.
2. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019;1450:15–31.
3. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28:S560-71.
4. Scott SP, Chen-Edinboro LP, Caulfield LE, Murray-Kolb LE. The impact of anemia on child mortality: An updated review. Nutrients. 2014;6:5915–32.
5. McCann JC, Ames BN. An overview of evidence for a causal relationship between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931–45.
6. Vos T, Abajobir AA, Abbafati C, Abbas KM, Abate KH, Abd-Allah F, Abdulle AM, Abebo TA, Abera SF, Aboyans V, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390:1211–59.
7. Pasricha S, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. Lancet. 2020;6736:1–16.
8. World Health Organization. Global Health Observatory data repository: Anaemia in children < 5 years [Internet]. 2017. Available from: https://apps.who.int/gho/data/view.main.ANEMIACHILDRENv
9. Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF International. Ghana Demographic and Health Survey 2014. Rockville, Maryland, USA; 2015.
10. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. Vitamin and Mineral Nutrition Information System. Geneva; 2011. Available from: http://www.who.int/vmnis/indicators/haemoglobin.pdf
11. Camaschella C. Iron-Deficiency Anemia. N Engl J Med. 2015;372:1832–48.
12. Kassebaum NJ. The global burden of anemia. Hematol Oncol Clin North Am. 2016;30:247–308.
13. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. Nat Rev Immunol. 2015;15:500–10.
14. Pasricha S-R, Armitage AE, Prentice AM, Drakesmith H. Reducing anaemia in low income countries: control of infection is essential. BMJ. 2018;3165:k3165.
15. Engle-Stone R, Aaron GJ, Huang J, Wirth JP, Namaste SM, Williams AM, Peerson JM, Rohner F, Varadhan R, Addo OY, et al. Predictors of anemia in preschool children: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106(Suppl):402S–15S.
16. Petry N, Olofin I, Hurrell RF, Boy E, Wirth JP, Moursi M, Angel MD, Rohner F. The proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: A systematic analysis of national surveys. Nutrients. 2016;8:1–17.
17. Wegmüller R, Bentil H, Wirth JP, Petry N, Tanumihardjo SA, Allen L, Williams TN, Selenje L, Mahama A, Amoaful E, et al. Anemia, micronutrient deficiencies, malaria, hemoglobinopathies and malnutrition in young children and non-pregnant women in Ghana: Findings from a national survey. PLoS One. 2020;15:1–19.
18. Petry N, Wirth JP, Adu-Afarwuah S, Wegmuller R, Woodruff BA, Tanumihardjo SA,

Bentil H, Donkor WES, Williams TN, Shahab-Ferdows S, et al. Risk factors for anaemia among Ghanaian women and children vary by population group and climate zone. Matern Child Nutr. 2020;1–10.
19. Ruel MT, Alderman H. Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition? Lancet. 2013;382:536–51.
20. SPRING GHS. Ghana: Landscape Analysis of Anemia and Anemia Programming. Arlington, VA; 2016.
21. Moorthy D, Merrill R, Namaste S, Iannotti L. The impact of nutrition-specific and nutrition-sensitive interventions on hemoglobin concentrations and anemia: A meta-review of systematic reviews. Adv Nutr. 2020;11:1631–45.
22. Ruel MT, Quisumbing AR, Balagamwala M. Nutrition-sensitive agriculture: What have we learned so far? Glob Food Sec. 2018;17:128–53.
23. Herrero M, Grace D, Njuki J, Johnson N, Enahoro D, Silvestri S, Rufino MC. The roles of livestock in developing countries. Animal. 2013;7:3–18.
24. Iannotti LL, Lutter CK, Bunn DA, Stewart CP. Eggs: The uncracked potential for improving maternal and young child nutrition among the world's poor. Nutr Rev. 2014;72:355–68.
25. Neumann C, Harris DM, Rogers LM. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutr Res. 2002;22:193–220.
26. Adesogan AT, Havelaar AH, McKune SL, Eilittä M, Dahl GE. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. Glob Food Sec. 2020;25.
27. Nordhagen S, Beal T, Haddad L. The role of animal-source foods in healthy, sustainable, and equitable food systems. Global Alliance for Improved Nutrition (GAIN). Discussion Paper Series #5. Geneva, Switzerland; 2020.
28. Jones AD, Colecraft EK, Awuah RB, Boatemaa S, Lambrecht NJ, Adjorlolo LK, Wilson ML. Livestock ownership is associated with higher odds of anaemia among preschool-aged children, but not women of reproductive age in Ghana. Matern Child Nutr. 2018;14:1–13.
29. Christian AK, Marquis GS, Colecraft EK, Lartey A, Soueida R. Household food insecurity but not dietary diversity is associated with children's mean micronutrient density adequacy in rural communities across Ghana. Nutrition. 2019;65:97–102.
30. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393:447–92.
31. Haider LM, Schwingshackl L, Hoffmann G, Ekmekcioglu C. The effect of vegetarian diets on iron status in adults: A systematic review and meta-analysis. Crit Rev Food Sci Nutr. 2018;58:1359–74.
32. Shapiro MJ, Downs SM, Swartz HJ, Parker M, Quelhas D, Kreis K, Kraemer K, West KP, Fanzo J. A systematic review investigating the relation between animal-source food consumption and stunting in children aged 6–60 months in low and middle-income countries. Adv Nutr. 2019;10:827–47.
33. Mosites E, Aol G, Otiang E, Bigogo G, Munyua P, Montgomery JM, Neuhouser ML, Palmer GH, Thumbi SM. Child height gain is associated with consumption of animal-source foods in livestock-owning households in Western Kenya. Public Health Nutr.

2016;20:336–45.
34. de Bruyn J, Thomson PC, Darnton-Hill I, Bagnol B, Maulaga W, Alders RG. Does village chicken-keeping contribute to young children's diets and growth? A longitudinal observational study in rural Tanzania. Nutrients. 2018;10:1799.
35. Headey D, Hirvonen K. Is exposure to poultry harmful to child nutrition? An observational analysis for rural Ethiopia. PLoS One. 2016;11:e0160590.
36. Broaddus-Shea ET, Manohar S, Thorne-Lyman AL, Bhandari S, Nonyane BAS, Winch PJ, West KP. Small-scale livestock production in Nepal is directly associated with children's increased intakes of eggs and dairy, but not meat. Nutrients. 2020;12:1–11.
37. Rawlins R, Pimkina S, Barrett CB, Pedersen S, Wydick B. Got milk? The impact of Heifer International's livestock donation programs in Rwanda on nutritional outcomes. Food Policy. 2014;44:202–13.
38. Hoddinott J, Headey D, Dereje M. Cows, missing milk markets, and nutrition in rural Ethiopia. J Dev Stud. 2015;51:958–75.
39. Choudhury S, Headey DD. Household dairy production and child growth: evidence from Bangladesh. Econ Hum Biol. 2018;30:150–61.
40. Dumas SE, Kassa L, Young SL, Travis AJ. Examining the association between livestock ownership typologies and child nutrition in the Luangwa Valley, Zambia. PLoS One. 2018;13:1–21.
41. Jin M, Iannotti LL. Livestock production, animal source food intake, and young child growth: The role of gender for ensuring nutrition impacts. Soc Sci Med. 2014;105:16–21.
42. Nyantakyi-Frimpong H, Colecraft EK, Awuah RB, Adjorlolo LK, Wilson ML, Jones AD. Leveraging smallholder livestock production to reduce anemia: A qualitative study of three agroecological zones in Ghana. Soc Sci Med. 2018;212:191–202.
43. Colecraft E, Marquis GS, Aryeetey R, Sakyi-Dawson O, Lartey A, Ahunu B, Canacoo E, Butler LM, Reddy MB, Jensen HH, et al. Constraints on the use of animal source foods for young children in Ghana: A participatory rapid appraisal approach. Ecol Food Nutr. 2006;45:351–77.
44. Delahoy MJ, Wodnik B, McAliley L, Penakalapati G, Swarthout J, Freeman MC, Levy K. Pathogens transmitted in animal feces in low- and middle-income countries. Int J Hyg Environ Health. 2018;221:661–76.
45. Watanabe K, Petri WA. Environmental enteropathy: elusive but significant subclinical abnormalities in developing countries. EBioMedicine. 2016;10:25–32.
46. Syed S, Ali A, Duggan C. Environmental enteric dysfunction in children. J Pediatr Gastroenterol Nutr. 2016;63:6–14.
47. Budge S, Parker AH, Hutchings PT, Garbutt C. Environmental enteric dysfunction and child stunting. Nutr Rev. 2019;77:240–53.
48. McCormick BJJ, Murray-Kolb LE, Lee GO, Schulze KJ, Ross AC, Bauck A, Lima AAM, Maciel BLL, Kosek MN, Seidman JC, et al. Intestinal permeability and inflammation mediate the association between nutrient density of complementary foods and biochemical measures of micronutrient status in young children: results from the MAL-ED study. Am J Clin Nutr. 2019;110:1015–25.
49. Fahim SM, Das S, Sanin KI, Gazi MA, Mahfuz M, Munirul Islam M, Ahmed T. Association of fecal markers of environmental enteric dysfunction with zinc and iron status among children at first two years of life in Bangladesh. Am J Trop Med Hyg. 2018;99:489–94.

50. Lauer JM, Ghosh S, Ausman LM, Webb P, Bashaasha B, Agaba E, Turyashemererwa FM, Tran HQ, Gewirtz AT, Erhardt J, et al. Markers of environmental enteric dysfunction are associated with poor growth and iron status in rural Ugandan infants. J Nutr. 2020;150:2175–82.
51. Jonker FAM, te Poel E, Bates I, Boele van Hensbroek M. Anaemia, iron deficiency and susceptibility to infection in children in sub-Saharan Africa, guideline dilemmas. Br J Haematol. 2017;177:878–83.
52. Murray-Kolb LE, Acosta AM, De Burga RR, Chavez CB, Flores JT, Olotegui MP, Pinedo SR, Salas MS, Trigoso DR, Vasquez AO, et al. Early childhood cognitive development is affected by interactions among illness, diet, enteropathogens and the home environment: findings from the MAL-ED birth cohort study. BMJ Glob Heal. 2018;3:e000752.
53. Kothari MT, Coile A, Huestis A, Pullum T, Garrett D, Engmann C. Exploring associations between water, sanitation, and anemia through 47 nationally representative demographic and health surveys. Ann N Y Acad Sci. 2019;1450:nyas.14109.
54. Yu EX, Addo OY, Williams AM, Engle-Stone R, Ou J, Huang W, Guo J, Suchdev PS, Young MF. Association between anemia and household water source or sanitation in preschool children: the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2020;112:488S-497S.
55. Penakalapati G, Swarthout J, Delahoy MJ, McAliley L, Wodnik B, Levy K, Freeman MC. Exposure to animal feces and human health: a systematic review and proposed research priorities. Environ Sci Technol. 2017;51:11537–52.
56. Fuhrmeister ER, Ercumen A, Pickering AJ, Jeanis KM, Ahmed M, Brown S, Arnold BF, Hubbard AE, Alam M, Sen D, et al. Predictors of enteric pathogens in the domestic environment from human and animal sources in rural Bangladesh. Environ Sci Technol. 2019;53:10023–33.
57. Ercumen A, Pickering AJ, Kwong LH, Arnold BF, Parvez SM, Alam M, Sen D, Islam S, Kullmann C, Chase C, et al. Animal feces contribute to domestic fecal contamination: evidence from *E. coli* measured in water, hands, food, flies, and soil in Bangladesh. Environ Sci Technol. 2017;51:8725–34.
58. Schiaffino F, Trigoso DR, Colston JM, Paredes Olortegui M, Shapiama Lopez W V., Garcia Bardales PF, Pisanic N, Davis MF, Penataro Yori P, Kosek MN. Associations among household animal ownership, infrastructure, and hygiene characteristics with source attribution of household fecal contamination in peri-urban communities of Iquitos, Peru. Am J Trop Med Hyg. 2020;104:372–81.
59. Wardrop NA, Hill AG, Dzodzomenyo M, Aryeetey G, Wright JA. Livestock ownership and microbial contamination of drinking-water: Evidence from nationally representative household surveys in Ghana, Nepal and Bangladesh. Int J Hyg Environ Health. 2018;221:33–40.
60. Ngure F, Gelli A, Becquey E, Ganaba R, Headey D, Huybregts L, Pedehombga A, Sanou A, Traore A, Zongo F, et al. Exposure to livestock feces and water quality, sanitation, and hygiene (WASH) conditions among caregivers and young children: formative research in rural Burkina Faso. Am J Trop Med Hyg. 2019;100:998–1004.
61. Ngure FM, Humphrey JH, Mbuya MNN, Majo F, Mutasa K, Govha M, Mazarura E, Chasekwa B, Prendergast AJ, Curtis V, et al. Formative research on hygiene behaviors and geophagy among infants and young children and implications of exposure to fecal bacteria. Am J Trop Med Hyg. 2013;89:709–16.

62. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJJ, McGrath M, Olortegui MP, Samie A, et al. Pathogen-specific burdens of community diarrhoea in developing countries: A multisite birth cohort study (MAL-ED). Lancet Glob Heal. 2015;3:e564–75.
63. Prendergast AJ, Gharpure R, Mor S, Viney M, Dube K, Lello J, Berger C, Siwila J, Joyeux M, Hodobo T, et al. Putting the "A" into WaSH: a call for integrated management of water, animals, sanitation, and hygiene. Lancet Planet Heal. 2019;3:e336–7.
64. Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2014;108:313–25.
65. George CM, Oldja L, Biswas SK, Perin J, Lee GO, Ahmed S, Haque R, Sack RB, Parvin T, Azmi IJ, et al. Fecal markers of environmental enteropathy are associated with animal exposure and caregiver hygiene in Bangladesh. Am J Trop Med Hyg. 2015;93:269–75.
66. Budge S, Barnett M, Hutchings P, Parker A, Tyrrel S, Hassard F, Garbutt C, Moges M, Woldemedhin F, Jemal M. Risk factors and transmission pathways associated with infant *Campylobacter spp.* prevalence and malnutrition: A formative study in rural Ethiopia. PLoS One. 2020;15:e0232541.
67. Lowenstein C, Vasco K, Sarzosa S, Salinas L, Torres A, Perry MJ, Simmens SJ, Trueba G, Eisenberg JNS, Graham JP. Determinants of childhood zoonotic enteric infections in a semirural community of Quito, Ecuador. Am J Trop Med Hyg. 2020;102:1269–78.
68. Vasco K, Graham JP, Trueba G. Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in Ecuador. Appl Environ Microbiol. 2016;82:4218–24.
69. Oberhelman RA, Gilman RH, Sheen P, Cordova J, Taylor DN, Zimic M, Meza R, Perez J, LeBron C, Cabrera L, et al. *Campylobacter* transmission in a Peruvian shantytown: a longitudinal study using strain typing of *Campylobacter* isolates from chickens and humans in household clusters. J Infect Dis. 2003;187:260–9.
70. El-Tras WF, Holt HR, Tayel AA, El-Kady NN. *Campylobacter* infections in children exposed to infected backyard poultry in Egypt. Epidemiol Infect. 2015;143:308–15.
71. Chard AN, Levy K, Baker KK, Tsai K, Chang HH, Thongpaseuth V, Sistrunk JR, Freeman MC. Environmental and spatial determinants of enteric pathogen infection in rural Lao People's Democratic Republic: A cross-sectional study. PLoS Negl Trop Dis. 2020;14:e0008180.
72. Lambrecht NJ, Wilson ML, Jones AD. Assessing the impact of animal husbandry and capture on anemia among women and children in low- and middle-income countries: A systematic review. Adv Nutr. 2019;10:331–44.
73. Jankowska MM, Lopez-Carr D, Funk C, Husak GJ, Chafe ZA. Climate change and human health: spatial modeling of water availability, malnutrition, and livelihoods in Mali, Africa. Appl Geogr. 2012;33:4–15.
74. Nathan MA, Fratkin EM, Roth EA. Sedentism and child health among Rendille pastoralists of Northern Kenya. Soc Sci Med. 1996;43:503–15.
75. Bechir M, Schelling E, Hamit MA, Tanner M, Zinsstag J. Parasitic infections, anemia and malnutrition among rural settled and mobile pastoralist mothers and their children in Chad. Ecohealth. 2012;9:122–31.
76. Custodio E, Descalzo MA, Roche J, Sánchez I, Molina L, Lwanga M, Bernis C, Villamor E, Baylin A. Nutritional status and its correlates in Equatorial Guinean preschool children:

results from a nationally representative survey. Food Nutr Bull. 2008;29:49–58.
77. Iannotti LL, Delnatus JR, Odom AR, Eaton JC, Griggs JJ, Brown S, Wolff PB. Determinants of anemia and hemoglobin concentration in Haitian school-aged children. Am J Trop Med Hyg. 2015;93:1092–8.
78. Christian AK, Wilson ML, Aryeetey RNO, Jones AD. Livestock ownership, household food security and childhood anaemia in rural Ghana. PLoS One. 2019;14:1–14.
79. Acharya Y, Yang D, Jones AD. Livestock ownership and anaemia in Sub-Saharan African households. Public Health Nutr. 2020;1–12.
80. Olney DK, Pedehombga A, Ruel MT, Dillon A, Bliznashka L, Pedehombga A, Dillon A, Ruel MT, Heckert J, Dillon A. A 2-year integrated agriculture and nutrition and health behavior change communication program targeted to women in Burkina Faso reduces anemia, wasting, and diarrhea in children 3 – 12.9 months of age at baseline: a cluster-randomized controlled trial. J Nutr. 2015;145:1317–24.
81. Osei AK, Pandey P, Spiro D, Adhikari D, Haselow N, De Morais C, Davis D. Adding multiple micronutrient powders to a homestead food production programme yields marginally significant benefit on anaemia reduction among young children in Nepal. Matern Child Nutr. 2015;11 Suppl 4:188–202.
82. Osei A, Pandey P, Nielsen J, Pries A, Spiro D, Davis D, Quinn V, Haselow N. Combining home garden, poultry, and nutrition education program targeted to families with young children improved anemia among children and anemia and underweight among nonpregnant women in Nepal. Food Nutr Bull. 2017;38:49–64.
83. Hillenbrand EP, Waid JL. Adapting food-based strategies to improve the nutrition of the landless: a review of HKI's homestead food production program in Bangladesh. Thompson, B and Amoroso L, editor. Improving diets and nutrition: food-based approaches. Wallingford: FAO; 2014.
84. Olney DK, Talukder A, Iannotti LL, Ruel MT, Quinn V. Assessing impact and impact pathways of a homestead food production program on household and child nutrition in Cambodia. Food Nutr Bull. 2009;30:355–69.
85. Talukder A, Osei AK, Haselow NJ, Kroeun H, Uddin A, Quinn V. Contribution of homestead food production to improved household food security and nutrition status - lessons learned from Bangladesh, Cambodia, Nepal and the Philippines. Thompson B, Amoroso L, editors. Improving Diets and Nutrition: Food-based Approaches. FAO; 2014.
86. Olney DK, Vicheka S, Kro M, Chakriya C, Kroeun H, Hoing LS, Talukder A, Quinn V, Iannotti L, Becker E, et al. Using program impact pathways to understand and improve program delivery, utilization, and potential for impact of Helen Keller International s Homestead Food Production Program in Cambodia. Food Nutr Bull. 2013;34:169–84.
87. Masset E, Haddad L, Cornelius A, Isaza-Castro J. Effectiveness of agricultural interventions that aim to improve nutritional status of children: systematic review. BMJ. 2012;344:d8222.
88. Webb Girard A, Self JL, McAuliffe C, Olude O. The effects of household food production strategies on the health and nutrition outcomes of women and young children: a systematic review. Paediatr Perinat Epidemiol. 2012;26 (Suppl. 1):205–22.
89. Herforth A, Ballard TJ. Nutrition indicators in agriculture projects: Current measurement, priorities, and gaps. Glob Food Sec. 2016;10:1–10.
90. Passarelli S, Ambikapathi R, Gunaratna NS, Madzorera I, Canavan CR, Noor AR, Worku A, Berhane Y, Abdelmenan S, Sibanda S, et al. A chicken production intervention and

additional nutrition behavior change component increased child growth in Ethiopia: A cluster-randomized trial. J Nutr. 2020;150:2806–17.
91. Wagner EG, Lanoix JN. Excreta disposal for rural areas and small communities. 1958;39.
92. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS. Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106:333S-347S.

CHAPTER 2 Associations between Livestock Ownership and Lower Odds of Anemia among Children 6-59 Months Old are not Mediated by Animal-Source Food Consumption in Ghana

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Abstract

Livestock ownership may mitigate anemia among young children by providing access to animal-source foods (ASFs), yet exacerbate anemia by exposing children to zoonotic pathogens. This study aimed to assess the associations between household livestock ownership and child anemia, and examine whether this relationship is mediated by child ASF consumption or by child morbidity and inflammation. We conducted a cross-sectional study of 470 children aged 6-59 months in Greater Accra, Ghana. Child blood samples were analyzed for hemoglobin (Hb) concentration, iron status biomarkers, and inflammatory biomarkers. Caregivers were asked about the child's consumption of meat, eggs, or milk in the past three months. Livestock ownership was categorized into five typologies to distinguish households by the number and combinations of species owned. In adjusted logistic regression, children from households in Type 5, owning cattle, small livestock (goats, sheep, or pigs), and poultry, had lower odds of anemia compared to those in Type 1, owning no livestock (OR (95% CI): 0.32 (0.14, 0.71)).

Although children from households that owned poultry were more likely to consume chicken meat and children from households with cattle were more likely to consume cow's milk, consumption of these ASFs did not mediate the observed association between livestock ownership and child anemia. There were no associations between livestock ownership and children's symptoms of illness or inflammation. Further research is needed to understand how ownership of certain livestock species, or a greater diversity of livestock species, may be associated with the risk of child anemia, including the role of dietary and income-based pathways.

Introduction

Anemia affects over half of all children aged 6-59 months in sub-Saharan Africa and South-East Asia (1). Anemia in childhood is associated with severe consequences, including deficits in motor, cognitive, and social-emotional development (2,3) and increased risk of childhood mortality (4). The causes of anemia are complex and interconnected, including several micronutrient deficiencies, infectious diseases, and genetic hemoglobin disorders (5,6). While addressing the proximal causes of anemia (e.g., iron deficiency, malaria, hookworm infection) is important for treating and preventing anemia in endemic regions, equally important is identifying factors that influence the underlying determinants of anemia and its etiological factors, for instance, food insecurity and unhygienic environments.

Many poor households in low- and middle-income countries (LMICs) keep small herds of livestock as a source of farm labor, income, insurance, and food (7,8). However, livestock are likely also an important source of infection and illness among children in LMICs (9,10). Because livestock ownership may simultaneously influence children's diets and exposure to infectious pathogens, understanding whether livestock ownership affects anemia and the pathways through

which it may do so is imperative in the development of programs that address childhood anemia. Animal-source foods (ASFs), which can be derived from own-produced livestock or through income from the sale of livestock and livestock products, contain nutrients which can mitigate anemia including heme iron, vitamin A, and vitamin B12 (11,12). Livestock ownership may therefore be negatively associated with anemia if there is a positive relationship between livestock ownership and ASF consumption. However, livestock ownership does not necessarily translate to greater ASF consumption if livestock are kept for financial or other non-food-related reasons. Instead, livestock ownership may be positively associated with anemia in children if livestock ownership increases exposure to pathogenic organisms. The pathogens that livestock carry, including soil-transmitted helminths and enteropathogenic bacteria (13,14), can lead to anemia by inducing blood loss, inflammation-induced iron sequestration in cells, and reduced iron absorption (15).

Several studies have examined the impacts of livestock on child growth outcomes within this nutrition-benefit and infection-risk framework (16–28), yet few studies have robustly examined impacts on anemia in young children. Intervention studies in South-East Asia promoting poultry and goat production, in combination with nutrition education and home gardening, showed little to no improvements in anemia among children under five years of age despite increased ASF consumption (29–34). Given the multi-component intervention designs of these studies, identifying the benefits derived specifically from livestock rearing in these studies is challenging. Three observational studies found associations between chicken ownership and higher odds of anemia (two in Ghanaian preschool-aged children (35,36), and one in Haitian school-aged children (37)). One of these studies (35) examined helminth infection as a potential mediator, but found no associations with chicken ownership. Both studies conducted in Ghana

postulated that exposure to pathogens in chicken feces may contribute to the higher likelihood of anemia observed among children from chicken-owning households.

There are still many unknowns regarding the relationships between livestock ownership and child anemia, including whether the species or quantity of livestock, the composition of herds, or the management of livestock are important factors. The aforementioned studies assessing livestock ownership and anemia outcomes used common livestock indicators including whether a household owned livestock, which species were owned, and the quantity of each species owned. However, households may simultaneously own several different types of species, and these different ownership patterns may influence how livestock are used for income or food, as well as the thresholds for infectious pathogen exposure. A recent study of livestock ownership and child growth (22) that sought to address this methodological question developed a livestock indicator that categorizes livestock ownership by specific types of livestock and numbers of livestock, which they defined as livestock typologies. This livestock indicator may better capture the utility and draw-back of owning different types of livestock in varying amounts.

The objective of the present study is to assess the relationship between household livestock ownership and anemia in young children living in the Greater Accra Region of Ghana, and determine if this relationship is mediated by child ASF consumption or by child morbidity and inflammation. To better understand the potential relationship between livestock ownership and anemia, we used common livestock indicators and the "typologies" approach of Dumas et al. (22). We also expanded on the work of Christian et al. (35) and Jones et al. (36) by examining iron status and inflammation biomarkers, and child morbidity and diets, to explore the potentially different pathways through which livestock may impact anemia in children in a low-income, high-infection context. We hypothesized that livestock ownership, particularly among

households that own chickens, would be positively associated with child anemia, mediated by children's symptoms of morbidity and elevated inflammation. We further hypothesized that ASF consumption from livestock would not substantially contribute to children's diets, and that there would therefore not be a negative association between livestock ownership and anemia.

Methods

Study area

This cross-sectional study was conducted in the Ga East and Shai Osudoku Districts in the Greater Accra Region, Ghana, during October and November 2018. Ghana is a West African country located on the Guinea Coast. It is divided into ten administrative regions (**Figure 2.1A**). The Greater Accra Region, located along Ghana's southern border and home to Ghana's capital city of Accra, is the smallest and most urban region of Ghana (38). The Ga East District, one of Greater Accra's sixteen districts, borders the urban Accra District at its southern border and is mostly grassland with some densely vegetated areas towards the northern part of the district (39). In 2019, the population of Ga East was 182,183 (40). Ninety percent of Ga East's population resides in urban areas, and the main economic activity is service and sales work. Only 5.5% of households in Ga East are engaged in agricultural activities, with one-quarter of those rearing livestock including chickens (51%), goats (11%), pigs (10%), sheep (8%), and cattle (7%) (39). The Shai Osudoku District, in the north-eastern part of the region, is also a dry grassland area, with denser vegetation and forest towards its eastern border (41). Shai Osudoku is the largest and least populated district in the Greater Accra Region, with 76.7% of its population living in rural areas. The 2019 population of Shai Osudoku was 64,000 (40). Shai Osudoku is predominantly agrarian, with 34% of its employed population engaged in crop farming, forestry, livestock

rearing, and fish farming. Among livestock farmers, most rear chickens (50%) and cattle (26%); other livestock include goats (8%), sheep (4%), pigs (2%), and guinea fowl (5%).

Ghana, located a few degrees above the Equator, has a tropical climate with variations in temperature and rainfall patterns between its North and South. The Greater Accra Region experiences two rainy seasons, the first from April to June and the second, milder rainy season from September to November (38). This study was conducted during the harvest season of several staple crops, including millet, sorghum, rice, and yams. Malaria is endemic in all of Ghana throughout the year, though transmission during the period when data collection activities occurred is typically lower than at other times during the year (42). In 2014, the prevalence of anemia among children 6-59 months old in the Greater Accra Region was 59.6% (38).

Study design

The study population was children 6-59 months of age. Inclusion criteria for households to participate in the study were: (1) the household had at least one child aged 6-59 months old and (2) the primary caregiver for that child had to be 18 years or older.

We estimated that a minimum sample size of 466 children (households) was needed to detect a difference of 0.4 g/dL in hemoglobin concentration between children living in livestock- and non-livestock-owning households. We based the sample size calculation on nationally representative data from the 2014 Ghana Demographic and Health Survey (38) using an independent two-sample t-test with a statistical significance level of 0.05 and a power of 0.80. However, post hoc analyses using a typologies-based approach were underpowered (<50% power) to detect a difference in child anemia.

Livestock-rearing communities in the Ga East and Shai Osudoku districts were identified with the assistance of district-level health and agricultural extension agencies. Communities

ranged in population size from small (less than 750 residents), medium (750 to 2,000 residents), to large (over 2,000 residents), and varied in the types of livestock being reared and the driving distances to Accra. Of 38 identified communities in the two districts, we purposefully selected eighteen communities to maximize variation in community size and types of livestock reared, and to minimize driving distance to Accra to less than 90 minutes (**Figure 2.1B**). Next, the study team conducted a census of all households in each community to identify households with at least one child 6-59 months of age and to gather initial information on any livestock being reared by the household. We used a stratified random sampling approach to maximize the number of households included in the sample that had a child 6 to 24 months old and that owned livestock. This approach was used because we hypothesized that children under two years old would be at higher risk of exposure to animal feces due to crawling and mouthing behaviors (43), and because there were fewer 6-to-23-month-old children in the census compared with 24-to-59-month-old children. Specifically, households were divided into four groupings based on child age (6-23 months old and 24-59 months old) and household livestock ownership status (owns livestock or does not own livestock), and then were randomized within each group. Households with children 6-23 months old and with livestock were approached for participation first, then those with children 6-23 months old and without livestock, then those with children 24-59 months old with livestock, and lastly those with children 24-59 months old without livestock. No more than 40 households per community were selected for participation. In households with more than one child between 6-59 months old, the youngest child was selected for recruitment.

Survey preparation and data collection

Six enumerators were trained in the study protocols, including administering the survey and collecting anthropometric measurements, using classroom and field sessions one week

before the start of the study. In the training period, enumerators practiced and agreed upon translations of the survey from English to local languages (Twi, Ga, Ewe, Dangme). Survey data were collected using electronic tablets (Samsung Galaxy Tab A, Model Number SM-T285) with the Qualtrics survey platform (Qualtrics, Provo, UT, USA). The study survey had been piloted in two non-study communities in July 2018, prior to enumerator training.

Enumerators interviewed the primary caregiver, usually the mother, asking about household sociodemographic characteristics, livestock ownership and management, and the child's diet and health. Household sociodemographic indicators included: 1) the number of total members and number of children under five years old in the household; 2) the sex of the head of household; 3) the age, religion, ethnic group, and education level of the head of household and mother of the child; and, 4) the household's assets, water source, and sanitation and hygiene practices. In instances where the child's mother was not living in the household, the primary caregiver's education level was recorded. Livestock ownership and management questions included the number and species of animals owned, who in the household owned and controlled resources from the animals, corralling practices during the day and night, and the purchase, sale, and consumption of the household's livestock and livestock products (44). Enumerators also conducted an observational hygiene spot-check, assessing the house structure, indicators of household cleanliness, the mother's and child's cleanliness, and the presence of human and animal feces in and around the household (25).

The child's diet was assessed using a 24-hour food group recall and a qualitative three-month food frequency questionnaire assessing consumption of various ASFs. Caregivers were asked about the child's breastfeeding status and whether the child had consumed various types of liquids and foods in the past day or night (45). Caregivers were then asked whether and how

often the child had consumed various ASFs in the past three months including, 1) flesh foods (i.e., beef, mutton, pork, goat, chicken, duck, turkey, guinea fowl, other domestic meat, bushmeat, small fish, other fish, shellfish, organ meats), 2) milk and milk products (i.e., fresh cow's milk, tinned or powdered milk, yogurt and other dairy foods), 3) eggs, and 4) other animal-source foods (i.e., insects, snails). The primary way that each consumed ASF was obtained (e.g., own production, hunting or fishing, purchased) was also recorded.

Caregivers were then asked to recall symptoms of illness, including whether the child experienced fever, cough or cold, or diarrhea (three or more loose stools in 24 hours) in the previous seven days. Additional questions about health and morbidity included whether the child had received an iron supplement in the past seven days, a vitamin A dose in the past six months, or any drug for intestinal worms in the past six months.

Blood sample collection and laboratory analysis

Blood samples from children were collected via a standard finger-prick procedure (46). Hemoglobin (Hb) concentration was measured in the field using a HemoCue® Hb 201+ portable hemoglobinometer (HemoCue AB, Sweden). HemoCue analyzers were checked weekly against low, normal, and high Hemoglobin Extended Controls (R&D Systems, Minneapolis, MN) to monitor the quality of values obtained from the instruments. Malaria parasitemia was measured using the SD Malaria Ag P.f (HRP2/pLDH) antigen rapid diagnostic test (RDT) (Standard Diagnostics Inc., Korea) according to the manufacturer's instructions. This malaria RDT detects antigens to histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH) of *Plasmodium (P.) falciparum*, which is useful in countries, like Ghana (47), where *P. falciparum* parasites with HRP2 deletions may be present. Positive *Plasmodium* infection was defined as the

presence of either one or both of the HRP2 and pLDH test bands and the presence of the control band.

Approximately 200uL of blood was collected in a Microvette® clotting activator/serum tube (Sarstedt, Germany). Samples were transported at room temperature to Noguchi Memorial Institute for Medical Research (Accra, Ghana) and immediately spun in a microcentrifuge at 5,000 rpm for 10 minutes. 50uL of serum was aliquoted into a 0.2mL PCR tube (Sarstedt, Germany) and stored at −20°C. Serum samples were shipped on dry ice to the VitMin Laboratory (Willstaett, Germany) for analysis of serum ferritin (SF), serum transferrin receptor (sTfR), retinol binding protein (RBP), C-reactive protein (CRP), and α-1-acid glycoprotein (AGP) using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) technique (48).

Stool sample collection and laboratory analysis

Caregivers were instructed to collect the child's morning stool in a sterile fecal container (Sarstedt, Germany). Stool samples were transported in a cold chain to the Noguchi Memorial Institute for Medical Research every afternoon. Upon arrival at the lab, two trained laboratory technicians independently analyzed stool samples using the Kato-Katz method for eggs of the helminths *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, and *Necator americanus*. Duplicate Kato-Katz thick smears were prepared for each stool sample with a 41.7 mg plastic template according to standard protocols (49). The number of helminth eggs for each species was counted by microscopy and then multiplied by 24 to estimate the number of eggs per gram of feces.

Anthropometry

A pair of trained enumerators collected duplicate anthropometric measurements on each child according to standard protocols (50). Height, for children 24-59 months old, and recumbent

length, for children 6-23 months old, was measured to the nearest 0.1 cm using a ShorrBoard® (Weigh and Measure, USA). Weight was measured to nearest 0.1 kg using a digital scale (Model WB-110A, Tanita, Japan). Length or height-for-age Z-scores (hereafter HAZ) and weight-for-length or -height Z-scores (hereafter WHZ) were calculated using the World Health Organization (WHO) Child Growth Standards against the reference standard for each child's respective age and sex using a STATA macro provided by the WHO, to assess stunting (HAZ < −2.0 SD) and wasting (WHZ < −2.0 SD), respectively (51).

Variable definitions

The primary independent variable in this study was household livestock ownership. We defined livestock ownership as owning or rearing cattle, goats, sheep, pigs, chickens, ducks, turkeys, guineafowl (*Numida meleagris*), grasscutter (*Thryonomys swinderianus*), rabbits, or rats at the time of the survey. Livestock ownership may affect child nutrition and health in distinct ways depending on the species and number of livestock a household owns. Thus, we examined livestock ownership using several different approaches, consistent with methods in previous studies (19,21,22,24,36). First, we created dichotomous variables for ownership of any livestock and distinct livestock species, as well as continuous variables for the number of animals owned by species. Next, we created variables reflecting the household's total livestock assets, including the household's total number of individual livestock and the household's tropical livestock unit score (TLU). TLU is a weighted measure of livestock ownership that accounts for the weight and market value of different livestock species to create a single unit variable (52). TLU was calculated as the sum of the number of each livestock species multiplied by its weight conversion factor (0.70 for cattle, 0.20 for pigs, 0.10 for goats and sheep, 0.03 for ducks, turkeys, and guinea fowl, 0.02 for grasscutter, rabbits, and rats, and 0.01 for chickens). Continuous variables were

log-base two transformed to normalize the distribution of animals owned by livestock-owning households and the resulting effect estimates are interpreted as the change in the outcome variable for a two-fold increase in the number of livestock owned. Lastly, we developed livestock typologies to represent the typical groupings of livestock species among this sample of Ghanaian households as this may better represent how household livestock ownership can impact child nutrition and health in such settings. We based our methods on the livestock typologies used in the analyses by Dumas et al. (22) and de Bruyn et al. (21), who used two methods to capture the asset value of owning smaller (e.g., poultry) versus larger (e.g., cattle) livestock. Dumas et al. (22) cross-tabulated households' TLU and the total number of animals owned to create five livestock typologies and de Bruyn et al. (21) created four typologies based on the concept of the "livestock ladder," which captures the hypothesis that households use livestock as an asset to get out of poverty, moving from chickens, the lowest rung on the ladder, to larger livestock, as they gain wealth. Most of the households in our sample owned chickens, so we created two categories for poultry ownership to distinguish households that owned more and less than the median number of poultry owned in our sample (12 birds). Ultimately, five typology categories were created to capture livestock ownership patterns in our sample:

a) Type 1 – No livestock;
b) Type 2 – Only poultry, less than 12;
c) Type 3 – Only poultry, equal to or more than 12;
d) Type 4 – Small livestock (goats, sheep, pigs) or rodents (grasscutter and other rats) or rabbits, with or without poultry;
e) Type 5 – Cattle, with or without small livestock or poultry.

The primary outcome variable was child anemia, defined as Hb<11.0 g/dL for children 6-59 months old (53). Mild, moderate, and severe anemia were defined as Hb 10.0-10.9 g/dL, 7.0-9.9 g/dL, and <7.0 g/dL, respectively (53). We analyzed iron deficiency as a secondary outcome. Iron deficiency was defined, respectively, as low serum ferritin (SF<12 ug/L), indicative of low iron stores, or high serum transferrin receptor (sTfR>8.3 mg/L), indicative of low availability of iron to tissues (54). As a positive acute-phase protein, SF is elevated in the presence of inflammation and does not accurately reflect true iron stores. Thus, iron deficiency may be underestimated in settings with high inflammation. Therefore, we adjusted SF concentrations for inflammation using the regression correction approach with the inflammation biomarkers CRP and AGP, as outlined by Namaste et al. (54).

Two sets of mediators were analyzed to examine the two pathways of interest that may mediate associations between livestock ownership and child anemia: diet and morbidity (55). We assessed child diet using both a dietary diversity indicator and reported ASF intake. The child's consumption of food types in the previous 24 hours was categorized into seven food groups: grains, roots, and tubers; legumes and nuts; dairy products; flesh foods (meat, fish, poultry, and organ meats); eggs; vitamin-A rich fruits and vegetables; and other fruits and vegetables. We created dichotomous variables for each food group and summed the food groups to create a dietary diversity score ranging from 0 to 7 (45). To assess ASF intake, we created dichotomous variables for any consumption of different categories of ASF in the past 24 hours (cow meat, other red meat, chicken meat, organ meats, eggs, animal milk) and past three months (cow meat, goat, sheep, or pig meat, chicken or other poultry meat, organ meats, eggs, fresh cow milk). Chicken meat was the primary poultry meat consumed in the past three months, so we use the term chicken meat moving forward, but note that this variable includes a small proportion (2.4%)

of children who also consumed duck, turkey, and guinea fowl meat. The number of times per month the ASF categories were consumed during the three months before the survey was calculated, ranging from 0 times per month (if the food group was not consumed) to 62 times per month (if the food group was consumed two or more times per day during a 31-day month). We assessed morbidity indicators and inflammation as proxies for child infection. Morbidity indicators included whether or not the child had a fever, diarrhea, or a cough or cold in the seven days before the survey. Inflammation was defined as CRP>5 mg/L and AGP>1 g/L (54). CRP is a biomarker for acute inflammation, peaking within 48 hours of infection, while AGP is a biomarker for chronic inflammation (56).

Several sociodemographic and child-level covariates were analyzed, identified *a priori* as theoretical determinants of anemia (57–59). Household sociodemographic variables included the number of children under five years of age in the household, the household head's sex, religion, and ethnic group, the mother's education level, the household's access to an improved water source, the household's type of sanitation facility, and an asset-based wealth index. An improved water source was defined according to the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation guidelines and includes piped water, public pipe/standpipe, tube well or borehole, protected dug well, protected spring, bottled water, sachet water, cart with tank, and rainwater (60). We developed a household wealth index using principal components analysis (PCA) of 26 assets (e.g., electricity, radio, color television, mobile phone, electric generator, computer, access to internet or data in any device, bicycle, car), excluding land holdings (61). The wealth index excluded livestock assets (our primary independent variable), and water, sanitation, and hygiene indicators, as these are reported separately. Asset scores from the first component were categorized into quintiles and used to characterize household wealth from

lowest to highest. Child-level variables included child sex, age, breastfeeding status, and malaria parasitemia. The child's date of birth was verified by enumerators using their clinical Child Health Records. If health records were not available and the child's month and year of birth, but not day of birth, were known, we imputed that the child was born on the 15th of the month (n=3).

Statistical analysis

Data cleaning and statistical analysis were conducted using Stata SE version 14.2 (StataCorp, College Station, TX, USA). Mediation analysis was done using the mediation package in R (62,63).

We conducted descriptive analyses of child- and household-level characteristics in the overall sample, by child anemia status, and by household livestock typologies. Differences in characteristics between anemic and non-anemic children were tested using chi-squared tests for proportions and t-tests for means. Differences between livestock typologies were tested using chi-squared tests for proportions and ANOVA for means. Bivariate associations between livestock typology and anemia, iron status, and potential mediating variables were calculated using chi-squared and ANOVA tests. We used logistic regression to model the association between livestock ownership typology and anemia. In our adjusted models, we controlled for the child- and household-level covariates described above. Adjusted models also included a fixed effect for the study district in order to account for unobserved differences between the two study districts. We excluded child breastfeeding status from the adjusted models, as it was strongly associated with child age. We also excluded the improved water source variable as there was little variation among the sample, and inclusion of this variable in the model did not alter effect estimates. Helminth infection was not included because none of the 415 stool samples tested (88%) were positive for any helminth species. As a sensitivity analysis, we analyzed unadjusted

and adjusted associations between additional indicators of livestock ownership and anemia using logistic regression. We also analyzed the unadjusted and adjusted associations between all of the livestock ownership variables and hemoglobin concentration using linear regression, as well as with iron deficiency indicators (i.e., SF and sTfR) using logistic regression. To test whether younger children may be at higher anemia risk from livestock exposure, we ran an adjusted logical regression model with an interaction term between livestock typology and child age (6-23 months versus 24-59 months). In all of the regression models, we calculated robust standard errors clustered at the community level.

To explore the hypothesized pathways between household livestock ownership and anemia, we tested for mediation by child ASF consumption and by child morbidity symptoms or inflammation using two approaches (**Figure 2.2**). First, we used multiple logistic regression models to analyze the association between livestock ownership typologies and the mediators. For relationships that showed evidence of an association between livestock ownership and one of the mediators, we then used the R mediation package to assess the total, direct, and indirect effects of typology on anemia through that mediator (62). Briefly, two logistic models were specified: the mediation model, which predicted the mediator conditional on livestock typology and select covariates, and the outcome model, which predicted anemia conditional on livestock typology, the mediator, and covariates. The average causal mediated effect (indirect effect) was then estimated for each typology in comparison to Type 1 (no livestock) using quasi-Bayesian Monte Carlo with 500 simulations and clustering set at the community level.

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) and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18). Before data collection, we met with local chiefs and key assemblypersons from the selected communities to brief them on the study and obtain their general approval. Then, at each selected household, enumerators explained the study to a parent or guardian of the participating child. The child's parent or guardian provided written, informed parental consent for the child's participation in the study. The child's primary caregiver, usually the mother, provided written informed consent for their own participation in the interview. The parent or guardian of the child also elected whether the child's collected blood and stool specimens could be used for future research. If the caregiver was illiterate, the enumerator read the consent documents aloud to the caregiver in their preferred language. In the presence of a witness, the caregiver provided a thumbprint, and the witness provided a signature certifying that the study benefits, risks, and procedures were explained. Households were given a non-monetary gift (e.g., washbasin) as compensation for participating in the study.

Results

Sample characteristics

In total, 484 households were consented and sampled. Of those, we excluded ten households (2.9%) that had missing information on the mother's education level (n=3), the child's date of birth (n=2), consumption of ASF (n=2), morbidity symptoms (n=1), or SF and sTfR measurements (n=2). Four households in the sample were poultry farms, rearing from 500 to more than 1000 poultry birds, of which most were foreign breeds. Children from these

households had Hb concentrations 0.5 g/dL higher than the sample average, a greater likelihood of consuming chicken meat and eggs, and placement in the highest wealth quintile. Given that we were interested in understanding small-scale livestock ownership, these four households were also excluded from the analyses. The final sample size for this study was 470 households.

Child and household characteristics

Approximately half of the children were 6-23 months of age (50.9%) and female (52.1%) (**Table 2.1**). Most households had access to basic electricity (87.5%), an improved water source (97.0%), and dwelling structures with cement walls (76.6%), non-thatched roofs (98.9%), and non-earthen floors (98.7%). However, 29.4% of households did not have a toilet facility and practiced open defecation, and 97.9% of households lacked a handwashing facility.

Overall, nearly half (47.9%) of children were anemic (**Table 2.1**). Of these, half (50.7%) were mildly anemic, just under half (47.1%) had moderate anemia, and 2.2% had severe anemia. The prevalence of anemia was substantially higher among children 6-23 months of age than those between 24-59 months of age. Iron deficiency was common, with 52.8% of all children presenting with elevated sTfR and 25.1% presenting with low SF. Iron deficiency was significantly higher among anemic children. The concentrations of the inflammatory biomarkers CRP and AGP, as well as malaria parasitemia, were also significantly higher among anemic children. Other symptoms of illness, including fever, diarrhea, and cough or cold, were not different among anemic and non-anemic children. None of the children tested for helminths had a detectable infection, though over one-quarter took a drug for helminths in the last six months.

Livestock ownership characteristics

Overall, 43.6% of households owned or reared livestock, which were classified into different livestock typology categories (**Table 2.2**). Of livestock-owning households, most

owned poultry, predominantly chickens, either alone or with other types of livestock. Among Type 2, households owned a median of five chickens while in Type 3 households owned a median of 20 chickens (**Figure 2.3**). Among households in Type 4, 71.7% owned goats, and 81.7% owned chickens, while ownership of sheep, pigs, and specialty rodents such as grasscutters was less common. Among the 15 households in Type 5 that reared cattle, 14 also reared chickens, and 10 reared goats, sheep, or pigs.

Among the livestock owners, most allowed their animals to roam freely during the daytime. Poultry were typically free-roaming around the household compound during the day, while cattle and a small proportion of small livestock free-roamed elsewhere (such as on land away from the household compound). Overall, the majority of chickens (88.1%), goats (71.4%), and sheep (68.4%) free-roamed within the household compound and yard. Most households confined their animals outside at night, and very few confined any of their animals inside their house dwelling structure.

Households reported keeping chickens for food or food and financial purposes, while larger livestock were primarily kept for financial reasons (**Figure 2.4**). Less than one-fifth (18.1%) of households reported selling poultry from their holdings, while over one-third (38.3%) had slaughtered poultry for household consumption in the past three months **(Table 2.3)**. The proportion of households that sold small livestock and cattle from their own holdings, in contrast, was higher than the proportion that consumed their animals. Across all categories of livestock (i.e., poultry, small livestock, cattle), the number of livestock sold was on average higher than the number that the household slaughtered for consumption. Poultry-owning households reported consuming eggs laid by their poultry (mostly from chickens), while the sale of eggs was very rare (1.0% of households). Most households with cattle sold milk from their cows and also

consumed some, but the majority reported consuming less than half or very little of the milk produced.

Child and household characteristics were analyzed by livestock typologies (**Table 2.4**). No key child characteristics were associated with the livestock typologies. Livestock ownership was more common in Shai Osudoku than in the Ga East district, and all households with cattle resided in this district. Household size, religion, ethnic group, occupation, and education were different between the typologies, especially amongst Type 5 households, who had on average more family members, were predominantly Muslim, of a minority ethnic group, employed in agricultural labor, and had the highest proportion of mothers with no or nursery-level education. A higher proportion of livestock-owning households compared to non-livestock owing households practiced open defecation, with the highest proportion among households in Type 5. While there were no significant differences in wealth quintile between the household typologies, the average wealth quintile was slightly lower among households in Types 2 and 5. In each livestock typology category, animal feces were observed in households' yards, including in the yards of households that did not own any livestock.

Livestock ownership and child anemia

In unadjusted analyses, children in Type 4 had lower odds of anemia compared to children in Type 1 (**Table 2.5**). After adjusting for child and household sociodemographic characteristics, the association between Type 4 and anemia was attenuated and marginally significant (OR (95% CI): 0.62 (0.36, 1.07)). Only children in Type 5 households had significantly lower odds of anemia in comparison to children with no livestock in the adjusted logistic regression model (0.32 (0.14, 0.71)). Among the covariates, the odds of anemia were notably higher among children that were 6-23 months old, were positive for malaria parasitemia,

and lived in the Shai Osudoku District. We evaluated whether child age modified the association between livestock ownership and anemia, but found no evidence for interaction when comparing children 6-23 and 24-59 months old ($X^2(4) = 5.40$, p=0.14) (**Table 2.6**).

As a sensitivity analysis, we further examined associations of livestock ownership overall, by species, and by quantity with anemia, hemoglobin concentration, and iron deficiency (**Table 2.7**). There were no associations between livestock ownership overall and anemia, or by species and anemia. However, among livestock owners, a two-fold increase in the number of total livestock owned, in the number of cattle owned, and in the number of goats, sheep, or pigs owned was associated with 20%, 14%, and 14% lower odds of anemia in children, respectively. In line with these results, for every two-fold increase in TLU score, children had 33% lower odds of anemia. Ownership of goats, sheep, or pigs was associated with a 0.39 g/dL higher Hb concentration, while owning more livestock in total was marginally associated with higher Hb concentrations. None of the livestock indicators was associated with iron deficiency.

Livestock ownership and child ASF consumption

Overall, 84.9% of children consumed ASF in the past 24 hours, and 97.7% of children consumed ASF at least once in the previous three months (**Table 2.1**). Fish was the primary ASF consumed, though many children also consumed meat, eggs, and dairy. For subsequent analyses, we only considered livestock-derived ASF and excluded fish. There was no difference in the consumption of meat, eggs, or milk in the past 24 hours among the livestock ownership typologies (**Table 2.8**). In the three months before the survey, few children consumed meat from sheep, pigs, and rodents or eggs from ducks or guineafowl. Chicken meat and chicken eggs were consumed in the past three months by the majority of children, on average, about once per week for chicken meat and twice per week for chicken eggs. Children in Type 3 households (with at

least 12 poultry but no other livestock) were 3.5 times more likely to have consumed chicken meat in the past three months compared to non-livestock-owning households (**Table 2.9 and Figure 2.5**). The odds of consuming chicken eggs were not higher among livestock-owning typologies versus the non-livestock owning typology; interestingly, children from Type 2 households (with less than 12 poultry birds and no other livestock) were less likely to consume chicken eggs than children in Type 1. While most children had consumed dairy products in the past three months, only 11.5% had consumed fresh cow's milk. Furthermore, fresh cow's milk was only consumed in the Shai Osudoku District. Children from households in Types 3, 4, and 5 had higher odds of consuming cow's milk in the past three months compared to children from non-livestock-owning households (Type 1). Among households with cattle, just under half of the children had consumed fresh cow's milk in the past three months, on average, about three times per week. Consumption of other types of meat was not different between livestock-owning and non-livestock-owning typologies.

Livestock ownership and child morbidity

Overall, 23.6% of children experienced a fever in the past seven days, 7.7% had diarrhea, and 22.6% had a cough or cold (**Table 2.1**). Sixteen percent of children had elevated levels of CRP, and 35.5% had elevated levels of AGP. Neither symptoms of illness in the past seven days nor inflammation was significantly different among the livestock typologies in bivariate analyses. However, there was a slightly higher proportion of children with fever among Type 2 and a lower proportion of children with diarrhea among Types 4 and 5 (**Table 2.8**).

Mediation analysis

Formal mediation analysis was conducted for chicken meat and cow milk consumption as these were the only hypothesized mediators associated with livestock ownership typology. There

were no mediating effects of either chicken meat or cow milk consumption in the association between livestock ownership typologies and anemia (**Table 2.10**).

Discussion

Livestock ownership in low-income countries may mitigate anemia among young children by providing a source of micronutrient-rich ASFs, yet concurrently exacerbate anemia by exposing children to zoonotic pathogens that cause illness and inflammation. Contrary to our hypothesis that livestock ownership would be associated with higher odds of anemia mediated by symptoms of morbidity and inflammation, we found no negative associations between livestock ownership and child anemia. Rather, in this cross-sectional study of 6-59-month-old children in Greater Accra, Ghana, children from households that owned cattle concurrently with small livestock (goats, sheep, or pigs) or poultry had lower odds of anemia than children from households without livestock. Among livestock owners, owning more cattle or small livestock was also associated with lower odds of anemia. Although some livestock ownership typologies were associated with consumption of select ASFs, children's consumption of those ASFs did not mediate the association between livestock ownership and anemia. Owning livestock did not necessarily translate to more ASF consumption, and less than half of households consumed meat or eggs from their livestock. Furthermore, despite clear associations between anemia and iron deficiency, there were no associations between livestock ownership and iron deficiency. Livestock may therefore provide some benefit other than ASF intake that lowers children's likelihood of anemia.

In LMICs, livestock are an important financial resource (8). Poultry, small livestock, and cattle serve different purposes, and households diversify their livestock holdings to utilize the advantages and disadvantages of each species. Poultry, which includes free-ranging, indigenous

chickens, guinea fowl, turkeys, and ducks, are affordable, have short reproductive cycles, and are readily sold or exchanged to meet regular household needs (64,65). Goats, sheep, and pigs are insurance that can be used to meet more substantial expenses, while still being relatively affordable for households to maintain (66). In contrast, large livestock, such as cattle, require more inputs and are far more expensive to maintain, yet are the most financially valuable (66). In this study, livestock ownership typologies represented different diversification patterns among livestock-owning households, ranging from households that owned less than 12 poultry to those that owned large herds of cattle plus several goats, sheep, and chickens. Among the typologies, Type 5, characterized by cattle and the largest average total herd sizes, was associated with lower odds of anemia when compared to households with no livestock. Type 4, characterized predominantly by goats and chicken ownership, was also marginally associated with lower odds of anemia. Among livestock owning households, those with higher TLU and greater total number of livestock were less likely to have a child with anemia. Finally, ownership of small livestock such as goats, sheep, or pigs was the only livestock indicator associated with higher hemoglobin concentrations in children. Interestingly, we found no evidence that consumption of meat, eggs, or milk mediated the association between owning livestock and lower odds of anemia. An alternative pathway, which this study was unable to test, is that livestock ownership lowers children's likelihood of anemia through an income-based pathway.

In line with literature on the financial value of different types of livestock, the livestock indicators that were associated with lower odds of having anemia in this study were those that represent higher livestock assets, and also greater opportunity for supplemental income from those livestock. Households in our study reported keeping livestock, particularly cattle, goats, sheep, and pigs, primarily for financial purposes, and over one-third of households reported

selling live animals in the past year, on average five goats, sheep, or pigs among small livestock-owning households and four cattle among cattle-owning households. The income earned from sales of these animals in one year equates to approximately $305 for the sale of five small livestock and $1,124 for the sale of four cattle, based on households' self-reported earnings per live animal sale. Qualitative studies conducted in Ghana have similarly found that households keep livestock, not for consumption, but foremost as a bank account, to be used to pay for school fees, healthcare costs, and farm labor (67,68). How livestock-derived financial capital may translate into reduced anemia warrants further investigation.

Several plausible pathways between livestock capital and lower child anemia may be considered. First, having livestock may enable households to access and utilize medical care for children who are ill. Second, children from households that can afford school fees may be more likely to receive healthcare services and nutritious meals provided at schools. Third, livestock-derived income may enable households to increase overall expenditures on food, improving children's nutrition. Alternatively, it is possible that the associations between lower anemia and larger, higher-value livestock holdings are confounded by greater household wealth. Some argue that incremental livestock diversification from poultry to small ruminants to cattle, known as moving up the "livestock ladder", is a pathway out of poverty (65,69,70). However, our analysis is not consistent with this. Associations between livestock ownership and anemia were present even after adjusting for several sociodemographic factors that proxy a household's wealth, and livestock ownership typologies were not associated with asset-based wealth. Similarly, other research has not found a consistent relationship between household wealth and livestock assets (66,71). Pica-Ciamarra et al. (71) concluded that the concept of the livestock ladder might be country-specific, and more dependent on small ruminant holdings, rather than ownership of

poultry or large ruminants. Thus, in our study region, livestock holdings may facilitate a form of dispensable wealth that positively impacts child anemia status, independent of wealth status.

Our study adds to the small body of literature investigating the association between livestock ownership and anemia in young children. Christian et al. (35) found that children from households that owned livestock had lower odds of anemia compared to those from households without livestock, yet higher odds of anemia with increasing numbers of sheep and goats, and marginally higher odds of anemia with increasing numbers of chickens. Jones et al. (36), using the nationally representative Ghana Demographic and Health Survey, found no associations between child anemia and owning cattle, goats, sheep, or pigs, but significantly higher odds of anemia among children living in households with chickens. Neither study found evidence that relationships between livestock ownership and anemia were mediated by ASF consumption, and neither investigated mediation by illness. Contrary to these studies, we did not observe any statistically significant associations between owning chickens, or the number of chickens owned, and anemia in children. Furthermore, we found no significant associations between any livestock indicator and higher odds of anemia. Conflicting findings in these studies and ours may be because of differences in how livestock ownership was assessed. Neither Christian et al. (35) nor Jones et al. (36) assessed the distribution and quantity of livestock species in study households, and as noted above, the household composition of flocks and herds may be an important factor in how households can utilize their livestock. Context may also be a key factor determining how livestock can impact child anemia. Our study, which was conducted in the wealthiest administrative region of Ghana located less than two hours from the capital city of Accra, may have differed from the other studies in how households depended on livestock for their

livelihoods, how animals were managed, and whether households had access to resources such as clinics and markets.

Although our study found no evidence of a mediation pathway between livestock ownership, consumption of ASFs, and anemia, ownership of poultry and cattle was positively associated with consumption of chicken meat and cow's milk, respectively. Other studies that investigated associations between livestock ownership and child ASF consumption have found inconsistent results, including no associations (22,36), positive associations when livestock are female-owned (28), or positive associations dependent on the type and number of livestock owned, as in our study (20,21,24,72). Also, similar to our findings, consumption of cow's milk has been found to be higher among households that own dairy cows (17,27,73). Because livestock are viewed as a financial asset, households preferentially purchase meat and eggs to feed their children, rather than source ASFs from their animals. In Ghana, as in other sub-Saharan African countries, poultry are occasionally slaughtered for consumption, often for special occasions or guests, but larger livestock such as goats and sheep are consumed only if the animal is sick or dies (67,68,74–76). Consumption of chicken eggs is also only occasional, as eggs are primarily viewed through their reproductive potential – eggs hatch into chickens, which can then be sold or used for further propagation of the flock. The lack of an indirect effect of chicken meat consumption between poultry ownership and anemia may be because of the overall infrequency with which children consumed chicken meat, limiting the impact on children's overall iron intake. Cow's milk is also unlikely to positively impact children's anemia status given that unfortified cow's milk is a poor source of iron (12), and milk consumption may increase iron deficiency among young children (77). Thus, even under conditions when

household livestock-derived ASFs were used for home consumption, the foods that were most likely to be consumed may have had little to no impact on child anemia status.

While there is some evidence that livestock ownership is associated with increased risk of morbidity in young children, findings to-date are mixed and show heightened risk of diarrhea in some countries but not others (18,21,25,78). Our study found no adverse associations between livestock ownership and fever, diarrhea, cough, or inflammation in this study. Different methods of animal management may influence risk of pathogenic exposure from livestock. Headey and Hirvonen (24), for example, found a negative association between poultry ownership and child growth in rural Ethiopia only if poultry were corralled indoors overnight, hypothesizing that the proximity of chickens to children may expose children to bacteria and viruses that negatively affect their growth. In our sample, over half of households confined poultry and other livestock at night, but very few did so inside their household dwelling, which may reduce children's exposure to the pathogens in animal feces. During the daytime, however, the majority of households in our sample did not confine their livestock, and over three-quarters of households reported that poultry roamed freely within the household compound. It is well-established that livestock feces can contain abundant enteropathogenic bacteria that may cause human illness (13); children, especially those under two years old, are susceptible to exposure through various direct and indirect pathways (9,43). Thus, another reason why we found no associations between livestock and illness may be because livestock feces are widely distributed across communities, resulting in community-level, rather than household-level, exposure. In our study, more than two-thirds (68%) of households that reported no livestock ownership had livestock feces in their yards, and three-quarters (76%) had chickens roaming in the yards during the study interview (data not shown). Thus, even in households without livestock, children are still likely exposed to

pathogenic organisms from livestock feces, because unconfined livestock roam among household yards.

Anemia in this sample of children was significantly associated with indicators of micronutrient status, inflammation, and infection, confirming the importance of both nutritional and infectious causes of anemia in low-income settings. Specifically, anemic children were more likely to have low levels of circulating and stored iron, have vitamin A deficiency, and have elevated levels of CRP and AGP, all of which is consistent with the literature on determinants of anemia in young children living in countries with high infection burdens (57). Malaria is a known, significant risk factor for anemia among children under five years old in Ghana (58,79), and our data shows that it remains a critical factor in addressing the anemia burden among this vulnerable population. Interestingly, we did not find any active soil-transmitted helminth infections in children, which are also a known infectious cause of anemia in young children in SSA (6,80). Research on helminth infection in children under five years in Ghana is limited, as infection control is primarily focused on school-aged children. Studies that have included children 2-5 years old, conducted in Accra (81) and the Eastern Region (35), have found low prevalence of soil-transmitted helminths (less than 5%) in these young children. Interestingly, over one-quarter of children in our study had taken an anti-helminthic drug in the last six months, and anemia prevalence among those who took the drug was lower, pointing to the need for further investigation of helminth infections in this young population.

Strengths of this study included detailed characterization of household livestock ownership and the use of iron and inflammatory biomarkers to examine potential pathways between livestock ownership and anemia. However, this study has several limitations. First, it was cross-sectional in design, which prevents us from determining a temporal link between

livestock ownership and anemia, or any causal inferences from our findings. Second, because this study was conducted during two months, we were unable to evaluate seasonality. Seasonal fluctuations in food availability may change how households utilize livestock during food shortages, and temperature and precipitation also may influence pathogen burdens and, consequently, children's morbidity. Third, hemoglobin concentrations measured using a finger-prick rather than venous blood draw are overestimated, and readings can vary based on temperature, humidity, and the technique used in obtaining blood (82,83). Although we took several precautions to minimize variability, including training technicians and calibrating instruments, there is the possibility that we measured higher hemoglobin concentrations, producing lower anemia prevalence, in an unknown systematic way that influenced our results. Fourth, the sample size of cattle owners, hence Type 5, was small and the typologies-based approach was underpowered to detect differences in child anemia. Nevertheless, small sample sizes increase the likelihood of false positives, thus power may not have been a limitation in this study since we detected a significant result even with a small number of cattle-owning households. Still, households in Type 5 differed in several characteristics (e.g., occupation, religion, household size) from the other typologies. Despite controlling for these factors in our analyses, there may be an unmeasured factor associated with these households driving the relationship with anemia. This limits the generalizability of our findings. We analyzed several other indicators of livestock ownership, which confirmed our findings, but it is possible that these indicators, especially TLU and the total quantity of livestock, were also influenced by the limited cattle owners in the study sample. This limits the generalizability of our findings. Lastly, we did not assess the quantity of ASFs consumed, nor was nutrient intake measured, which may explain why we did not see any mediation through diet.

Conclusion

Anemia among children under five years old remains an intractable public health problem in many LMICs. Our findings in Ghana suggest that diversified ownership of livestock, such as cows or goats and sheep along with poultry, may be an accessible nutrition-sensitive approach to reducing anemia among young children, but that this is not the result of higher consumption of ASF. Households were unlikely to consume ASF from their own livestock, and there is little existing evidence that ASF consumption reduces anemia or iron deficiency in children aged 6 to 59 months (84). Rather, owning a diverse and sufficiently large herd of livestock may provide households with a financial resource that benefits their children's health. Although we found that livestock were kept as a financial asset, we were unable to explore income-based pathways in this study. Further research elucidating non-dietary pathways between livestock ownership and anemia in Ghana would help to clarify how households decide to consume or sell livestock, and how livestock-derived income is used. Research on the frequency and quantity of ASFs needed to impact child micronutrient status and Hb concentrations would also help livestock-development programs identify feasibility of dietary benefits from household livestock ownership. Finally, given the negative associations found between chickens and anemia in prior studies, further evaluation is needed to determine whether livestock-derived infections in children increase their risk of anemia, and whether animal management strategies can mitigate such risks. Identifying the importance of financial, dietary, and infectious disease pathways between livestock ownership and child anemia will help livestock development programs clarify policy priorities to maximize the benefits and minimize risks of livestock for low and middle-income households.

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Ethical Statement

This study was approved by the University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board (protocol no. HUM00145171) and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18).

References

1. World Health Organization. Global Health Observatory Data Repository: Anaemia in Children <5 Years by Region. [Internet]. 2017 [cited 2020 Apr 20]. Available from: https://apps.who.int/gho/data/view.main-amro.ANEMIACHILDRENREGv?lang=en
2. McCann JC, Ames BN. An overview of evidence for a causal relationship between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931–45.
3. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28:S560-71.
4. Scott SP, Chen-Edinboro LP, Caulfield LE, Murray-Kolb LE. The impact of anemia on child mortality: An updated review. Nutrients. 2014;6:5915–32.
5. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. Lancet. 2011;378:2123–35.
6. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019;1450:15–31.
7. Randolph TF, Schelling E, Grace D, Nicholson CF, Leroy JL, Cole DC, Demment MW, Omore A, Zinsstag J, Ruel M. Invited review: role of livestock in human nutrition and health for poverty reduction in developing countries. J Anim Sci. 2007;85:2788–800.
8. Herrero M, Grace D, Njuki J, Johnson N, Enahoro D, Silvestri S, Rufino MC. The roles of livestock in developing countries. Animal. 2013;7:3–18.
9. Penakalapati G, Swarthout J, Delahoy MJ, McAliley L, Wodnik B, Levy K, Freeman MC. Exposure to animal feces and human health: a systematic review and proposed research priorities. Environ Sci Technol. 2017;51:11537–52.
10. Prendergast AJ, Gharpure R, Mor S, Viney M, Dube K, Lello J, Berger C, Siwila J, Joyeux M, Hodobo T, et al. Putting the "A" into WaSH: a call for integrated management of water, animals, sanitation, and hygiene. Lancet Planet Heal. 2019;3:e336–7.
11. Iannotti LL, Lutter CK, Bunn DA, Stewart CP. Eggs: The uncracked potential for improving maternal and young child nutrition among the world's poor. Nutr Rev. 2014;72:355–68.
12. Neumann C, Harris DM, Rogers LM. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutr Res. 2002;22:193–220.
13. Delahoy MJ, Wodnik B, McAliley L, Penakalapati G, Swarthout J, Freeman MC, Levy K. Pathogens transmitted in animal feces in low- and middle-income countries. Int J Hyg Environ Health. 2018;221:661–76.
14. Anchang KY, Avery L, Pertiwiningrum A. A commentary on occupational infectious diseases due to agricultural practices in Sub-Saharan Africa. Biomass and Bioenergy. 2014;70:99–111.
15. Jonker FAM, te Poel E, Bates I, Boele van Hensbroek M. Anaemia, iron deficiency and susceptibility to infection in children in sub-Saharan Africa, guideline dilemmas. Br J Haematol. 2017;177:878–83.
16. Azzarri C, Zezza A, Haile B, Cross E. Does livestock ownership affect animal source foods consumption and child nutritional status? Evidence from rural Uganda. J Dev Stud. 2015;51:1034–59.
17. Choudhury S, Headey DD. Household dairy production and child growth: evidence from Bangladesh. Econ Hum Biol. 2018;30:150–61.
18. Kaur M, Graham JP, Eisenberg JNS. Livestock ownership among rural households and child morbidity and mortality: an analysis of Demographic Health Survey data from 30

Sub-Saharan African countries (2005–2015). Am J Trop Med Hyg. 2017;96:741–8.
19. Mosites EM, Rabinowitz PM, Thumbi SM, Montgomery JM, Palmer GH, May S, Rowhani-Rahbar A, Neuhouser ML, Walson JL. The relationship between livestock ownership and child stunting in three countries in Eastern Africa using national survey data. PLoS One. 2015;10:e0136686.
20. Mosites E, Aol G, Otiang E, Bigogo G, Munyua P, Montgomery JM, Neuhouser ML, Palmer GH, Thumbi SM. Child height gain is associated with consumption of animal-source foods in livestock-owning households in Western Kenya. Public Health Nutr. 2016;20:336–45.
21. de Bruyn J, Thomson PC, Darnton-Hill I, Bagnol B, Maulaga W, Alders RG. Does village chicken-keeping contribute to young children's diets and growth? A longitudinal observational study in rural Tanzania. Nutrients. 2018;10:1799.
22. Dumas SE, Kassa L, Young SL, Travis AJ. Examining the association between livestock ownership typologies and child nutrition in the Luangwa Valley, Zambia. PLoS One. 2018;13:1–21.
23. Fierstein JL, Eliasziw M, Rogers BL, Forrester JE. Nonnative cattle ownership, diet, and child height-for-age: Evidence from the 2011 Uganda demographic and health survey. Am J Trop Med Hyg. 2017;96:74–82.
24. Headey D, Hirvonen K. Is exposure to poultry harmful to child nutrition? An observational analysis for rural Ethiopia. PLoS One. 2016;11:e0160590.
25. Headey D, Nguyen P, Kim S, Rawat R, Ruel M, Menon P. Is exposure to animal feces harmful to child nutrition and health outcomes? A multicountry observational analysis. Am J Trop Med Hyg. 2017;96:961–9.
26. Hetherington JB, Wiethoelter AK, Negin J, Mor SM. Livestock ownership, animal source foods and child nutritional outcomes in seven rural village clusters in Sub-Saharan Africa. Agric Food Secur. 2017;6:1–11.
27. Hoddinott J, Headey D, Dereje M. Cows, missing milk markets, and nutrition in rural Ethiopia. J Dev Stud. 2015;51:958–75.
28. Jin M, Iannotti LL. Livestock production, animal source food intake, and young child growth: The role of gender for ensuring nutrition impacts. Soc Sci Med. 2014;105:16–21.
29. Hillenbrand EP, Waid JL. Adapting food-based strategies to improve the nutrition of the landless: a review of HKI's homestead food production program in Bangladesh. In: Thompson B, Amoroso L, editors. Improving diets and nutrition: food-based approaches. Wallingford: FAO; 2014. p. 173–93.
30. Olney DK, Talukder A, Iannotti LL, Ruel MT, Quinn V. Assessing impact and impact pathways of a homestead food production program on household and child nutrition in Cambodia. Food Nutr Bull. 2009;30:355–69.
31. Olney DK, Pedehombga A, Ruel MT, Dillon A. A 2-year integrated agriculture and nutrition and health behavior change communication program targeted to women in Burkina Faso reduces anemia, wasting, and diarrhea in children 3 – 12.9 months of age at baseline: a cluster-randomized controlled trial. J Nutr. 2015;145:1317–24.
32. Osei AK, Pandey P, Spiro D, Adhikari D, Haselow N, De Morais C, Davis D. Adding multiple micronutrient powders to a homestead food production programme yields marginally significant benefit on anaemia reduction among young children in Nepal. Matern Child Nutr. 2015;11 Suppl 4:188–202.
33. Osei A, Pandey P, Nielsen J, Pries A, Spiro D, Davis D, Quinn V, Haselow N. Combining

home garden, poultry, and nutrition education program targeted to families with young children improved anemia among children and anemia and underweight among nonpregnant women in Nepal. Food Nutr Bull. 2017;38:49–64.
34. Talukder A, Osei AK, Haselow NJ, Kroeun H, Uddin A, Quinn V. Contribution of homestead food production to improved household food security and nutrition status - lessons learned from Bangladesh, Cambodia, Nepal and the Philippines. Thompson B, Amoroso L, editors. Improving Diets and Nutrition: Food-based Approaches. FAO; 2014.
35. Christian AK, Wilson ML, Aryeetey RNO, Jones AD. Livestock ownership, household food security and childhood anaemia in rural Ghana. PLoS One. 2019;14:1–14.
36. Jones AD, Colecraft EK, Awuah RB, Boatemaa S, Lambrecht NJ, Adjorlolo LK, Wilson ML. Livestock ownership is associated with higher odds of anaemia among preschool-aged children, but not women of reproductive age in Ghana. Matern Child Nutr. 2018;14:1–13.
37. Iannotti LL, Delnatus JR, Odom AR, Eaton JC, Griggs JJ, Brown S, Wolff PB. Determinants of anemia and hemoglobin concentration in Haitian school-aged children. Am J Trop Med Hyg. 2015;93:1092–8.
38. Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF International. Ghana Demographic and Health Survey 2014. Rockville, Maryland, USA; 2015.
39. Ghana Statistical Service. 2010 Population & Housing Census: District Analytical Report - Ga East Municipality. Accra; 2014.
40. Ghana Statistical Service. Population by regions: Greater Accra [Internet]. 2019 [cited 2020 Apr 5]. Available from: https://www.statsghana.gov.gh/regionalpopulation.php?population=MTM0NTk2MjQzOS4yMDE1&&Greater Accra&regid=3
41. Ghana Statistical Service. 2010 Population & Housing Census: District Analytical Report - Shai Osudoku District. Accra; 2014.
42. Ayanful-Torgby R, Quashie NB, Boampong JN, Williamson KC, Amoah LE. Seasonal variations in *Plasmodium falciparum* parasite prevalence assessed by varying diagnostic tests in asymptomatic children in southern Ghana. PLoS One. 2018;13:e0199172.
43. Ngure FM, Humphrey JH, Mbuya MNN, Majo F, Mutasa K, Govha M, Mazarura E, Chasekwa B, Prendergast AJ, Curtis V, et al. Formative research on hygiene behaviors and geophagy among infants and young children and implications of exposure to fecal bacteria. Am J Trop Med Hyg. 2013;89:709–16.
44. Zezza A, Pica-Ciamarra U, Mugera HK, Mwisomba T, Okello P. Measuring the Role of Livestock in the Household Economy: A Guidebook for Designing Household Survey Questionnaires [Internet]. 2016. Available from: http://www.fao.org/3/a-i6739e.pdf
45. World Health Organization. Indicators for assessing infant and young child feeding practices: Part 2 measurement. Geneva, Switzerland.WHO; 2010.
46. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. Geneva, Switzerland; 2010.
47. Amoah LE, Abankwa J, Oppong A. *Plasmodium falciparum* histidine rich protein-2 diversity and the implications for PfHRP 2: Based malaria rapid diagnostic tests in Ghana. Malar J. BioMed Central; 2016;15:1–8.
48. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich Enzyme-Linked Immunosorbent Assay

Technique. J Nutr. 2004;134:3127–32.
49. World Health Organization. Bench aids for the diagnosis of intestinal parasites. 1994.
50. Cogill B. Anthropometric indicators measurement guide. Food and Nutrition Technical Assistance Project, Academy for Educational Development, Washington, D.C. 2003.
51. World Health Organization. WHO Anthro Survey Analyser and other tools [Internet]. Available from: https://www.who.int/childgrowth/software/en/)
52. Njuki J, Poole J, Johnson N, Baltenweck I, Pali P, Lokman Z, Mburu S. Gender, Livestock and Livelihood Indicators. Nairobi, Kenya; 2011.
53. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. Vitamin and Mineral Nutrition Information System. Geneva; 2011. Available from: http://www.who.int/vmnis/indicators/haemoglobin.pdf
54. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS. Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106:333S-347S.
55. Lambrecht NJ, Wilson ML, Jones AD. Assessing the Impact of Animal Husbandry and Capture on Anemia among Women and Children in Low- and Middle-Income Countries: A Systematic Review. Adv Nutr. 2019;10:331–44.
56. Thurnham DI, Mccabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization Report: Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva: World Health Organization; 2012.
57. Engle-Stone R, Aaron GJ, Huang J, Wirth JP, Namaste SM, Williams AM, Peerson JM, Rohner F, Varadhan R, Addo OY, et al. Predictors of anemia in preschool children: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106(Suppl):402S–15S.
58. SPRING GHS. Ghana: Landscape Analysis of Anemia and Anemia Programming. Arlington, VA; 2016.
59. Shenton L, Jones AD, Wilson ML. Factors associated with anemia status among children aged 6-59 months in Ghana, 2003-2014. Matern Child Health J. 2020; 24:483-502.
60. World Health Organization. Safely managed drinking water - thematic report on drinking water 2017. Geneva, Switzerland; 2017.
61. Vyas S, Kumaranayake L. Constructing socio-economic status indices: How to use principal components analysis. Health Policy Plan. 2006;21:459–68.
62. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. J Stat Softw. 2014;59:1–38.
63. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2019. Available from: https://www.r-project.org/
64. Alders RG, Dumas SE, Rukambile E, Magoke G, Maulaga W, Jong J, Costa R. Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. Matern Child Nutr. 2018;14:1–14.
65. Wong JT, de Bruyn J, Bagnol B, Grieve H, Li M, Pym R, Alders RG. Small-scale poultry and food security in resource-poor settings: A review. Glob Food Sec. 2017;15:43–52.
66. Otte J, Costales A, Dijkman J, Pica-Ciamarra U, Robinson T, Ahuja V, Ly C, Roland-

Holst D. Livestock sector development for poverty reduction: an economic and policy perspective – Livestock's many virtues. Rome: FAO; 2012.
67. Nyantakyi-Frimpong H, Colecraft EK, Awuah RB, Adjorlolo LK, Wilson ML, Jones AD. Leveraging smallholder livestock production to reduce anemia: A qualitative study of three agroecological zones in Ghana. Soc Sci Med. 2018;212:191–202.
68. Aboe PAT, Boa-Amponsem K, Okantah SA, Dorward PT, Bryant MJ. Free-range village chickens on the Accra Plains, Ghana: Their contribution to households. Trop Anim Health Prod. 2006;38:223–34.
69. Todd H. Women climbing out of poverty through credit; or what do cows have to do with it? Livest Res Rural Dev. 1998;10:86–95.
70. Udo HMJ, Aklilu HA, Phong LT, Bosma RH, Budisatria IGS, Patil BR, Samdup T, Bebe BO. Impact of intensification of different types of livestock production in smallholder crop-livestock systems. Livest Sci. 2011;139:22–9.
71. Pica-Ciamarra U, Tasciotti L, Otte J, Zezza A. Livestock in the household economy: Cross-country evidence from microeconomic data. Dev Policy Rev. 2015;33:61–81.
72. Broaddus-Shea ET, Manohar S, Thorne-Lyman AL, Bhandari S, Nonyane BAS, Winch PJ, West KP. Small-scale livestock production in Nepal is directly associated with children's increased intakes of eggs and dairy, but not meat. Nutrients. 2020;12:1–11.
73. Rawlins R, Pimkina S, Barrett CB, Pedersen S, Wydick B. Got milk? The impact of Heifer International's livestock donation programs in Rwanda on nutritional outcomes. Food Policy. 2014;44:202–13.
74. Colecraft E, Marquis GS, Aryeetey R, Sakyi-Dawson O, Lartey A, Ahunu B, Canacoo E, Butler LM, Reddy MB, Jensen HH, et al. Constraints on the use of animal source foods for young children in Ghana: A participatory rapid appraisal approach. Ecol Food Nutr. 2006;45:351–77.
75. Bundala N, Kinabo J, Jumbe T, Rybak C, Sieber S. Does homestead livestock production and ownership contribute to consumption of animal source foods? A pre-intervention assessment of rural farming communities in Tanzania. Sci African. 2020;7.
76. Haileselassie M, Redae G, Berhe G, Henry CJ, Nickerson MT, Tyler B, Mulugeta A. Why are animal source foods rarely consumed by 6-23 months old children in rural communities of Northern Ethiopia? A qualitative study. PLoS One. 2020;15:e0225707.
77. Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. Nutr Rev. 2011;69:37–42.
78. Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2014;108:313–25.
79. Ehrhardt S, Burchard GD, Mantel C, Cramer JP, Kaiser S, Kubo M, Otchwemah RN, Bienzle U, Mockenhaupt FP. Malaria, anemia, and malnutrition in African children—defining intervention priorities. J Infect Dis. 2006;194:108–14.
80. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. N Engl J Med. 2004;351:799–807.
81. Forson AO, Arthur I, Ayeh-Kumi PF. The role of family size, employment and education of parents in the prevalence of intestinal parasitic infections in school children in Accra. PLoS One. 2018;13:1–10.
82. Whitehead RD, Mei Z, Mapango C, Jefferds MED. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. Ann N Y Acad Sci.

2019;1450:147–71.
83. Boghani S, Mei Z, Perry GS, Brittenham GM, Cogswell ME. Accuracy of capillary hemoglobin measurements for the detection of anemia among U.S. low-income toddlers and pregnant women. Nutrients. 2017;9.
84. Eaton JC, Rothpletz-Puglia P, Dreker MR, Iannotti L, Lutter C, Kaganda J, Rayco-Solon P. Effectiveness of provision of animal-source foods for supporting optimal growth and development in children 6 to 59 months of age. Cochrane database Syst Rev. 2019;2:1–69.

Figures and Tables

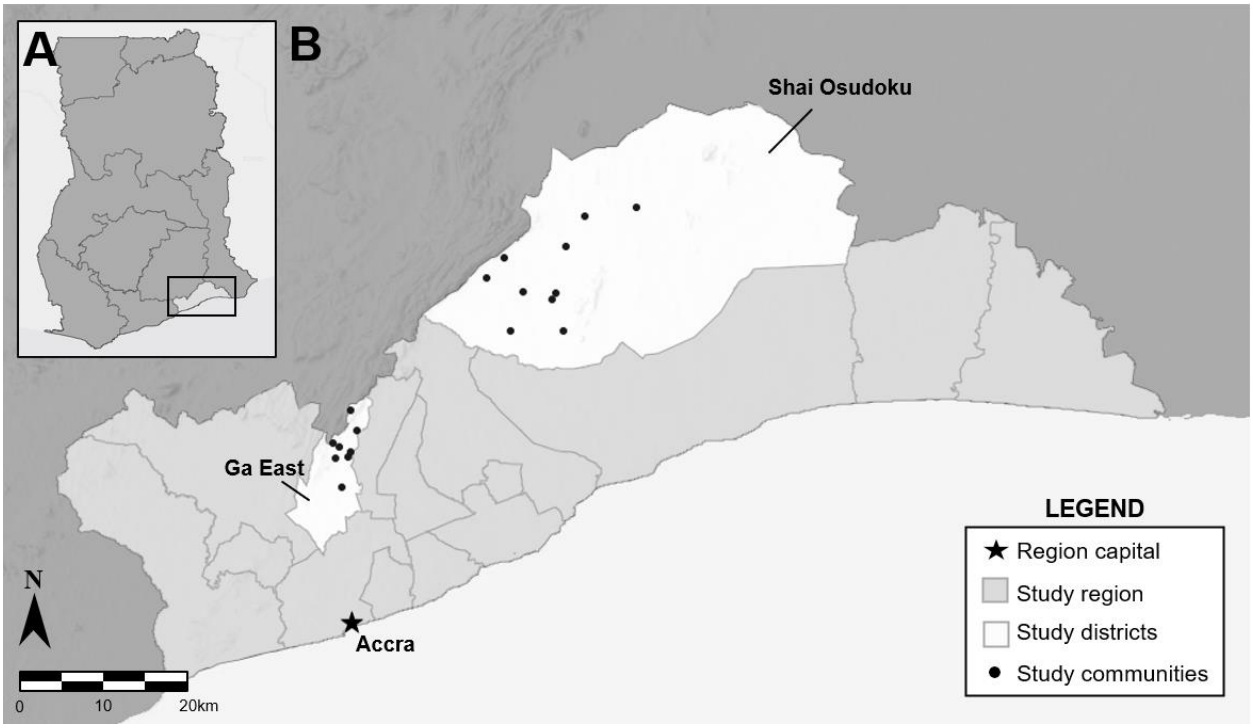

Figure 2.1 Map of (A) study region in Ghana and (B) study districts and communities.

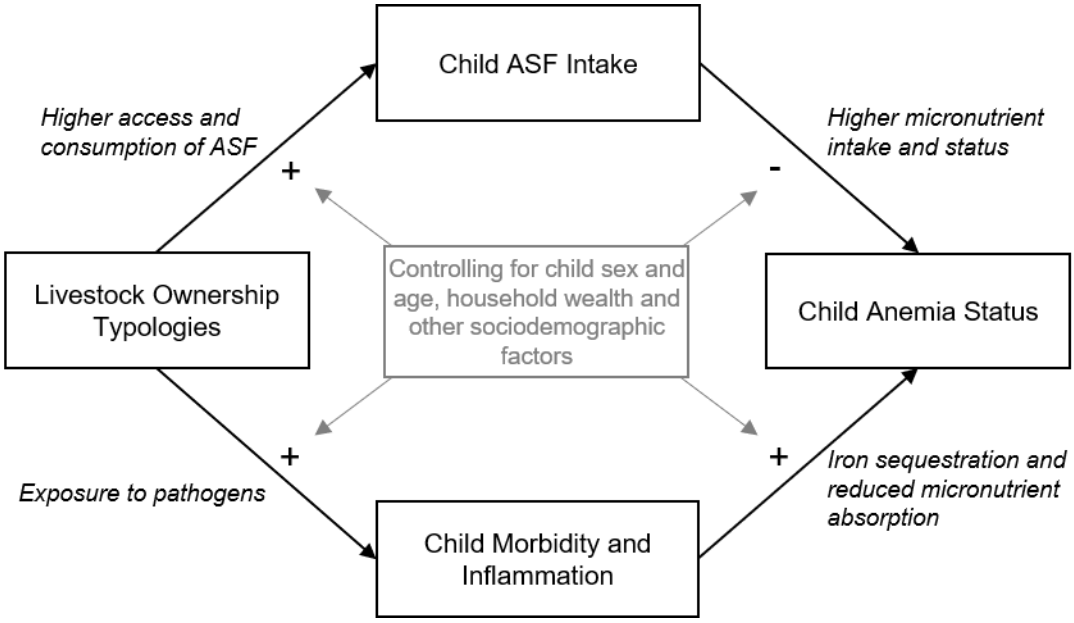

Figure 2.2 Mediation analysis pathway model of livestock ownership and child anemia, mediated by ASF intake, morbidity symptoms, or inflammation. Livestock ownership typologies were defined as five categories including: no livestock; only poultry (<12); only poultry (≥12); small livestock +/- poultry; and cattle +/- small livestock or poultry. Child ASF intake was modeled separately for consumption of each types of ASF (cow meat; goat, sheep or pig meat; chicken meat; organ meats; chicken eggs; cow milk) in the past three months. Child morbidity and illness was modeled separately for each type of illness (fever; diarrhea; cough/cold) experienced in the past seven days and elevated concentrations of each inflammatory biomarker (CRP>5 mg/L; AGP>1 g/L). Anemia was defined as hemoglobin <11.0 g/dL.

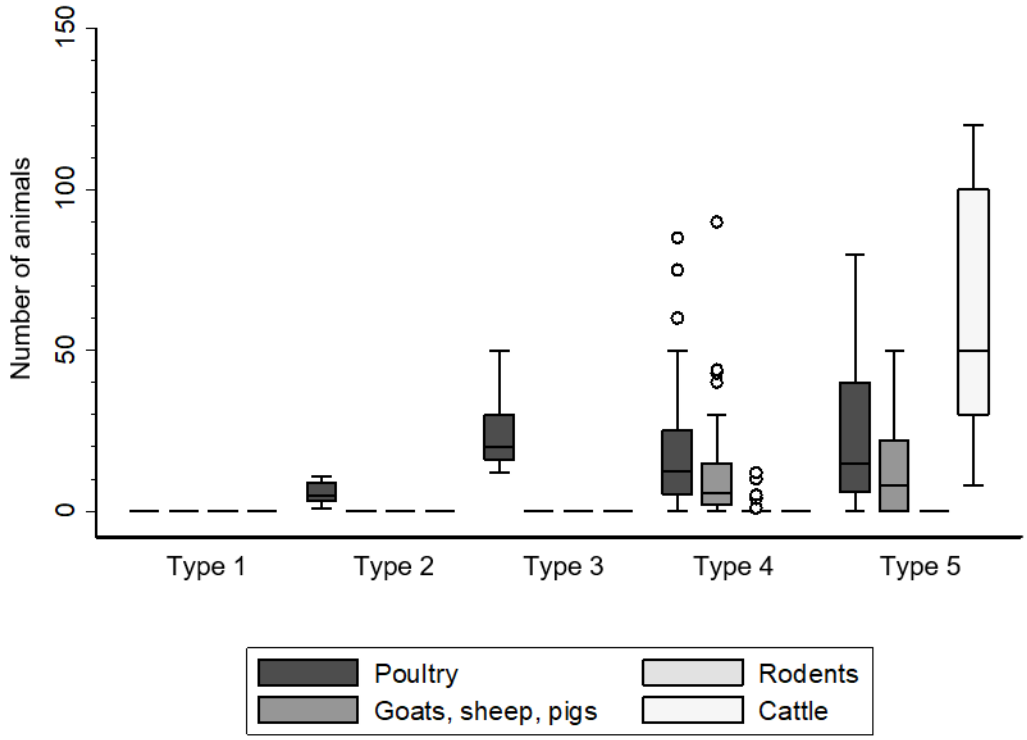

Figure 2.3 Box plots of the number of animals reared stratified by livestock species and livestock ownership typology among 470 households sampled during October-November 2018 in Greater Accra Region, Ghana. 265 households were categorized in Type 1, 71 in Type 2, 59 in Type 3, 60 in Type 4, and 15 in Type 5. Top and bottom whiskers represent the highest and lowest values, top and bottom of boxes represent the 75th and 25th percentiles, middle lines of boxes represent medians, and circles represent outlying values.

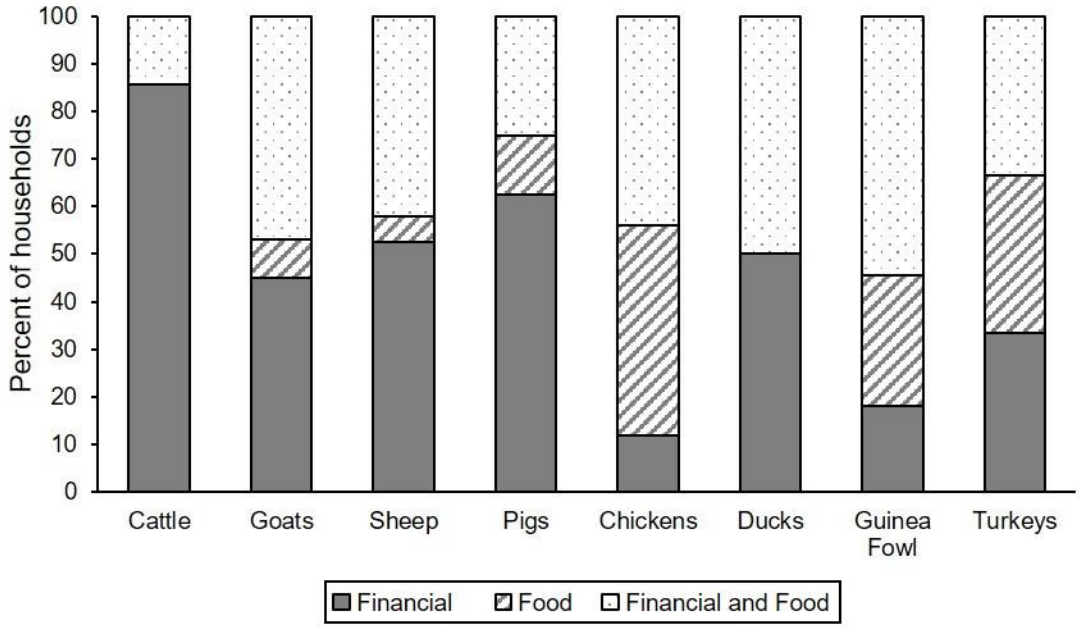

Figure 2.4 Respondent-reported primary reasons for why households owned or reared animals, categorized by livestock species in Greater Accra, Ghana. The financial reasons category includes sale of live animals or livestock products, income, and savings/insurance. The food reasons category includes food for the family. The financial and food reasons category includes all of the above.

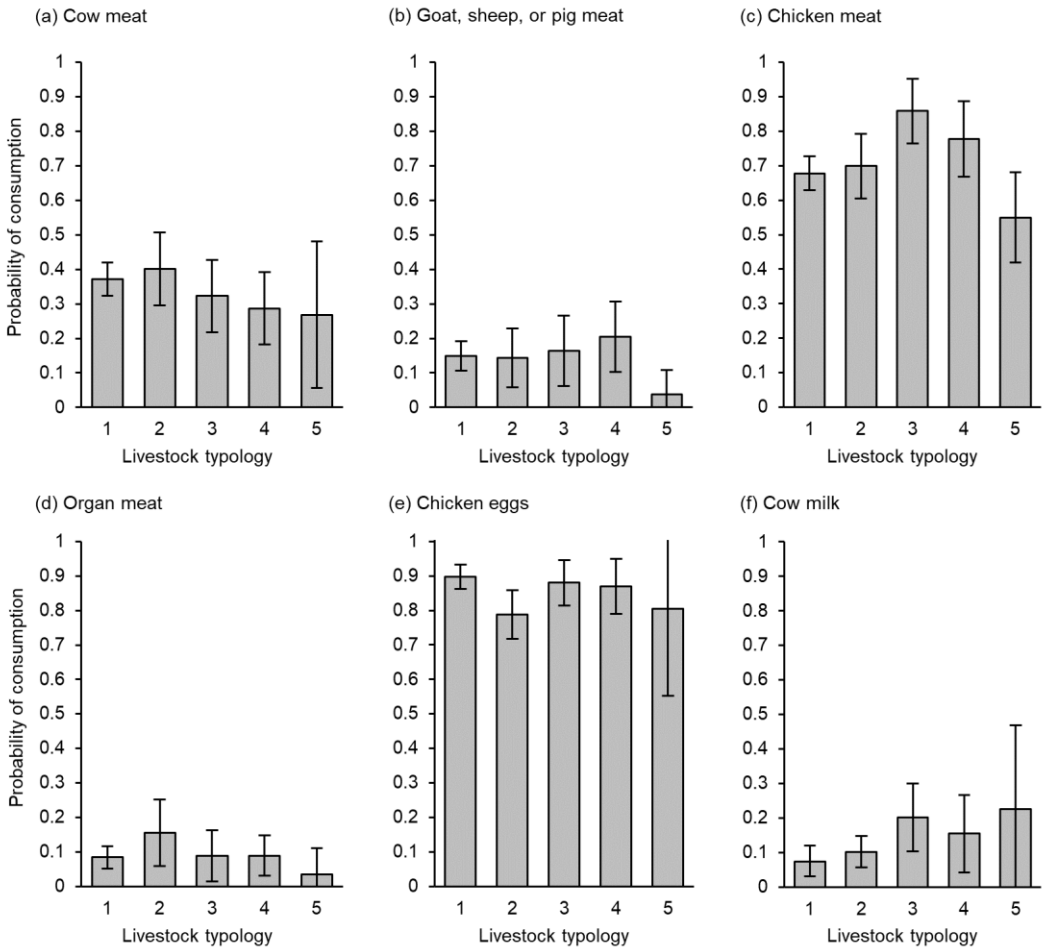

Figure 2.5 Predicted probability estimates (with 95% confidence interval) for child's consumption of (a) cow meat, (b) goat, sheep, or pig meat, (c) chicken meat, (d) organ meat, (e) chicken eggs, and (f) cow milk by household livestock typology categories. Probabilities hold covariates from adjusted logistic regression models in Table 3.8 constant. Animal source food consumption is modeled as dichotomous consumption of the food in the three months preceding survey administration, amongst children 6-59 months old in Greater Accra Region, Ghana (n=470). Livestock typology categories: 1 – no livestock; 2 – only poultry (<12); 3 – only poultry (≥12); 4 – small livestock +/- poultry; and 5 – cattle +/- small livestock or poultry.

Table 2.1 Bivariate comparisons of child and household characteristics with anemia status among 6-59-month-old children in Greater Accra Region, Ghana, October-November 2018. Values are % or mean ± SD.

Indicator	Overall	Anemic (Hb<11.0g/dL)	Not anemic (Hb≥11.0g/dL)	P-value[3]
Number of households	**470**	**225**	**245**	
Child characteristics				
Female sex, %	52.1	54.2	50.2	0.38
Age, months	26.6 ± 14.0	23.2 ± 12.4	29.6 ± 14.8	**<0.001**
Age (6-23 months old), %	50.9	60.9	41.6	**<0.001**
Health Indicators				
Hemoglobin, g/dL	10.9 ± 1.4	9.7 ± 1.0	12.0 ± 0.7	**<0.001**
Iron deficiency (SF<12µg/L), %	25.1	40.0	11.4	**<0.001**
Serum ferritin, µg/L[1]	29.13 ± 23.27	24.26 ± 23.50	33.61 ± 22.18	**<0.001**
Iron deficiency (sTfR>7.3mg/L), %	52.8	71.1	35.9	**<0.001**
Serum transferrin receptor, mg/L	10.08 ± 5.17	12.12 ± 6.43	8.21 ± 2.45	**<0.001**
Vitamin A deficiency (RBP<0.70 µmol/L), %	16.0	19.6	12.7	**0.04**
Retinol binding protein, µmol/L	0.95 ± 0.26	0.91 ± 0.26	0.98 ± 0.25	**0.003**
Inflammation (CRP>5mg/L), %	16.0	17.8	14.3	0.30
C-reactive protein, mg/L	4.08 ± 11.32	5.43 ± 14.47	2.85 ± 7.12	**0.01**
Inflammation (AGP>1g/L), %	35.5	38.7	32.7	0.17
α-1-acid glycoprotein, g/L	0.95 ± 0.54	1.01 ± 0.56	0.90 ± 0.50	**0.02**
Stunted (HAZ < −2), % [n=466]	12.2	13.4	11.2	0.46
HAZ [n=466]	−0.69 ± 1.15	−0.71 ± 1.22	−0.67 ± 1.09	0.72
Wasted (WHZ < −2), % [n=463]	6.5	7.2	5.8	0.56
WHZ [n=463]	−0.53 ± 1.08	−0.57 ± 1.12	−0.49 ± 1.04	0.47
Recent infection or illness				
Fever in past 7 days, %	23.6	24.4	22.9	0.69
Diarrhea in past 7 days, %	7.7	8.9	6.5	0.34
Cough/cold in past 7 days, %	22.6	21.8	23.3	0.70
Malaria parasitemia, %	8.5	12.4	4.9	**0.003**
Helminth infection, % [n=415]	0	0	0	.
Helminth drug in last 6mo, % [n=468]	28.9	23.7	33.6	**0.02**
Took antibiotics in past 7d, %	15.5	16.0	15.1	0.79
Dietary Indicators				
Currently breastfeeding, %	38.3	46.2	31.0	**0.001**
ASF consumption in past 24h[2], %	84.9	82.2	87.4	0.12
Meat, %	20.6	17.8	23.3	0.14
Eggs, %	26.6	22.7	30.2	0.07
Fish, %	64.0	63.6	64.5	0.83
Dairy, %	32.6	32.9	32.2	0.88
ASF consumption in past 3mo[2], %	97.7	96.9	98.4	0.29
Meat, %	75.1	72.9	77.1	0.29
Eggs, %	87.5	83.1	91.4	**0.007**
Fish, %	92.8	92.0	93.5	0.54
Dairy, %	87.5	83.1	91.4	**0.007**
Dietary diversity score (24h)	3.4 ± 1.6	3.3 ± 1.6	3.6 ± 1.5	**0.02**
Met minimum dietary diversity (≥4 food groups), %	52.6	48.0	56.7	0.06
Iron supplement in past 7d, % [n=465]	14.8	17.9	12.0	0.08
Vitamin A supplement in past 6mo, % [n=466]	83.5	88.0	79.3	**0.01**
Household characteristics				
Household size	5.0 ± 1.9	4.9 ± 1.8	5.0 ± 2.0	0.44

Indicator	Overall	Anemic (Hb<11.0g/dL)	Not anemic (Hb≥11.0g/dL)	P-value[3]
Number of children <5y	1.4 ± 0.6	1.4 ± 0.6	1.3 ± 0.5	**0.01**
Head of household sex (female), %	21.9	24.0	20.0	0.30
Head of household religion, %				**0.03**
Christian	85.7	83.1	88.2	
Muslim	11.5	15.1	8.2	
Other	2.8	1.8	3.7	
Head of household ethnic group, %				**0.008**
Ga-Dangme	42.8	45.8	40.0	
Akan	15.7	16.0	15.5	
Ewe	27.7	20.9	33.9	
Other	13.8	17.3	10.6	
Head of household occupation, %				**0.03**
Unemployed/unpaid work	9.6	11.6	7.8	
Agricultural labor	19.2	16.0	22.0	
Self-employed	60.0	57.8	62.0	
Salaried employment	11.3	14.7	8.2	
Maternal education, %				0.67
None or nursery	21.3	21.3	21.2	
Primary	23.0	25.3	20.8	
Junior	41.3	39.1	43.3	
Senior or higher	14.5	14.2	14.7	
Dwelling				
Access to electricity, %	87.5	88.0	86.9	0.73
Access to improved water source, %	97.0	97.3	96.7	0.70
Type of sanitation, %				0.54
Flush or pour flush	12.6	11.1	13.9	
Pit latrine	58.1	60.4	55.9	
Open defecation	29.4	28.4	30.2	
Handwashing ladder, %				0.52
Basic	1.5	0.9	2.0	
Limited	0.6	0.4	0.8	
No facility	97.9	98.7	97.1	
Mud floor, %	1.3	1.3	1.2	0.92
Thatch roof, %	1.1	0.4	1.6	0.21
Cement walls, %	76.6	75.6	77.6	0.61
Animal feces in yard or compound, % [n=463]	78.2	78.3	78.1	0.96
Child slept under mosquito bed net in previous night, %	59.4	60.9	58.0	0.52
Wealth quintile, %				0.61
Lowest	19.2	20.9	17.6	
Low	20.6	20.4	20.8	
Middle	20.9	19.6	22.0	
High	20.4	22.2	18.8	
Highest	18.9	16.9	20.8	
District, %				**0.007**
Shai Osudoku	58.5	64.9	52.7	
Ga East	41.5	35.1	47.4	
Livestock				
Ownership of any livestock, %	43.6	40.9	46.1	0.25
Cattle, %	3.2	2.2	4.1	0.25
Goats, sheep, or pigs, %	14.3	10.2	18.0	**0.02**
Grasscutter, rabbits, or rats, %	1.1	0.0	2.0	**0.03**

Indicator	Overall	Anemic (Hb<11.0g/dL)	Not anemic (Hb≥11.0g/dL)	P-value[3]
Poultry (chickens, turkeys, ducks, or guinea fowl), %	41.1	38.7	43.3	0.31
Livestock typology, %				0.17
Type 1 – No livestock	56.4	59.1	53.9	
Type 2 – Poultry (<12)	15.1	16.4	13.9	
Type 3 – Poultry (≥12)	12.6	12.9	12.2	
Type 4 – Small livestock +/- poultry	12.8	9.3	15.9	
Type 5 – Cattle +/- small livestock or poultry	3.2	2.2	4.1	
Tropical livestock units	1.7 ± 8.8	1.0 ± 6.3	2.3 ± 10.6	0.10
Total number of livestock	10.9 ± 24.3	8.2 ± 18.4	13.3 ± 28.5	**0.02**

[1]Serum ferritin concentrations are adjusted for inflammation (CRP and AGP) using the regression correction approach recommended by the Biomarkers Reflection Inflammation and Nutritional Determinants of Anemia (BRINDA) project (54).
[2]Animal-source foods (ASF) include meat (beef, other red meat, organ meat, chicken meat, other poultry meat), fish (fresh or dried fish or shellfish), eggs, and dairy (milk, cheese, yogurt, or other foods made from milk).
[3]P- values calculated from chi-square test for categorical values and t-test for continuous variables.
Abbreviations: HAZ – Length/height-for-age Z-score; SF – serum ferritin; sTfR – serum transferrin receptor; RBP – retinol binding protein; CRP – C-reactive protein; AGP - α-1-acid glycoprotein; WHZ – Weight-for-length/height Z-score; ASF – animal source food

Table 2.2 Distribution and number of livestock species reared by households in each livestock typology category (n=470). Values are the number (%) of households that own each livestock species and the median (range) number of livestock owned within each typology[1].

	Type 1	**Type 2**		**Type 3**		**Type 4**		**Type 5**	
	No livestock (n=265)	**Poultry (<12) (n=71)**		**Poultry (≥12) (n=59)**		**Small livestock +/- poultry (n=60)**		**Cattle +/- small livestock or poultry (n=15)**	
	Number (%)	Number (%)	Median (range)	Number (%)	Median (range)	Number (%)	Median (range)	Number (%)	Median (range)
Any livestock	0 (0%)	71 (100%)	5 (1-11)	59 (100%)	20 (12-50)	60 (100%)	20 (2-175)	15 (100%)	90 (20-212)
Chickens	·	71 (100%)	5 (1-11)	58 (98.3%)	20 (0-50)	49 (81.7%)	11 (0-50)	14 (93.3%)	12 (0-60)
Ducks	·	0 (0%)	0	5 (8.5%)	0 (0-32)	8 (13.3%)	0 (0-20)	3 (20.0%)	0 (0-27)
Guinea fowl	·	1 (1.4%)	0 (0-4)	2 (3.4%)	0 (0-21)	4 (6.7%)	0 (0-40)	4 (26.7%)	0 (0-15)
Turkeys	·	0 (0%)	0	0 (0%)	0	2 (3.3%)	0 (0-10)	1 (6.7%)	0 (0-5)
Goats	·	0 (0%)	0	0 (0%)	0	43 (71.7%)	2 (0-60)	6 (40.0%)	0 (0-50)
Sheep	·	0 (0%)	0	0 (0%)	0	13 (21.7%)	0 (0-40)	6 (40.0%)	0 (0-45)
Pigs	·	0 (0%)	0	0 (0%)	0	6 (10.0%)	0 (0-41)	2 (13.3%)	0 (0-10)
Grasscutter	·	0 (0%)	0	0 (0%)	0	4 (6.7%)	0 (0-12)	0 (0%)	0
Rabbits or rats	·	0 (0%)	0	0 (0%)	0	2 (3.3%)	0 (0-4)	0 (0%)	0
Cattle	·	0 (0%)	0	0 (0%)	0	0 (0%)	0	15 (100%)	50 (8-120)
TLU, median (min-max)[1]	0	0.05 (0.01-0.13)		0.24 (0.12-0.98)		0.73 (0.10-10.65)		38.60 (5.72-85.02)	

[1]Multiple species of livestock may be present in a single household; thus, the categories of species are not mutually exclusive.
Abbreviations: TLU – tropical livestock unit score

Table 2.3 Self-reported sale and consumption of livestock and livestock products from own livestock holdings among livestock-rearing households, reported by livestock category (poultry, small livestock, cattle)[1].

	Households with poultry (n=193)		Households with small livestock (n=67)		Households with cattle (n=15)	
	Sold[2]	**Consumed**	**Sold[3]**	**Consumed**	**Sold[4]**	**Consumed**
Percent of households that sold or consumed livestock/livestock products from own livestock holdings						
Poultry (chickens, ducks, turkeys, guinea fowl), in past 3 months	18.1%	38.3%	·	·	·	·
Poultry eggs[5], in past 3 months	1.0%	37.8%	·	·	·	·
Small livestock (goats, sheep, pigs), in past 12 months	·	·	34.3%	19.4%	·	·
Cattle, in past 12 months	·	·	·	·	40.0%	6.7%
Cow's milk[6], in milk-producing months	·	·	·	·	86.6%	60.0%
Mean (range) number of livestock that were sold or consumed per household						
Poultry (chickens, ducks, turkeys, guinea fowl), in past 3 months	6 (1-35)	3 (1-10)	·	·	·	·
Small livestock (goats, sheep, pigs), in past 12 months	·	·	5 (1-30)	2 (1-6)	·	·
Cattle, in past 12 months	·	·	·	·	4 (1-7)	1 (1)

[1]Households may own multiple categories of animals (e.g., 33% of households with poultry also own other livestock). Households that own more than one category of livestock are represented within each category of livestock that they own.
[2]Average self-reported income from sales of live poultry in this sample was GHC 40 ($7) per animal. Live poultry sales were made up of 77% chickens, 10% ducks, 3% turkeys, and 10% guinea fowl.
[3]Average self-reported income from sales of live small livestock (goats, sheep, pigs) in this sample was GHC 342 ($61) per animal. Live small livestock sales were made up of 52% goats, 28% sheep, and 20% pigs.
[4]Average self-reported income from sales of live cattle in this sample was GHC 1,572 ($281) per animal.
[5]Poultry birds laid on average 15 eggs per week. Households that consumed eggs (n=73) reported consuming on average 7 eggs per week in the past 3 months. Eggs for consumption were primarily sourced from chickens (92%), rather than ducks (3%), turkeys (1%), or guinea fowl (4%).
[6]Of households with cattle that milked their cows in the past 12 months (n=14), one household reported consuming "all or nearly all (90-100%)" of the milk produced, three households reported consuming "less than half (10-40%)" of the milk produced, five reported consuming "a small amount (1-10%)" of the milk produced, and five reported that they "did not consume" any of the milk produced.

Table 2.4 Bivariate comparisons of child and household characteristics with livestock typology among children 6-59 months old in Greater Accra Region, Ghana, October-November 2018 (n=470). Values are % or mean ± SD.

Indicator	Type 1	Type 2	Type 3	Type 4	Type 5	P-value[2]
	No livestock	Poultry (<12)	Poultry (≥12)	Small livestock +/- poultry	Cattle +/- small livestock or poultry	
Child characteristics						
Female sex, %	57.4	43.7	45.8	46.7	46.7	0.15
Age, months	25.5	26.3	26.5	31.2	27.5	0.09
Age (6-23 months old), %	55.5	45.1	54.2	38.3	33.3	0.06
Health Indicators						
Stunted (HAZ < −2), % [n=466]	11.7	11.4	13.8	13.6	13.3	0.99
Wasted (WHZ < −2), % [n=463]	4.6	11.3	6.9	10.2	0.0	0.16
Recent infection or illness						
Malaria parasitemia, %	9.8	7.0	5.1	6.7	13.3	0.67
Helminth infection, % [n=415]	0.0	0.0	0.0	0.0	0.0	.
Helminth drug in last 6mo, % [n=468]	28.5	23.9	32.2	35.0	20.0	0.58
Took antibiotics in past 7d, %	15.9	23.9	11.9	10.0	6.7	0.15
Dietary Indicators						
Currently breastfeeding, %	43.0	35.2	37.3	25.0	26.7	0.09
Met minimum dietary diversity (≥4 food groups), %	51.3	45.1	59.3	58.3	60.0	0.41
Iron supplement in past 7d, % [n=465]	17.9	15.5	10.2	6.9	6.7	0.15
Vitamin A supplement in past 6mo, % [n=466]	82.8	87.3	81.0	81.7	93.3	0.68
Household characteristics						
Household size	4.5 ± 1.5	4.9 ± 2.0	5.7 ± 2.3	5.8 ± 2.0	7.3 ± 2.9	**<0.001**
Number of children <5y	1.3 ± 0.5	1.4 ± 0.6	1.3 ± 0.5	1.4 ± 0.7	1.5 ± 0.8	0.94
Head of household sex (female), %	24.2	28.2	11.9	20.0	0.0	**0.04**
Head of household religion, %						**<0.001**
Christian	88.3	85.9	88.1	81.7	46.7	
Muslim	9.1	11.3	10.2	13.3	53.3	
Other	2.6	2.8	1.7	5.0	0.0	
Head of household ethnic group, %						**<0.001**
Ga-Dangme	43.8	38.0	33.9	55.0	33.3	
Akan	20	16.9	11.9	3.3	0.0	
Ewe	24.2	33.8	39	28.3	13.3	
Other	12.1	11.3	15.3	13.3	53.3	
Head of household occupation, %						**<0.001**
Unemployed/unpaid work	9.4	11.3	11.9	8.3	0.0	

Indicator	Type 1	Type 2	Type 3	Type 4	Type 5	P-value[2]
	No livestock	Poultry (<12)	Poultry (≥12)	Small livestock +/- poultry	Cattle +/- small livestock or poultry	
Agricultural labor	10.9	16.9	25.4	33.3	93.3	
Self-employed	64.9	63.4	55.9	51.7	6.7	
Salaried employment	14.7	8.5	6.8	6.7	0.0	
Maternal education, %						**<0.001**
None or nursery	18.1	11.3	30.5	28.3	60	
Primary	23.0	32.4	18.6	21.7	0.0	
Junior	42.3	32.4	45.8	45.0	33.3	
Senior or higher	16.6	23.9	5.1	5.0	6.7	
Access to electricity, %	87.2	84.5	88.1	91.7	86.7	0.81
Access to improved water source, %	97.7	95.8	100	93.3	93.3	0.19
Type of sanitation, %						**0.006**
Flush or pour flush	14.3	2.8	13.6	16.7	6.7	
Pit latrine	62.3	62.0	50.9	48.3	33.3	
Open defecation	23.4	35.2	35.6	35.0	60.0	
Handwashing ladder, %						0.84
Basic	1.1	1.4	1.7	3.3	0.0	
Limited	1.1	0.0	0.0	0.0	0.0	
No facility	97.7	98.6	98.3	96.7	100	
Mud floor, %	1.5	0.0	0.0	0.0	13.3	**0.001**
Thatch roof, %	0.4	2.8	0.0	1.7	6.7	0.07
Cement walls, %	83.8	66.2	64.4	75.0	53.3	**<0.001**
Animal feces in yard or compound, % [n=463]	68.3	88.7	89.7	91.7	100.0	**<0.001**
Wealth quintile, %						
Lowest	18.5	28.2	13.6	15.0	26.7	0.90
Low	18.9	22.5	23.7	23.3	20.0	
Middle	21.5	16.9	20.3	25	13.3	
High	20.8	18.3	23.7	18.3	20.0	
Highest	20.4	14.1	18.6	18.3	20.0	
Wealth quintile	3.1 ± 1.4	2.7 ± 1.4	3.1 ± 1.3	3.0 ± 1.3	2.9 ± 1.6	0.32
District, %						**<0.001**
Shai Osudoku	50.9	67.6	69.5	60.0	100.0	
Ga East	49.1	32.4	30.5	40.0	0.0	

[1]P- values calculated from chi-square statistics for comparisons of proportions and F-statistics using ANOVA for comparisons of means.
Abbreviations: HAZ – Length/height-for-age Z-score; WHZ – Weight-for-length/height Z-score

Table 2.5 Unadjusted and adjusted logistic regression of the association of household livestock ownership typology and anemia in children aged 6-59 months in Greater Accra Region, Ghana, October-November 2018 (n=470)[1].

Indicator	Unadjusted Model		Adjusted Model	
	OR	95% CI	OR	95% CI
Livestock ownership typology (Ref: No livestock)				
Type 2 – Poultry (<12)	1.08	(0.72, 1.62)	1.14	(0.74, 1.76)
Type 3 – Poultry (≥12)	0.96	(0.57, 1.60)	1.07	(0.63, 1.82)
Type 4 – Small livestock +/- poultry	**0.53***	**(0.31, 0.93)**	0.62+	(0.36, 1.07)
Type 5 – Cattle +/- small livestock or poultry	0.50	(0.19, 1.29)	**0.32****	**(0.14, 0.71)**
Child sex (Ref: Male)				
Female			1.17	(0.95, 1.44)
Child age (Ref: 24-59 months)				
6-23 months			**2.56*****	**(1.68, 3.92)**
Child has malaria			**3.04****	**(1.48, 6.25)**
Number of children under 5y in household			**1.52****	**(1.17,1.97)**
Head of household sex (Ref: Male)				
Female			1.13	(0.69, 1.84)
Head of household religion (Ref: Christian)				
Muslim			1.73	(0.48, 6.25)
Traditional or no religion			0.79	(0.26, 2.38)
Head of household ethnic group (Ref: Ga-Dangme)				
Akan			1.11	(0.65, 1.89)
Ewe			**0.51****	**(0.33, 0.77)**
Other			1.28	(0.52, 3.20)
Maternal education (Ref: None or nursery)				
Primary			1.61+	(0.96, 2.71)
Junior			1.19	(0.64, 2.23)
Senior or higher			1.30	(0.49, 3.42)
Household sanitation (Ref: Open defecation)				
Pit latrine			1.78	(0.61, 5.16)
Flush or pour flush			1.54	(0.83, 2.87)
Household wealth quintile (Ref: Lowest)				
Low			0.85	(0.46, 1.57)
Middle			0.81	(0.50, 1.33)
High			1.05	(0.48, 2.28)
Highest			0.68	(0.31, 1.46)
District (Ref: Ga East)				
Shai Osudoku			**2.35*****	**(1.49, 3.70)**

[1]Values are OR (odds ratios) and 95% CI (confidence intervals) from multiple logistic regression models between livestock ownership typology and anemia. Robust standard errors are adjusted for community cluster. Anemia is defined as hemoglobin <11.0 g/dL. Marginal significance (0.05≤p<0.1) indicated by +. Statistical significance indicated by * for p<0.05, ** for p<0.01, and *** for p<0.001.

Table 2.6 Adjusted logistic regression model testing for the interaction between livestock ownership typology and child age on anemia (n=470)[1].

	Child age	
	6-23 months	**24-59 months**
	OR	**OR**
Livestock ownership typology		
Typology 1 – No livestock (reference)	1	1
Typology 2 – Poultry (<12)	1.24	1.02
Typology 3 – Poultry (≥12)	1.02	1.16
Typology 4 – Small livestock and poultry	1.45	**0.30***
Typology 5 – Cattle, small livestock and poultry	**0.21****	**0.36***

Likelihood ratio test between model without and with interaction dummies

$X^2(4) = 5.40$

P-value = 0.1448

[1]Values are OR (odds ratios) and 95% CI (confidence intervals) from multiple logistic regression between livestock ownership typology and anemia. Model adjusted for child sex, child malaria, number of children under five years in household, head of household sex, head of household religion, head of household ethnic group, maternal education, household sanitation type, household wealth quintile, and district. Robust standard errors are adjusted for community cluster. Anemia is defined as hemoglobin <11.0 g/dL. Marginal significance (0.05≤p<0.1) indicated by $^+$. Statistical significance indicated by * for p<0.05, ** for p<0.01, and *** for p<0.001.

Table 2.7 Adjusted associations between measures of household livestock ownership and anemia, hemoglobin, and iron deficiency in children aged 6-59 months in Greater Accra Region, Ghana, October-November 2018 (n=470)[1].

Indicator	**Anemia**		**Hemoglobin (g/dL)**		**Low SF**		**High sTfR**	
	OR	95% CI	β	95% CI	OR	95% CI	OR	95% CI
Ownership of any livestock (yes/no)	0.88	(0.67, 1.15)	0.03	(-0.20, 0.26)	0.97	(0.64, 1.49)	1.03	(0.69, 1.54)
Total number of livestock owned among livestock owners (log-2 scale)	**0.80***	**(0.67, 0.95)**	0.14+	(-0.00, 0.27)	0.98	(0.89, 1.10)	0.99	(0.89, 1.10)
TLU	0.97*	(0.95, 1.00)	0.01	(-0.00, 0.02)	0.98	(0.95, 1.02)	1.00	(0.98, 1.02)
TLU (log-2 scale)	**0.77****	**(0.66, 0.91)**	0.09	(-0.04, 0.23)	0.87	(0.65, 1.17)	0.98	(0.80, 1.20)
Ownership of cattle (yes/no)[2]	0.42+	(0.17, 1.02)	0.32	(-0.44, 1.09)	0.38	(0.08, 1.78)	0.95	(0.42, 2.13)
Number of cattle owned among cattle owners (log-2 scale)	**0.86***	**(0.74, 0.99)**	0.05	(-0.07, 0.17)	0.83	(0.63, 1.11)	1.03	(0.89, 1.20)
Ownership of goats, sheep, or pigs (yes/no)[2]	0.58+	(0.32, 1.06)	**0.39***	**(0.10, 0.69)**	0.85	(0.44, 1.64)	0.92	(0.52, 1.61)
Number of goats, sheep, or pigs owned among goat, sheep, and pig owners (log-2 scale)	**0.86***	**(0.74, 1.00)**	0.08	(-0.02, 0.18)	1.00	(0.82, 1.24)	0.94	(0.77, 1.16)
Ownership of poultry (yes/no)[2]	1.09	(0.79, 1.51)	-0.10	(-0.36, 0.15)	1.08	(0.69, 1.67)	1.10	(0.69, 1.75)
Number of poultry owned among poultry owners (log-2 scale)	0.91	(0.76, 1.09)	0.09	(-0.04, 0.23)	0.95	(0.85, 1.07)	0.93	(0.74, 1.16)
Livestock typology								
Typology 1 – No livestock (reference)	-	-	-	-	-	-	-	-
Typology 2 – Poultry (<12)	1.14	(0.74, 1.76)	-0.14	(-0.79, 0.21)	0.97	(0.56, 1.69)	1.22	(0.68, 2.18)
Typology 3 – Poultry (≥12)	1.07	(0.63, 1.82)	-0.07	(-0.44, 0.31)	1.27	(0.81, 2.01)	0.92	(0.44, 1.90)
Typology 4 – Small livestock and poultry	0.62+	(0.36, 1.07)	0.24	(-0.06, 0.55)	0.87	(0.43, 1.78)	0.95	(0.55, 1.66)
Typology 5 – Cattle, small livestock and poultry	**0.32****	**(0.14, 0.71)**	0.48	(-0.29, 1.24)	0.36	(0.08, 1.64)	0.95	(0.38, 2.38)
Livestock typology (ordinal)	**0.86***	**(0.75, 0.99)**	0.07	(-0.03, 0.16)	0.94	(0.78, 1.14)	0.98	(0.82, 1.18)

[1]Models adjusted for child age and sex, malaria, number of children under 5 in household, sex of head of household, head of household ethnic group and religion, maternal education, type of toilet facility, wealth quintile, and district. Robust standard errors are adjusted for community cluster. Marginal significance (0.05≤p<0.1) indicated by +. Statistical significance indicated by * for p<0.05, ** for p<0.01, and *** for p<0.001.

[2]Controlling for ownership of other livestock species (i.e., cattle; goats, sheep, or pigs; poultry).

Abbreviations: SF – serum ferritin; sTfR – serum transferrin receptor; TLU – tropical livestock unit score

Table 2.8 Unadjusted associations between livestock ownership typology and indicators of illness and diet among children 6-59 months old in Greater Accra Region, Ghana, October-November 2018 (n=470). Values are % or mean ± SD.

	Type 1	Type 2	Type 3	Type 4	Type 5	P-value[3]
	No livestock (n=265)	Poultry (<12) (n=71)	Poultry (≥12) (n=59)	Small livestock +/- poultry (n=60)	Cattle +/- small livestock or poultry (n=15)	
Illness indicators						
Fever in past 7 days, %	23.8	35.2	18.6	15.0	20.0	0.07
Diarrhea in past 7 days, %	9.8	7.0	8.5	0.0	0.0	0.09
Cough/cold in past 7 days, %	24.2	26.8	18.6	15.0	20.0	0.46
Inflammation (CRP>5mg/L), %	17.7	11.3	15.2	15.0	13.3	0.75
Inflammation (AGP>1g/L), %	34.7	31.0	40.7	35.0	53.3	0.48
Dietary Indicators						
Dietary diversity score	3.4 ± 1.6	3.4 ± 1.4	3.7 ± 1.4	3.5 ± 1.7	3.5 ± 2.0	0.67
ASF consumption in past 24 hours						
Cow meat, %	4.9	2.8	5.1	6.7	6.7	0.88
Other red meat, %	0.8	1.4	0.0	3.3	0.0	0.40
Chicken meat, %	16.2	19.7	15.3	15.0	0.0	0.45
Organ meats, %	0.4	1.4	1.7	0.0	0.0	0.65
Eggs, %	29.1	16.9	28.8	23.3	33.3	0.28
Animal milk[1], %	20.0	14.1	22.0	23.3	40.0	0.22
ASF consumption in past 3 months						
Cow meat, %	36.6	38.0	30.5	31.7	40.0	0.83
Cow meat frequency, times/month	1.6 ± 3.2	1.6 ± 3.2	1.6 ± 3.4	1.2 ± 3.3	2.3 ± 4.9	0.83
Goat meat, %	10.2	12.7	8.5	11.7	6.7	0.92
Goat meat frequency, times/month	0.3 ± 1.2	0.4 ± 1.5	0.3 ± 1.3	0.4 ± 1.6	0.1 ± 0.3	0.88
Sheep meat, %	1.5	4.2	0.0	5.0	0.0	0.20
Sheep meat frequency, times/month	0.0 ± 0.4	0.0 ± 0.3	0.0 ± 0.0	0.1 ± 1.0	0.0 ± 0.0	0.54
Pig meat, %	4.2	0.0	3.4	8.3	0.0	0.15
Pig meat frequency, times/month	0.1 ± 0.5	0.0 ± 0.0	0.1 ± 0.5	0.3 ± 1.2	0.0	0.09
Chicken meat, %	66.0	73.2	84.8	80.0	60.0	**0.017**
Chicken meat frequency, times/month	5.1 ± 6.2	4.9 ± 5.2	5.0 ± 4.5	4.2 ± 4.0	2.7 ± 3.6	0.46
Chicken eggs, %	89.1	81.7	88.1	88.3	80.0	0.46
Chicken eggs frequency, times/month	8.4 ± 6.7	7.3 ± 7.1	8.0 ± 7.5	6.9 ± 4.7	7.1 ± 8.3	0.48
Cow milk[2], %	6.4	11.3	18.6	18.3	46.7	**<0.001**
Cow milk frequency, times/month	0.6 ± 3.1	0.1 ± 0.5	1.0 ± 2.8	1.9 ± 8.4	13.1 ± 19.2	**<0.001**

[1]Animal milk includes fresh milk, tinned milk, or powdered milk.
[2]Cow milk includes only fresh cow's milk.
[3]P- values calculated from chi-square statistics for comparisons of proportions and F-statistics using ANOVA for comparisons of means.
Abbreviations: ASF – animal source foods; CRP – C-reactive protein; AGP – α-1-acid glycoprotein

Table 2.9 Multivariate logistic regression of the associations between livestock ownership typology and child consumption of animal-source foods in the past three months in Greater Accra Region, Ghana, October-November 2018 (n=470). Values are adjusted odds ratios[1].

Indicator	Cow meat	Goat, sheep, or pig meat	Chicken meat[2]	Organ meats	Chicken eggs	Cow milk
Livestock ownership typology (Ref: No livestock)	-	-	-	-	-	-
Type 2 – Poultry (<12)	1.15	0.95	1.13	2.21	**0.36***	1.54
Type 3 – Poultry (≥12)	0.77	1.14	**3.47****	1.06	0.81	**4.39****
Type 4 – Small livestock +/- poultry	0.64	1.58	1.84	1.07	0.72	**2.86***
Type 5 – Cattle +/- small livestock or poultry	0.57	0.18	0.50^{+}	0.36	0.41	**5.32***
Child sex (Ref: Male)						
Female	**1.58***	1.57^{+}	**1.66***	**1.70***	0.71	0.83
Child age (Ref: 24-59 months)						
6-23 months	**0.28*****	**0.31*****	**0.14*****	0.49^{+}	**0.13*****	**0.22*****
Number of children under 5y in household	1.08	1.47	1.18	0.98	0.87	0.89
Head of household sex (Ref: Male)						
Female	1.19	1.18	1.73^{+}	0.58	2.25	1.74
Head of household religion (Ref: Christian)						
Muslim	**1.79**	2.66	0.91	1.28	0.50	**8.43****
Traditional or no religion	0.27	**5.11***	3.53^{+}	1.51	0.36^{+}	0.72
Head of household ethnic group (Ref: Ga-Dangme)						
Akan	0.75	1.02	1.06	2.81^{+}	2.34^{+}	0.14^{+}
Ewe	0.70^{+}	0.68	1.49	1.65	**3.27****	0.88
Other	2.00^{+}	2.32	1.89^{+}	3.10	4.29	0.49
Maternal education (Ref: None or nursery)						
Primary	1.00	1.48	1.39	**0.33***	1.52	1.46
Junior	1.41	1.00	1.07	0.39^{+}	0.94	1.09
Senior or higher	1.58	**4.45***	1.70	**0.14***	1.47	0.58
Household wealth quintile (Ref: Lowest)						
Low	**2.04***	0.77	1.62	2.67	**2.62***	0.54
Middle	**2.46****	1.61	1.21	**6.94****	**3.64****	0.80
High	**2.44***	1.28	1.28	**6.06*****	**3.65****	1.80
Highest	**3.85*****	2.61	1.61	**15.82*****	2.13	0.67

[1]Animal-source food consumption variables are dichotomous (yes/no) for any consumption in the past three months. Models adjusted for listed covariates. Robust standard errors are adjusted for community cluster. Marginal significance (0.05≤p<0.1) indicated by $^{+}$. Statistical significance indicated by * for p<0.05, ** for p<0.01, and *** for p<0.001.

[2]The category for chicken meat includes consumption of other poultry meats (turkey, guinea fowl, and duck meat) in the past three months. 99.7% of children consumed chicken meat, 2.1% of children consumed other poultry meat in addition to chicken meat, and 0.3% of children consumed other poultry meat but not chicken meat in the past three months.

Table 2.10 Estimates of the average causal mediated effect (indirect effect) of chicken meat and cow milk consumption in the association between livestock ownership typologies and anemia among children 6-59 months old in Greater Accra, Ghana (n=470)[1].

Indicator	Chicken meat[2]			Cow milk		
	ACME	95% CI	p-value	ACME	95% CI	P-value
Livestock typology						
Type 1 – No livestock (reference)	.	.	.	.	.	.
Type 2 – Poultry (<12)	0.0008	(-0.007, 0.01)	0.82	-0.0005	(-0.0052, 0.00)	0.82
Type 3 – Poultry (≥12)	0.0107	(-0.005, 0.03)	0.18	-0.0023	(-0.0188, 0.02)	0.76
Type 4 – Small livestock +/- poultry	0.0058	(-0.0046, 0.02)	0.30	-0.0017	(-0.0152, 0.01)	0.77
Type 5 – Cattle +/- small livestock or poultry	-0.0068	(-0.0244, 0.00)	0.25	-0.0026	(-0.0308, 0.02)	0.77

[1]R mediation package (62) was used to the average causal mediated effect (ACME) of chicken meat and cow milk consumption on anemia for each typology in comparison to Typology 1 (no livestock) using quasi-Bayesian Monte Carlo with 500 simulations and clustering set at the community level. Chicken meat and cow milk consumption were hypothesized mediators based on significance in Table 2.8. Covariates in mediation models: child age (binary), child sex, number of children under 5, head of household sex, head of household religion, head of household ethnic group, maternal education category, and wealth quintile. Covariates in outcome models: child age (binary), child sex, number of children under 5, head of household sex, head of household religion, head of household ethnic group, maternal education category, wealth quintile, malaria, and toilet facility.

[2]The category for chicken meat includes consumption of other poultry meats (turkey, guinea fowl, and duck meat) in the past three months. 99.7% of children consumed chicken meat, 2.1% of children consumed other poultry meat in addition to chicken meat, and 0.3% of children consumed other poultry meat but not chicken meat in the past three months.

CHAPTER 3 Ruminant-Related Risk Factors are Associated with Shiga Toxin-Producing *Escherichia coli* Infection in Children in Southern Ghana

Abstract

Livestock can provide benefits to households in low- and middle-income countries, including supplemental income and food, 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 ownership of animals, exposure to animal feces, and consumption of animal-source foods, were associated with bacterial zoonotic enteropathogen infections in children 6 to 59 months old in two districts of 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 qPCR. Adjusted logistic regression was used to model associations between livestock-related risk factors and the odds of enteropathogen infection in children. 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. Household ownership of goats or sheep was associated with STEC detection in children [Odds Ratio (95% CI): 4.30 (1.32, 14.08)]. Positive detection of STEC in chicken feces [7.85 (2.54, 24.30)] and consumption of fresh cow's milk at least once per week [3.03 (1.75, 5.24)] were also associated with higher odds of STEC detection in children. None of the livestock-related risk factors were associated with aEPEC or *C. jejuni/coli* detection in children,

and associations with *Salmonella* were not examined because of the low prevalence of infections. 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 livestock feces. Further research is also needed to identify context-appropriate household and food hygiene practices that mitigate foodborne transmission of pathogens from dairy products.

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 that can cause diarrhea and long-term health detriments in children (4,5). Recent household-level water, sanitation, and hygiene (WASH) efficacy trials were ineffective in preventing enteric infections in children (6) or eliminating *Escherichia coli* (*E. coli*) contamination of the household environment (7), prompting calls for greater attention to domestic animal fecal contamination as an important source of enteropathogen exposure (8).

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) (9). The transmission of zoonotic enteropathogens from animal feces occurs

via similar pathways (10): 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. 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 (10–13). 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 (14–19). Young children may be particularly at risk of enteropathogen infection from environmental contamination given their frequent hand-to-mouth and exploratory behaviors (20). Indeed, direct observational studies have shown that infants engage in geophagy and even consumption of chicken feces in normal day-to-day behavior (21–25).

Bacterial pathogens commonly found in livestock feces, including *Salmonella*, *Campylobacter*, and certain *E. coli* pathotypes, are important contributors to the global burden of childhood diarrhea (4,26). 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 *Salmonella* and *Campylobacter* have many animal reservoirs (primarily poultry and cattle) and are transmitted through food-borne routes, mainly via consumption of contaminated poultry meat and dairy products (4,26). *Campylobacter* can also be transmitted via direct contact with infected

ruminants and poultry and by ingestion of contaminated water (31). While *Salmonella* can be spread by person-to-person fecal-oral transmission, *Campylobacter* transmission is predominantly zoonotic (26).

A growing body of evidence has linked household livestock ownership in LMICs to enteropathogen infections in young children, including in Ecuador (29,30,32), Egypt (33), Ethiopia (13), and Lao People's Democratic Republic (34). Four of these studies found that exposure to household chickens and their feces was associated with *Campylobacter jejuni* infection in children (13,29,32,33), 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 (30,34). In Ghana, where our study was conducted, household livestock ownership is common, yet research to date on zoonotic transmission from livestock has focused on infections in adult farmers (35). The present study builds on this 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 specific 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.

Methods

Study design

This cross-sectional study of children between 6 to 59 months old in the Greater Accra Region, Ghana was conducted from October to November 2018. 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, community size, and distance from the capital city of Accra. To be eligible for inclusion in the study, children had to be 6 to 59 months old, and their primary caregiver, usually their mother, had to be at least 18 years old. Characteristics of the study sites and participant recruitment methods are described in further detail in Chapter 2. Briefly, Ga East is a primarily urban district with about 6% of its population engaged in agriculture (36). Three-quarters of the population of Shai Osudoku lives in rural areas, with 34% of its population is engaged in agricultural activities (37).

A subsample of children from the sample described in Chapter 2 was selected for inclusion in this study (**Figure 3.1**). From the main study sample of 484 children, 430 (88.8%) had a stool sample collected. Of those with available stool samples, financial constraints limited fecal analysis to 265 (61.6%), which were selected in a two-step process. 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 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) and the Qualtrics survey platform (Qualtrics, Provo, UT, USA), as described in Chapter 2. 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 (38). 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 how frequently the index child consumed certain animal-source foods (e.g., fresh cow's milk, chicken meat) in the prior three months, and whether the child had diarrhea in the prior seven days, as described in Chapter 2. During and following 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 instructions and a stool collection kit, which included a sterile fecal container (Sarstedt, 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 (**Figure 3.2**). 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 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 2mL cryovial 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 250mg of sample was used for extraction, while approximately 115mg of sample was used for extraction from chicken stools. In the final extraction step, 100µL of DNA was eluted into an Eppendorf™ DNA LoBind microcentrifuge tube (Eppendorf, Hamburg, Germany). An extraction blank which 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, USA). DNA samples were then transported to the University of Michigan (Ann Arbor, MI, USA) for 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. (39) and validated for specificity using the NIH Nucleotide Basic Local Alignment Search Tool (BLASTn) program (**Appendix 1**). Child and chicken DNA samples and extraction blanks were diluted 1:10 and run single-plex on 384-well plates using the QuantStudio™ 5 System (Applied Biosystems™, Foster City, CA, USA). Samples were run in quadruplicate for the *cadF*, *bfpA*, and *eae* gene targets, and in triplicate for *stx1* and *stx2*. Each amplification well contained 4.5µL 1:10-diluted DNA, 5.0µL TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Foster City, CA, USA), and 0.5µL of primer-probe mixture at a final concentration of 500:250nM 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 which replaced DNA with ddH_2O to control for reagent contamination. 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 min. For the *cadF* gene, annealing and extension was at 58°C for 1 min.

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 Platform (Thermo Fisher Scientific, Carlsbad, CA, USA). 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 plate to ensure comparability between plates for each gene target. Technical replicates for each sample were inspected if the standard deviation 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 ≤50% amplification of replicates were classified as negative for that gene. None of the extraction blanks or water controls amplified. A Ct cut-off of ≤35 was applied to define positive detection of a gene for child samples. This cut-off 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 cut-off (Ct≤40) was applied to chicken samples given that chicken samples had on average higher Ct values than child samples (see **Appendix 2 and 3**), possibly due to lower initial sample input and lower purity of the samples (child A260/280 mean ± 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*), *Salmonella* (*ttr*) (see **Appendix 4**).

The PCR amplification efficiency for each target gene was calculated by pooling at minimum four known positive child samples and running eight four-fold dilutions of the pooled sample with four technical replicates at each dilution. Linear regression of the average Ct against the log10-dilution was used to estimate the slope ($\beta \pm$ 95% confidence interval) and R^2 of the

standard curve. The PCR efficiency value was calculated from the slope of the standard curve. PCR efficiencies, ideally between 90-110%, ranged from 87.3% to 112.8% (Appendix 1).

Statistical analysis

Descriptive analysis was conducted of child- and household-level characteristics of the study sample, and of livestock management, livestock observations, and household hygiene behaviors. Means and standard deviations (SD) were calculated for normally distributed continuous variables, while 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≤35) of *C. jejuni/coli*, aEPEC, and STEC in children. Because *Salmonella* was detected in <1% of children, it was not analyzed. 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 food (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 three months. Pig ownership was not examined as a predictor as ownership was rare.

Potential confounding variables were selected a priori based on the literature (30,34), and included child sex and age, caregiver highest attained education, number of household members, and the household's 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 water source, defined according to the WHO/UNICEF Joint Monitoring Programme 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 (40). We created a household asset-based wealth index score using principal components analysis (PCA) of 26 assets, excluding land holdings, livestock, and water, sanitation, and hygiene indicators (41). 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. Due to the low number of observations in certain wealth quintiles for models predicting *C. jejuni/coli* and STEC infection, asset-based wealth quintile was also not included in the final adjusted models. 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 above, as well as a fixed effect for district. Multi-level logistic models were examined with community as a random effect and district as a fixed effect, however, this model structure was not used because the logistic models with clustered standard errors had better predictive value (lower overall p-value). Adjusted models examining cow's milk consumption as the main predictor also included cow 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, USA). 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 & 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, non-monetary 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, 265 children were selected in the subsample for enteropathogen testing, of which 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 3.1**). Most households reported using an improved water source for drinking water, primarily a public tap/standpipe (45.2%) or packaged (sachet) water (44.0%), while seven households (2.7%) used

an unimproved drinking water source, mainly surface water. Almost one-third (30.5%) of households practiced open defecation, while 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, while over half had completed junior education or higher.

Household livestock ownership and management characteristics

Sixty-one percent of households owned livestock (**Table 3.1**). Of households that owned livestock, 62.9% owned only chickens and other poultry (guinea fowl, turkeys, ducks) while the others owned poultry as well as small ruminants, pigs, or cattle. Livestock-owning households reared a median of 12 poultry (interquartile range [IQR]: 6, 25), five goats (IQR: 2, 10), ten sheep (IQR: 5, 18), and 70 cattle (IQR: 42, 100). Cattle were raised solely in the Shai Osudoku district. Small ruminants and poultry were raised in both districts, though the number of sheep and goats per household was higher in Shai Osudoku (median: 7, IQR: 2, 16) compared to 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 (**Table 3.2**). 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 most household yards, even if the household reported owning no livestock. 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 livestock 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 (**Table 3.2**). 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, while 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 3.3**). In particular, aEPEC was detected in 45.6% of stool samples, *C. jejuni/coli* in 11.6%, and STEC in 4.3%. *Salmonella* was detected in only two children's stool samples. Children's diarrheal prevalence in the preceding seven 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 seven 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) (**Table 3.4**). Conversely, children positive for STEC were on average 36 months old, compared to 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 compared to those using a pit latrine or flush toilet (**Table 3.4)**.

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 3.3**). *C. jejuni/coli* was most prevalent, detected in 44.9% of chicken stool samples. aEPEC was detected in 38.5% of chicken stool samples, while 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 3.3. Household cattle ownership was positively associated with STEC detection in chicken stool (OR: 3.85, 95% CI: 1.48, 10.05; p=0.006), 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 (**Figure 3.3**, **Table 3.5**). 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. Among the 156 households in which

chicken stools were tested, detection of STEC in chicken stool was associated with 7.85 times higher odds of STEC detection in a child's stool (95% CI: 2.54, 24.30; p-value<0.001).

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 confounders. 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) (**Table 3.6**). Among the nineteen 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 cases, caregivers reported that children consumed milk boiled, while 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, while 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, though most infections were not associated with recent diarrhea. In particular, STEC infection in

children was associated with owning ruminants, consuming fresh cow's milk, and living in a household in which chicken feces were positive for STEC. 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 contributes to STEC infection in Ghanaian children. According to the 2010 Ghana census survey, there were one-tenth as many cattle reared in Ga East (~2,600) (36) compared to Shai Osudoku (~28,300) (37), 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). Nevertheless, small ruminants are also important reservoirs of STEC in addition to cattle (42,43), 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 (44). 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. Secondary transmission of STEC can also result from asymptomatic adults and children shedding STEC in their feces (43). 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. STEC exposure due to open defecation could also have contributed to the

attenuated effect of cattle ownership after adjusting for confounders, as ownership of cattle was marginally correlated with open defecation.

Our finding that STEC detection in chicken feces was associated with STEC infection in children living in the same household is notable given that chickens are not considered to be a major zoonotic reservoir of STEC (43,45). In our study, STEC was found in 12% of chicken fecal samples (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 (46). Although chickens are not natural reservoir hosts of STEC (i.e., they are not able to maintain STEC colonization in the absence of re-exposure) (43), they are potential spillover hosts that harbor and shed STEC in their feces (42,45). As with STEC spillover to wild animals living near livestock operations (45), 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. STEC infection in children and STEC detection in chicken feces remained significantly associated independent of cattle ownership, which suggests that chicken fecal contamination may be 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 (31,47,48). 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 (27,49), and was identified as a risk factor for STEC infection in children under three years old in Germany (50). In Accra, Ghana, a recent study of commonly consumed milk and milk products sold from vendors found high levels of fecal coliforms in raw wagashie (unfermented soft cheese) and brukina (a mixture of millet and fermented milk), with lower levels in boiled milk and other heat-treated milk products (51). Another study of urban Accra dairy products supplied by Fulani herders found equal concentrations of fecal coliforms and *E. coli* in raw and boiled milk (52). That study detected *E. coli* O157:H7 in boiled milk and various cow milk products from Fulani dairy farms, while an earlier 2007 study of informally marketed cow milk did not detect STEC O157:H7 in raw milk samples from the Greater Accra Region (53). These investigations, along with our findings, suggest that informal sharing and selling of fresh cow's milk, even if milk is boiled, is 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 (31), and many other epidemiologic studies have found associations between chicken ownership and *C. jejuni* infection in children (5,13,29,30,33). 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 five 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. (13) found that keeping animals indoors at night was associated with higher odds of *Campylobacter* infection in Ethiopian infants. Also, in Ethiopia, Headey and Hirvonen (11) found positive associations between poultry ownership and height-for-age Z-scores (HAZ) in children under five 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 (26), 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 also some evidence of aEPEC transmission between animals and humans (29,54), 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 identified previously unexplored ruminant-related risk factors for STEC detection among young children in southern Ghana. Strengths of this study included detailed data collection on livestock ownership and management. In addition, it is among few studies in the region to assess enteropathogenic bacterial infections in children using molecular diagnostic testing. However, this study also has several important limitations. Because the design is cross-sectional, we cannot infer causality between the examined livestock risk factors and child infection. In addition, we only tested fecal samples of chickens, and not ruminants, which limits confidence in attributing STEC in children to these animals, despite the biologic plausibility. Future investigations should also test ruminant feces and other potential environmental sources, such as drinking water and household soil, to evaluate other possible 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 (55). Nevertheless, our testing of household chicken feces demonstrated certain zoonotic enteropathogens to which these children may be exposed. Lastly, we purposefully studies communities rearing livestock, so the prevalence of zoonotic enteropathogens in children in our sample may over-estimate that of other children in the region.

Although poultry ownership was not associated with enteropathogen infections in children, chickens were determined to carry several zoonotic pathogens. Livestock ownership was not measured for all households in the community, but rather only those households with children under five, thus we may have missed community-level livestock exposures. Although statistical models that included community as a random effect did not improve model fit, neighboring livestock could still play a role in enteropathogen exposure. While almost all livestock-owning households had chickens and livestock feces present, over two-thirds of

households that did not own livestock also had chickens and animal feces in their yards. Particularly in peri-urban communities of the Ga East District, where home dwellings are close together and yard spaces are shared, children are likely to be exposed to zoonotic pathogens even if their household does not own animals. Observations of free-roaming chickens and of animal feces in household yards represent only a proxy for livestock exposures, and does not take into account the density of animals or feces, which may modify the risk of transmission to children. As with some sanitation research, future infection risk studies could more carefully evaluate larger spatial patterns of exposure, including whether neighboring household or community-level livestock ownership or density are as or more important than household-level exposures (56,57).

Conclusions

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 (58,59). 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 (15,17,19), 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 (60,61), further research is 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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Ethical Statement

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 & Use Committee (protocol no. 00008493), and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18).

References

1. Herrero M, Grace D, Njuki J, Johnson N, Enahoro D, Silvestri S, Rufino MC. The roles of livestock in developing countries. Animal. 2013;7:3–18.
2. Alders RG, Dumas SE, Rukambile E, Magoke G, Maulaga W, Jong J, Costa R. Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. Matern Child Nutr. 2018;14:1–14.
3. Ruel MT, Quisumbing AR, Balagamwala M. Nutrition-sensitive agriculture: What have we learned so far? Glob Food Sec. 2018;17:128–53.
4. Delahoy MJ, Wodnik B, McAliley L, Penakalapati G, Swarthout J, Freeman MC, Levy K. Pathogens transmitted in animal feces in low- and middle-income countries. Int J Hyg Environ Health. 2018;221:661–76.
5. Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2014;108:313–25.
6. Rogawski McQuade ET, Platts-Mills JA, Gratz J, Zhang J, Moulton LH, Mutasa K, Majo FD, Tavengwa N, Ntozini R, Prendergast AJ, et al. Impact of water quality sanitation, handwashing, and nutritional interventions on enteric infections in rural Zimbabwe: the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial. J Infect Dis. 2020;221:1379–86.
7. Pickering AJ, Null C, Winch PJ, Mangwadu G, Arnold BF, Prendergast AJ, Njenga SM, Rahman M, Ntozini R, Benjamin-Chung J, et al. The WASH Benefits and SHINE trials: interpretation of WASH intervention effects on linear growth and diarrhoea. Lancet Glob Heal. 2019;7:e1139–46.
8. Prendergast AJ, Gharpure R, Mor S, Viney M, Dube K, Lello J, Berger C, Siwila J, Joyeux M, Hodobo T, et al. Putting the "A" into WaSH: a call for integrated management of water, animals, sanitation, and hygiene. Lancet Planet Heal. 2019;3:e336–7.
9. Wagner EG, Lanoix JN. Excreta disposal for rural areas and small communities. 1958;39.
10. Penakalapati G, Swarthout J, Delahoy MJ, McAliley L, Wodnik B, Levy K, Freeman MC. Exposure to animal feces and human health: a systematic review and proposed research priorities. Environ Sci Technol. 2017;51:11537–52.
11. Headey D, Hirvonen K. Is exposure to poultry harmful to child nutrition? An observational analysis for rural Ethiopia. PLoS One. 2016;11:e0160590.
12. George CM, Oldja L, Biswas SK, Perin J, Lee GO, Ahmed S, Haque R, Sack RB, Parvin T, Azmi IJ, et al. Fecal markers of environmental enteropathy are associated with animal exposure and caregiver hygiene in Bangladesh. Am J Trop Med Hyg. 2015;93:269–75.
13. Budge S, Barnett M, Hutchings P, Parker A, Tyrrel S, Hassard F, Garbutt C, Moges M, Woldemedhin F, Jemal M. Risk factors and transmission pathways associated with infant *Campylobacter spp.* prevalence and malnutrition: A formative study in rural Ethiopia. PLoS One. 2020;15:e0232541.
14. Fuhrmeister ER, Ercumen A, Pickering AJ, Jeanis KM, Ahmed M, Brown S, Arnold BF, Hubbard AE, Alam M, Sen D, et al. Predictors of enteric pathogens in the domestic environment from human and animal sources in rural Bangladesh. Environ Sci Technol. 2019;53:10023–33.
15. Ercumen A, Pickering AJ, Kwong LH, Arnold BF, Parvez SM, Alam M, Sen D, Islam S, Kullmann C, Chase C, et al. Animal feces contribute to domestic fecal contamination:

evidence from *E. coli* measured in water, hands, food, flies, and soil in Bangladesh. Environ Sci Technol. 2017;51:8725–34.
16. Schiaffino F, Trigoso DR, Colston JM, Paredes Olortegui M, Shapiama Lopez W V., Garcia Bardales PF, Pisanic N, Davis MF, Penataro Yori P, Kosek MN. Associations among household animal ownership, infrastructure, and hygiene characteristics with source attribution of household fecal contamination in peri-urban communities of Iquitos, Peru. Am J Trop Med Hyg. 2020;104:372–81.
17. Hamzah L, Boehm AB, Davis J, Pickering AJ, Wolfe M, Mureithi M, Harris A. Ruminant fecal contamination of drinking water introduced post-collection in rural kenyan households. Int J Environ Res Public Health. 2020;17:1–23.
18. Wardrop NA, Hill AG, Dzodzomenyo M, Aryeetey G, Wright JA. Livestock ownership and microbial contamination of drinking-water: Evidence from nationally representative household surveys in Ghana, Nepal and Bangladesh. Int J Hyg Environ Health. 2018;221:33–40.
19. Harris AR, Pickering AJ, Harris M, Doza S, Islam MS, Unicomb L, Luby S, Davis J, Boehm AB. Ruminants contribute fecal contamination to the urban household environment in Dhaka, Bangladesh. Environ Sci Technol. 2016;50:4642–9.
20. Ngure FM, Reid BM, Humphrey JH, Mbuya MN, Pelto G, Stoltzfus RJ. Water, sanitation, and hygiene (WASH), environmental enteropathy, nutrition, and early child development: making the links. Ann N Y Acad Sci. 2014;1308:118–28.
21. Reid B, Orgle J, Roy K, Pongolani C, Chileshe M, Stoltzfus R. Characterizing potential risks of fecal–oral microbial transmission for infants and young children in Rural Zambia. Am J Trop Med Hyg. 2018;98:816–23.
22. Ngure FM, Humphrey JH, Mbuya MNN, Majo F, Mutasa K, Govha M, Mazarura E, Chasekwa B, Prendergast AJ, Curtis V, et al. Formative research on hygiene behaviors and geophagy among infants and young children and implications of exposure to fecal bacteria. Am J Trop Med Hyg. 2013;89:709–16.
23. Ngure F, Gelli A, Becquey E, Ganaba R, Headey D, Huybregts L, Pedehombga A, Sanou A, Traore A, Zongo F, et al. Exposure to livestock feces and water quality, sanitation, and hygiene (WASH) conditions among caregivers and young children: formative research in rural Burkina Faso. Am J Trop Med Hyg. 2019;100:998–1004.
24. Marquis GS, Ventura G, Gilman RH, Porras E, Miranda E, Carbajal L, Pentafiel M. Fecal contamination of shanty town toddlers in households with non-corralled poultry, Lima, Peru. Am J Public Health. 1990;80:146–9.
25. George, Christine Marie Oldja L, Biswas S, Perin J, Lee GO, Kosek M, Sack RB, Ahmed S, Haque R, Parvin T, Azmi IJ, et al. Geophagy is associated with environmental enteropathy and stunting in children in rural Bangladesh. Am J Trop Med Hyg. 2015;92:1117–24.
26. Goering R V., Dockrell HM, Zuckerman M, Chiodini PL. Gastrointestinal tract infections. MIMS' Medical Microbiology and Immunology. Sixth Ed. Elsevier; 2019. p. 265–77.
27. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev. 2013;26:822–80.
28. Gyles CL. Shiga toxin-producing *Escherichia coli*: An overview. J Anim Sci. 2007;85:E45–62.
29. Vasco K, Graham JP, Trueba G. Detection of zoonotic enteropathogens in children and

domestic animals in a semirural community in Ecuador. Appl Environ Microbiol. 2016;82:4218–24.
30. Lowenstein C, Vasco K, Sarzosa S, Salinas L, Torres A, Perry MJ, Simmens SJ, Trueba G, Eisenberg JNS, Graham JP. Determinants of childhood zoonotic enteric infections in a semirural community of Quito, Ecuador. Am J Trop Med Hyg. 2020;102:1269–78.
31. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of *Campylobacter* infection. Clin Microbiol Rev. 2015;28:687–720.
32. Oberhelman RA, Gilman RH, Sheen P, Cordova J, Taylor DN, Zimic M, Meza R, Perez J, LeBron C, Cabrera L, et al. *Campylobacter* transmission in a Peruvian shantytown: a longitudinal study using strain typing of *Campylobacter* isolates from chickens and humans in household clusters. J Infect Dis. United States; 2003;187:260–9.
33. El-Tras WF, Holt HR, Tayel AA, El-Kady NN. *Campylobacter* infections in children exposed to infected backyard poultry in Egypt. Epidemiol Infect. 2015;143:308–15.
34. Chard AN, Levy K, Baker KK, Tsai K, Chang HH, Thongpaseuth V, Sistrunk JR, Freeman MC. Environmental and spatial determinants of enteric pathogen infection in rural Lao People's Democratic Republic: A cross-sectional study. PLoS Negl Trop Dis. 2020;14:e0008180.
35. Amissah-Reynolds PK. Zoonotic risks from domestic animals in Ghana. Int J Pathog Res. 2020;4:17–31.
36. Ghana Statistical Service. 2010 Population & Housing Census: District Analytical Report - Ga East Municipality. Accra; 2014.
37. Ghana Statistical Service. 2010 Population & Housing Census: District Analytical Report - Shai Osudoku District. Accra; 2014.
38. Zezza A, Pica-Ciamarra U, Mugera HK, Mwisomba T, Okello P. Measuring the Role of Livestock in the Household Economy: A Guidebook for Designing Household Survey Questionnaires. Washington DC; 2016.
39. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, Mduma E, Platts-Mills J, Boisen N, Nataro J, et al. Optimization of quantitative PCR Methods for enteropathogen detection. Chan KH, editor. PLoS One. 2016;11:e0158199.
40. World Health Organization. Safely managed drinking water - thematic report on drinking water 2017. Geneva, Switzerland; 2017.
41. Vyas S, Kumaranayake L. Constructing socio-economic status indices: How to use principal components analysis. Health Policy Plan. 2006;21:459–68.
42. Ferens WA, Hovde CJ. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. Foodborne Pathog Dis. 2011;8:465–87.
43. Persad AK, LeJeune JT. Animal reservoirs of Shiga Toxin-Producing *Escherichia coli*. Microbiol Spectr. 2014;2:1–14.
44. Conrad CC, Stanford K, Narvaez-Bravo C, Callaway T, McAllister T. Farm fairs and petting zoos: A review of animal contact as a source of zoonotic enteric disease. Foodborne Pathog Dis. 2017;14:59–73.
45. Kim JS, Lee MS, Kim JH. Recent updates on outbreaks of Shiga toxin-producing *Escherichia coli* and its potential reservoirs. Front Cell Infect Microbiol. 2020;10:1–10.
46. Kagambèga A, Martikainen O, Siitonen A, Traoré AS, Barro N, Haukka K. Prevalence of diarrheagenic *Escherichia coli* virulence genes in the feces of slaughtered cattle, chickens, and pigs in Burkina Faso. Microbiol Open. 2012;1:276–84.
47. Chauret C. Survival and control of *Escherichia coli* O157:H7 in foods, beverages, soil and

water. Virulence. 2011;2:593–601.
48. Claeys WL, Cardoen S, Daube G, De Block J, Dewettinck K, Dierick K, De Zutter L, Huyghebaert A, Imberechts H, Thiange P, et al. Raw or heated cow milk consumption: Review of risks and benefits. Food Control. 2013;31:251–62.
49. Baker CA, Rubinelli PM, Park SH, Carbonero F, Ricke SC. Shiga toxin-producing *Escherichia coli* in food: Incidence, ecology, and detection strategies. Food Control. 2016;59:407–19.
50. Werber D, Behnke SC, Fruth A, Merle R, Menzler S, Glaser S, Kreienbrock L, Prager R, Tschäpe H, Roggentin P, et al. Shiga toxin-producing *Escherichia coli* infection in Germany - Different risk factors for different age groups. Am J Epidemiol. 2007;165:425–34.
51. Parry-Hanson Kunadu A, Aboagye EF, Colecraft EK, Otoo GE, Adjei MYB, Acquaah E, Afrifa-Anane E, Amissah JGN. Low consumption of indigenous fresh dairy products in Ghana attributed to poor hygienic quality. J Food Prot. 2019;82:276–86.
52. Parry-Hanson Kunadu A, Holmes M, Miller EL, Grant AJ. Microbiological quality and antimicrobial resistance characterization of *Salmonella spp*. in fresh milk value chains in Ghana. Int J Food Microbiol. 2018;277:41–9.
53. Addo KK, Mensah GI, Aning KG, Nartey N, Nipah GK, Bonsu C, Akyeh ML, Smits HL. Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. Trop Med Int Heal. 2011;16:227–32.
54. Hernandes RT, Elias WP, Vieira MAM, Gomes TAT. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiol Lett. 2009;297:137–49.
55. Goddard FGB, Ban R, Barr DB, Brown J, Cannon J, Colford JM, Eisenberg JNS, Ercumen A, Petach H, Freeman MC, et al. Measuring environmental exposure to enteric pathogens in low-income settings: review and recommendations of an interdisciplinary working group. Environ Sci Technol. 2020;54:11673–91.
56. Fuller JA, Villamor E, Cevallos W, Trostle J, Eisenberg JNS. I get height with a little help from my friends: Herd protection from sanitation on child growth in rural Ecuador. Int J Epidemiol. 2016;45:460–9.
57. Larsen DA, Grisham T, Slawsky E, Narine L. An individual-level meta-analysis assessing the impact of community-level sanitation access on child stunting, anemia, and diarrhea: Evidence from DHS and MICS surveys. PLoS Negl Trop Dis. 2017;11:1–13.
58. Pires SM, Majowicz S, Gill A, Devleesschauwer B. Global and regional source attribution of shiga toxin-producing *Escherichia coli* infections using analysis of outbreak surveillance data. Epidemiol Infect. 2019;147.
59. Lupindu AM. Epidemiology of Shiga toxin-producing *Escherichia coli* O157:H7 in Africa in review. South African J Infect Dis. 2018;33:24–30.
60. Platts-Mills JA, Liu J, Rogawski ET, Kabir F, Lertsethtakarn P, Siguas M, Khan SS, Praharaj I, Murei A, Nshama R, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. Lancet Glob Heal. 2018;6:e1309–18.
61. Amour C, Gratz J, Mduma ER, Svensen E, Rogawski ET, McGrath M, Seidman JC, McCormick BJJ, Shrestha S, Samie A, et al. Epidemiology and impact of *Campylobacter* infection in children in 8 low-resource settings: Results from the MAL-ED study. Clin Infect Dis. 2016;63:1171–9.

Figures and Tables

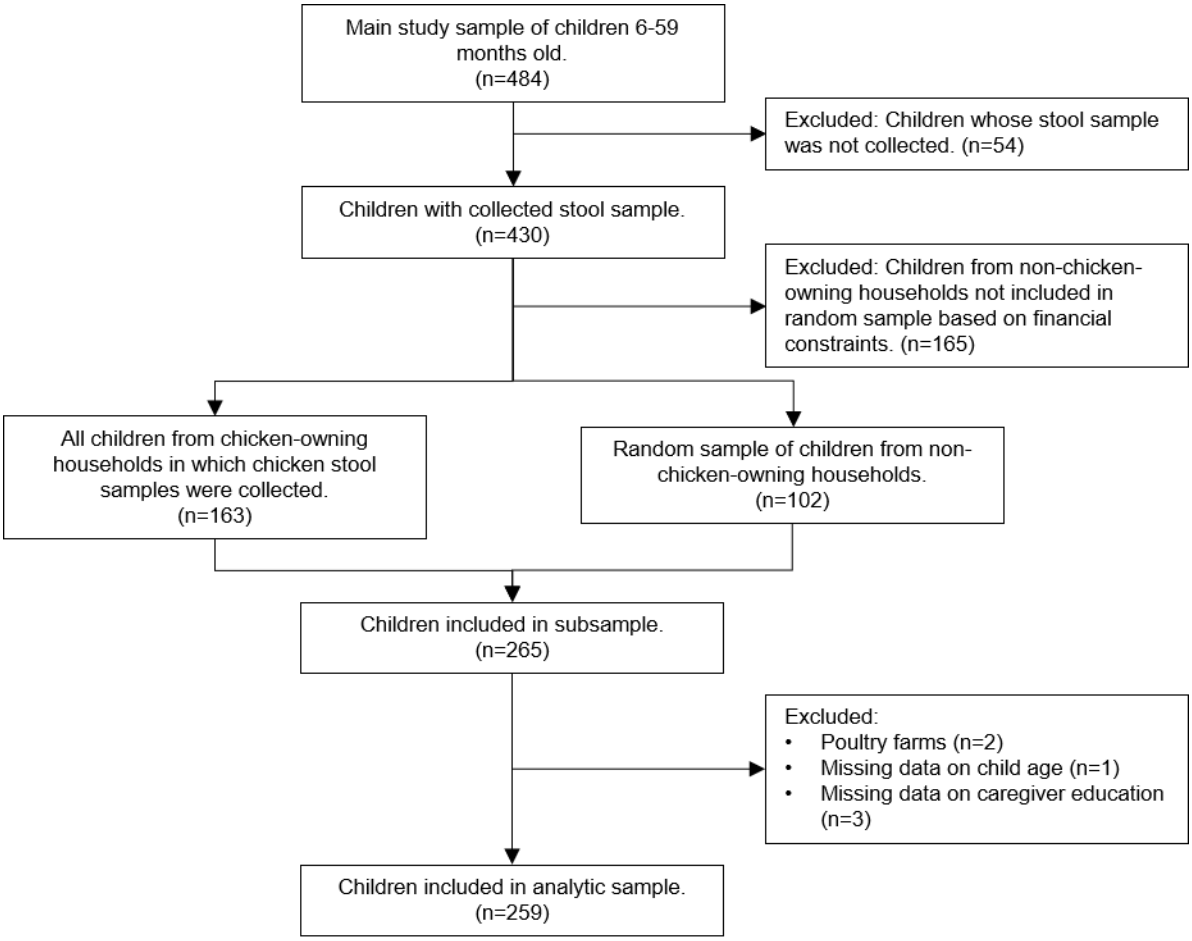

Figure 3.1 Flow chart of sub-sample selection from the main study sample in Chapter 2.

Figure 3.2 Chicken from a household's flock entering a stool sample collection pen.

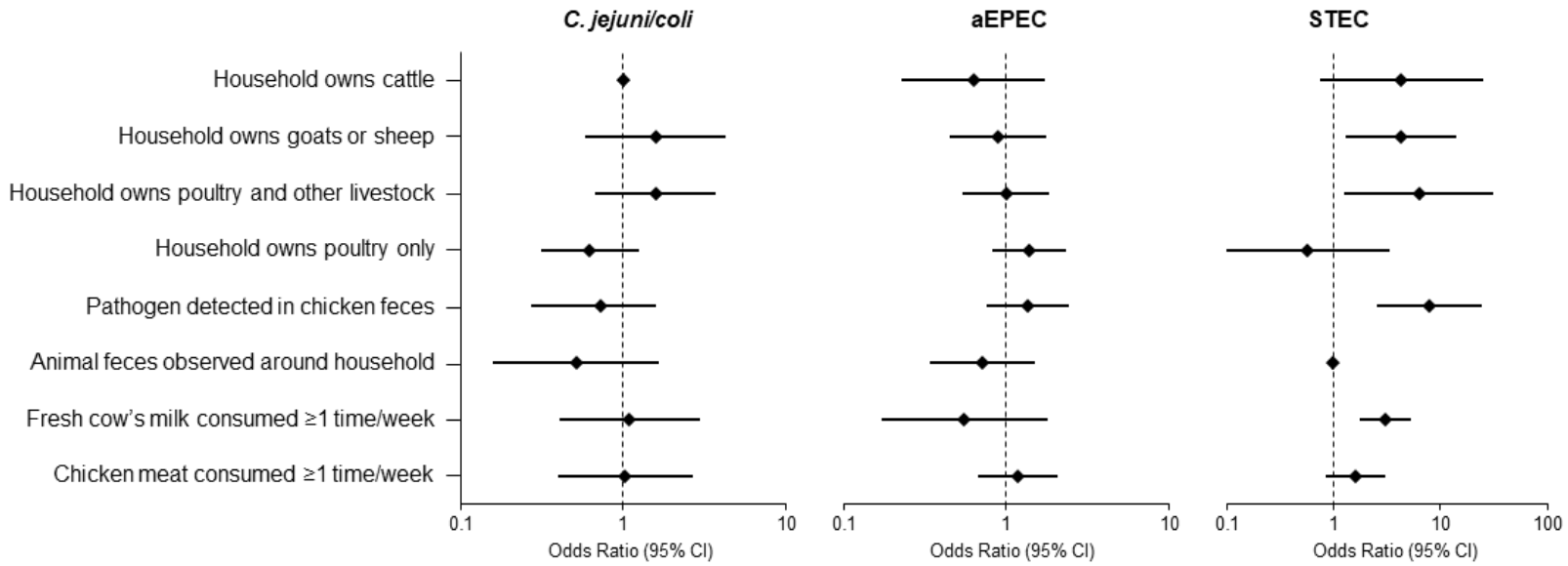

Figure 3.3 Odds Ratios (95% Confidence Intervals) of the association between livestock-related risk factors and zoonotic enteropathogen infection in children 6-59 months old in Greater Accra Region, Ghana. ORs and 95% CI are derived from adjusted logistic regression models shown in Table 3.5. ◆ with no CI indicates that the logistic model could not run due to complete separation. aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; *C. jejuni/coli*, *Campylobacter jejuni* or *Campylobacter coli;* STEC, Shiga toxin-producing *E. coli.*

Table 3.1 Child and household-level characteristics of study sample in Greater Accra Region Ghana, October-November 2018 (n=259)[1]

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]

[1]Abbreviations: SD, standard deviation; IQR, interquartile range.

Table 3.2 Livestock management and household hygiene characteristics among livestock-owning and non-livestock-owning households in Greater Accra, Ghana[1]

Indicator	N	Overall	Livestock-owning households	Non-livestock-owning households
Livestock management[2]				
Livestock free-roam in the yard during the day				
Chickens	157	N/A	141 (89.8%)	N/A
Goats	39	N/A	27 (69.2%)	N/A
Sheep	15	N/A	10 (66.7%)	N/A
Cattle	13	N/A	1 (7.7%)	N/A
Livestock are confined in a pen/corral/coop at night				
Chickens	157	N/A	107 (68.2%)	N/A
Goats	39	N/A	21 (53.9%)	N/A
Sheep	15	N/A	10 (66.7%)	N/A
Cattle	13	N/A	12 (92.3%)	N/A
Livestock observations during the interview[3]				
Livestock observed entering the household living quarters	253	127 (50.2%)	**92 (59.0%)**	**35 (36.1%)*** **
Chickens observed roaming in the yard	254	227 (89.4%)	**153 (97.5%)**	**74 (76.3%)*** **
Animal feces observed in the yard	256	214 (83.6%)	**148 (93.7%)**	**66 (67.4%)*** **
Household hygiene characteristics and behaviors				
Human feces observed in yard[3]	256	13 (5.1%)	10 (6.3%)	3 (3.1%)
Rubbish observed in yard[3]	257	112 (43.6%)	**78 (49.1%)**	**34 (34.7%)***
Gray (waste) water observed in yard[3]	255	30 (11.8%)	18 (11.4%)	12 (12.4%)
Primary caregiver wearing shoes[3]	253	185 (73.1%)	109 (70.3%)	76 (77.6%)
Child wearing shoes[3]	213	86 (40.4%)	52 (40.9%)	34 (39.5%)
Household cooking location[2]	259			
Inside the house or a separate building		112 (43.2%)	67 (42.1%)	45 (45.0%)
Outdoors		147 (56.8%)	92 (57.9%)	55 (55.0%)
Where index child spends time during the day[2]	259			
Inside household dwelling		22 (8.5%)	10 (6.3%)	12 (12.0%)
In the yard		127 (49.0%)	80 (50.3%)	47 (47.0%)
At daycare, nursery, or school		65 (25.1%)	42 (26.4%)	23 (23.0%)
Other location[4]		45 (17.4%)	27 (17.0%)	18 (18.0%)

[1]Values are n (%). Chi-squared test used to compare proportions between livestock-owning and non-livestock-owning households. *p<0.05, **p<0.01, ***p<0.001
[2]Answers based on caregiver-reported responses to questions during the interview.
[3]Answers based on enumerator observations during and after the interview.
[4]Other locations include: at another household (close to the index household), at another household (away from the index household, in the same community), with the mother/other household member (around in the community), with the mother/other household member (outside the community), in the field/farming (with mother/household member)

Table 3.3 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[1]

Pathogen	Children (n=259)	Chickens (n=156)	Child and chicken pairs[2]
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

[1]Values are n (%). Ct cut-off for enteropathogen detection in children's stool is Ct≤35 and in chicken's stool is Ct≤40.
[2]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, the sampled child and chicken both tested positive for aEPEC).
Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; STEC, Shiga toxin-producing *E. coli*.

Table 3.4 Descriptive child and household characteristics by child enteropathogen infection and associated p-values[1]

		C. jejuni/coli			aEPEC			STEC		
Characteristics	**N**	**Yes**	**No**	**p-value**	**Yes**	**No**	**p-value**	**Yes**	**No**	**p-value**
Child age (months), mean (SD)	259	21.7 (10.8)	28.3 (14.1)	**0.013**	27.3 (14.3)	27.7 (13.6)	0.805	36.4 (16.4)	27.2 (13.7)	**0.030**
Child sex, %										
Female	125	12.0	88.0	0.839	44.0	56.0	0.626	4.0	96.0	0.849
Male	134	11.2	88.8		47.0	53.0		4.5	95.5	
Household size, mean (SD)	259	4.7 (1.6)	5.2 (2.2)	0.182	5.0 (1.7)	5.3 (2.4)	0.197	5.5 (2.5)	5.2 (2.1)	0.667
Caregiver highest-attained education, %										
Primary, nursery, or no education	119	10.9	89.1	0.760	43.7	56.3	0.579	6.7	93.3	0.069
Junior or higher	140	12.1	87.9		47.1	52.9		2.1	97.9	
Household improved drinking water source				0.332			0.092			**0.001**
No	7	0.0	100.0		14.3	85.7		28.6	71.4	
Yes	252	11.9	88.1		46.4	53.6		3.6	96.4	
Household latrine facility, %										
Pit latrine or flush toilet	180	11.7	88.3	0.949	43.3	56.7	0.277	1.7	98.3	**0.002**
No toilet/open defecation	79	11.4	88.6		50.6	49.4		10.1	89.9	
District										
Ga East	87	10.3	89.7	0.658	56.3	43.7	**0.013**	0.0	100.0	**0.016**
Shai Osudoku	172	12.2	87.8		40.1	59.9		6.4	93.6	
Asset-based wealth quintile										
Lowest	51	17.7	82.4	**0.049**	41.2	58.8	0.951	2.0	98.0	0.528
Low	52	5.8	94.2		46.1	53.9		3.9	96.2	
Middle	52	19.2	80.8		55.8	44.2		5.8	94.2	
High	52	3.9	96.2		48.1	51.9		1.9	98.1	
Highest	52	11.5	88.5		48.1	51.9		7.7	92.3	

[1]P-values calculated from two-sided t-tests for continuous variables and chi-square tests for categorical variables. Abbreviations: atypical enteropathogenic *E. coli*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; STEC, Shiga toxin-producing *E. coli*.

Table 3.5 Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (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)[1]

Risk factors	*C. jejuni/coli*		aEPEC		STEC	
	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 ≥1 time/week[2]	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 ≥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)

[1]Values are OR (95% CI) using logistic regression models. Adjusted logistic regression models control for child sex, child age (months), household use of improved drinking water source (aEPEC and STEC only), 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. **p<0.05, **p<0.01, ***p<0.001* Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli*; *C. jejuni/coli*, *Campylobacter jejuni* or *Campylobacter coli*; STEC, Shiga toxin-producing *E. coli*.

[2]Adjusted for covariates above and cattle and goat or sheep ownership in aEPEC and STEC models.

Table 3.6 Full adjusted logistic regression models predicting odds of enteropathogen detection in children 6-59 months old by livestock-associated risk factors[1]

	Cattle ownership (n=259)	Goat or sheep ownership (n=259)	Poultry ownership, with other livestock (n=259)	Poultry ownership only (n=259)	Pathogen detected in chicken stool (n=156)	Animal feces observed around household (n=256)	Fresh cow's milk consumed ≥1 time/week (n=259)	Chicken meat consumed ≥1 time/week (n=259)
Models predicting odds of C. jejuni/coli detection[2]								
Livestock-associated risk factor	-	1.59 (0.59, 4.29)	1.58 (0.67, 3.73)	0.63 (0.31, 1.26)	0.73 (0.27, 1.96)	0.51 (0.16, 1.65)	1.10 (0.41, 2.94)	1.03 (0.40, 2.66)
Child sex (ref: male)		1.04 (0.53, 2.04)	1.05 (0.53, 2.06)	0.99 (0.49, 2.02)	1.13 (0.54, 2.38)	1.01 (0.49, 2.06)	1.03 (0.51, 2.05)	1.02 (0.52, 2.01)
Child age (months)		**0.95* (0.91, 0.99)**	**0.95* (0.91, 1.00)**	**0.96* (0.92, 1.00)**	0.93 (0.87, 1.00)	0.96 (0.92, 1.00)	0.95 (0.91, 1.00)	0.95 (0.91, 1.00)
Household practices open defecation (ref: no)		0.90 (0.25, 3.27)	0.89 (0.24, 3.23)	1.00 (0.26, 3.82)	0.53 (0.10, 2.77)	0.99 (0.27, 3.68)	0.95 (0.25, 3.59)	0.96 (0.27, 3.35)
Caregiver primary or no education (ref: junior or higher)		0.90 (0.39, 2.07)	0.90 (0.39, 2.08)	0.87 (0.37, 2.03)	1.02 (0.28, 3.74)	0.87 (0.40, 1.90)	0.90 (0.39, 2.07)	0.90 (0.38, 2.16)
District (ref: Ga East)		1.82 (0.65, 5.07)	1.80 (0.64, 5.02)	1.85 (0.66, 5.19)	1.93 (0.50, 7.44)	2.03 (0.67, 6.16)	1.79 (0.63, 5.05)	1.80 (0.63, 5.13)
Models predicting odds of aEPEC detection								
Livestock-associated risk factor	0.63 (0.23, 1.72)	0.89 (0.45, 1.76)	1.00 (0.54, 1.84)	1.37 (0.81, 2.32)	1.36 (0.76, 2.41)	0.71 (0.34, 1.50)	0.55 (0.17, 1.80)	1.17 (0.67, 2.05)
Child sex (ref: male)	0.92 (0.59, 1.42)	0.91 (0.59, 1.41)	0.92 (0.59, 1.42)	0.93 (0.60, 1.46)	0.87 (0.40, 1.88)	0.88 (0.55, 1.41)	0.90 (0.59, 1.38)	0.89 (0.57, 1.40)
Child age (months)	1.01 (0.99, 1.02)	1.01 (.99, 1.03)	1.01 (0.99, 1.02)	1.01 (0.99, 1.03)	1.00 (0.98, 1.03)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.00 (0.98, 1.03)
Household uses unimproved drinking water source (ref: no)	**0.20*** (0.11, 0.38)**	**0.20*** (0.10, 0.40)**	**0.20*** (0.10, 0.37)**	**0.20*** (0.11, 0.39)**	**0.35* (0.15, 0.80)**	**0.20*** (0.11, 0.37)**	**0.22*** (0.11, 0.44)**	**0.19*** (0.10, 0.37)**
Household practices open defecation (ref: no)	**1.99* (1.16, 3.41)**	**1.98* (1.16, 3.38)**	**1.96* (1.14, 3.37)**	**1.93* (1.13, 3.30)**	**1.77* (1.01, 3.10)**	**1.94* (1.13, 3.34)**	**2.12** (1.33, 3.36)**	**2.01* (1.15, 3.51)**
Caregiver primary or no education (ref: junior or higher)	0.90 (0.61, 1.32)	0.90 (0.61, 1.32)	0.90 (0.61, 1.31)	0.91 (0.62, 1.34)	0.83 (0.45, 1.52)	0.90 (0.59, 1.38)	0.91 (0.63, 1.32)	0.91 (0.61, 1.35)
District (ref: Ga East)	**0.43** (0.23, 0.81)**	**0.42** (0.22, 0.78)**	**0.42** (0.23, 0.78)**	**0.41** (0.22, 0.76)**	**0.41* (0.18, 0.89)**	**0.46** (0.26, 0.82)**	**0.44* (0.23, 0.83)**	**0.42** (0.23, 0.78)**
Cattle ownership (ref: no)							0.70 (0.21, 2.37)	
Goat or sheep ownership (ref: no)							0.97 (0.47, 1.98)	
Models predicting odds of STEC detection[3]								

Livestock-associated risk factor	4.32 (0.74, 25.10)	**4.30* (1.32, 14.08)**	**6.31* (1.27, 31.22)**	0.57 (0.09, 3.38)	**7.85*** (2.54, 24.30)**	-	**3.03*** (1.75, 5.24)**	1.60 (0.84, 3.03)
Child sex (ref: male)	1.04 (0.33, 3.28)	1.22 (0.47, 3.19)	1.28 (0.50, 3.32)	1.10 (0.40, 3.07)	1.28 (0.39, 4.14)		1.28 (0.44, 3.69)	1.11 (0.42, 2.95)
Child age (months)	1.04 (0.99, 1.09)	1.03 (0.98, 1.08)	1.03 (0.98, 1.08)	1.03 (0.98, 1.08)	**1.06* (1.00, 1.12)**		1.03 (0.97, 1.09)	1.03 (0.97, 1.08)
Household uses unimproved drinking water source (ref: no)	**5.09** (1.87, 13.89)**	**5.20** (1.87, 14.44)**	**6.98*** (2.68, 18.22)**	**5.77** (1.90, 17.49)**	**15.82*** (6.58, 38.05)**		**4.30** (1.50, 12.34)**	**5.78** (1.80, 18.56)**
Household practices open defecation (ref: no)	**3.99** (1.77, 9.00)**	**4.19** (1.54, 11.38)**	**3.85** (1.47, 10.07)**	**4.66** (1.71, 12.72)**	**6.90** (2.25, 21.15)**		**3.10* (1.31, 7.32)**	**5.01*** (2.14, 11.76)**
Caregiver primary or no education (ref: junior or higher)	2.20 (0.81, 5.99)	2.35 (0.93, 5.97)	2.26 (0.83, 6.13)	2.06 (0.66, 6.42)	3.46 (0.66, 18.12)		2.18 (0.84, 5.64)	2.20 (0.61, 7.92)
Cattle ownership (ref: no)							1.34 (0.15, 11.62)	
Goat or sheep ownership (ref: no)							**3.71* (1.26, 10.89)**	

[1] Values are adjusted odds ratios (OR) and 95% confidence intervals (CI) using logistic regression models controlling for listed covariates. **p<0.05, **p<0.01, ***p<0.001.* aEPEC, atypical enteropathogenic *Escherichia coli; C. jejuni/coli*, *Campylobacter jejuni* or *Campylobacter coli;* STEC, Shiga toxin-producing *E. coli*

[2] Cattle ownership logistic model did not run due to perfect prediction of no *C. jejuni/coli* detection. Improved drinking water source not included as a covariate in the models because of perfect prediction of no *C. jejuni/coli* detection. *C. jejuni/coli*, *Campylobacter jejuni* or *Campylobacter coli.*

[3] Observation of animal feces around household logistic model did not run due to perfect prediction of STEC detection. District was not included as a covariate in the models because of perfect prediction of STEC detection in the Shai Osudoku district.

CHAPTER 4 Bacterial Enteropathogens are Differentially Associated with Systemic Inflammation, Iron Status, and Anemia in Preschool-Age Children in Southern Ghana

Abstract

Anemia remains a pervasive public health problem among preschool-age children in Ghana. Enteric pathogen infections may contribute to anemia by reducing nutrient absorption or inducing systemic inflammation that alters iron homeostasis. The aim of this cross-sectional study was to investigate the associations between bacterial enteropathogen infections and inflammation, iron deficiency, and anemia in children 6-59 months old in Greater Accra, Ghana. Stool samples from 262 children were analyzed for bacterial enteropathogens using qPCR. Logistic regression was used to estimate associations between detection of each enteropathogen and two systemic inflammation biomarkers (elevated C-reactive protein (CRP) and α-1-acid glycoprotein (AGP)), two iron deficiency biomarkers (low serum ferritin (SF) and high serum transferrin receptor (sTfR)), and anemia. Enteropathogens were detected in 87.0% of children's stool and were predominantly subclinical. *Campylobacter jejuni/coli* was significantly associated with elevated CRP [Odds Ratio (95% CI): 3.49 (1.45, 8.41)] and elevated AGP [4.27 (1.85, 9.84)]. Enteroaggregative *Escherichia coli* (EAEC) infection was associated with higher odds of anemia [1.69 (1.01, 2.84)]. Enteroinvasive *E. coli/Shigella* spp. (EIEC/*Shigella*) infection was also associated with anemia [2.34 (1.15, 4.76)], as well as low SF [2.55 (1.23, 5.29)]. Subclinical enteropathogen infections, particularly involving enteroinvasive bacteria, appear to contribute to

anemia in young children. Further research is needed to understand the mechanisms by which each of these bacteria may contribute to anemia in children.

Introduction

Despite a 26-percentage point decrease in the prevalence of anemia among children 6 to 59 months of age in Ghana during the two decades from 1996 to 2016 (from 81% to 67%), the condition remains an important public health concern (1). In Ghana's Greater Accra Region, anemia affected 60% of preschool-age children in 2014, while other administrative regions had anemia burdens up to 82% (2). Anemia is a condition in which there is inadequate oxygen delivery to tissues due to insufficient red blood cells or functional hemoglobin. Anemic children are at risk of impaired cognitive and motor development, as well as increased mortality (3–5). A variety of factors can lead to anemia including inadequate intake or absorption of hematopoietic nutrients (e.g., iron, vitamin B12, folic acid, vitamin A), genetic hemoglobin disorders, and infectious diseases (e.g., malaria, helminthiasis) (6,7). While iron-deficiency is the primary cause of anemia in preschool-age children globally (8), the proportion of anemia attributable to iron deficiency varies by contextual factors, including infectious disease burdens (9). Analysis of the 2017 Ghana Micronutrient Survey found that 35% of total anemia in 6-59-month-old children was associated with iron deficiency (10). In the Southern Belt of Ghana (including the Greater Accra, Central, Volta, and Western Regions), one-fifth of child anemia was attributable to malaria parasitemia, one-third to inflammation, and one-fifth to fever (11). Almost half of Ghanaian children presented with some level of inflammation (10). Infectious diseases that commonly contribute to anemia among Ghanaian children include malaria and helminthiasis (12). Some evidence also suggests that poor water and sanitation conditions, which can expose children to bacterial enteric infections, may contribute to anemia in low-resource contexts

(13,14), though little is known about the associations of specific bacterial enteropathogens and anemia.

Recurrent infection with a range of protozoal, viral, and bacterial enteric pathogens is common among young children in low- and middle-income countries (LMIC) (15,16). The MAL-ED (Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health) multi-site birth cohort study of children under two years old across eight sites in three continents found at least one enteropathogen in 77% of diarrheal stool samples (16). Furthermore, 65% of non-diarrheal stool samples also had at last one enteropathogen, indicating that the burden of enteric infections is substantial even among asymptomatic children (16). Other studies have linked subclinical carriage of enteric pathogens to linear growth faltering and delays in cognitive development, which are posited to occur, in part, through a condition of gastrointestinal inflammation known as environmental enteric dysfunction (EED) (17–22). EED is characterized by blunted gut villi, increased intestinal permeability, nutrient malabsorption, and increased intestinal and systemic inflammation (23,24). Recent studies from the MAL-ED cohort have found that asymptomatic enteropathogen detection (25) and intestinal permeability and inflammation (26) in the first two years of life are associated with lower hemoglobin concentrations and higher anemia risk, respectively. Additional observational studies have found negative associations between EED biomarkers and iron status and hemoglobin concentrations in infants (27–29).

There are several mechanisms by which repeated exposure to bacterial enteric infections can lead to iron deficiency and anemia. First, morphological changes of the intestinal villi characteristic of EED may result in reduced absorption of iron and other hematopoietic nutrients (23,24). Second, enteropathogen infection may promote a systemic inflammatory response which

can alter iron homeostasis (30). In response to increased inflammatory cytokines, hepcidin-mediated signaling induces iron sequestration and reduces intestinal iron absorption, effectively starving the pathogen of iron at the cost of body iron homeostasis (7,31,32). Over time, this "functional iron deficiency" – in which the body has sufficient iron stores but iron is not available to tissues – can lead to reduced erythropoiesis and anemia (33). The evidence to date suggests that EED plays a role in iron deficiency and anemia. However, aside from one study in which authors measured the average detection rate of parasitic, viral, and bacterial pathogens in stool (25), it is not known whether bacterial carriage in asymptomatic children affects iron status and anemia, nor whether there are pathogen-specific effects on iron status and anemia.

Diet may also play a role in susceptibility to infection, as nutrients including vitamins A and D, zinc, and amino acids are important in immune function and for maintaining the gut mucosal barrier (34–36). In children under two years old, greater nutrient intake from complementary foods was associated with lower rates of enteropathogen infection and illness, as well as reduced gut permeability and inflammation (25,26). On the other hand, iron supplementation has been shown to increase intestinal inflammation, adversely affect the gut microbiome, and increase pathogen proliferation and survival (37,38), all of which presents risks in using iron supplementation to treat anemia (39).

In this study, we assessed the associations between bacterial enteropathogen infections and elevated inflammation, iron deficiency, and anemia in children 6-59 months old in Greater Accra, Ghana. A secondary aim was to determine whether children's diet quality, proxied by dietary diversity, modifies the associations between enteropathogens and anemia. We hypothesized that detection of enteropathogens would be associated with higher odds of systemic inflammation, iron deficiency, and anemia. We did not have *a priori* predictions about the

independent effect of bacterial pathogens on iron status and anemia, and thus analyzed each pathogen with all the outcomes of interest. We further hypothesized that the associations between enteropathogens and anemia would be attenuated in children with higher dietary diversity.

Methods

Study design

This cross-sectional study of preschool-age children was conducted in eighteen communities in the Ga East and Shai Osudoku Districts of the Greater Accra Region, Ghana, during October through November 2018. To be included in the study, children had to be 6-59 months old and their primary caregiver had to be at least 18 years old. Further details on study site selection and participant recruitment are included in Chapter 2.

A subsample of 265 children from the sample of 484 children described in Chapter 2 was selected for inclusion in this study based on financial constraints to conduct enteropathogen analysis. Methods for selecting the subsample are described in Chapter 3.

Survey data collection

Enumerators conducted interviews with each index child's primary caregiver, usually the mother, using electronic tablets (Samsung Galaxy Tab A, Model Number SM-T285) with the Qualtrics survey platform. Data were collected on the household's sociodemographic characteristics and the index child's age, health, and diet. The child's date of birth was verified using their clinical Child Health Records booklet. The caregiver was asked whether the child experienced any symptoms of illness in the preceding seven days (e.g., diarrhea, fever, illness with a cough, vomiting, nausea) and whether the child had received health services such as intestinal worm treatment in the past six months. The caregiver was also asked about the child's

consumption of specific liquids (e.g., breastmilk, animal milk, juice) and solid foods (e.g., green leafy vegetables, beef, eggs) in the preceding day and night (40).

Blood sample collection and analysis

Child capillary blood samples were collected using a finger-prick (41). After wiping away the first drop of blood, hemoglobin concentration (Hb) was measured using a HemoCue® Hb 201+ portable hemoglobinometer (HemoCue AB, Sweden) and malaria parasitemia was measured using the SD Malaria Ag P.f (HRP2/pLDH) antigen rapid diagnostic test (RDT) (Standard Diagnostics Inc., Korea). Approximately 200µL of additional blood was collected in a Microvette® clotting activator/serum tube (Sarstedt, Germany). Blood samples were spun in a microcentrifuge at the Noguchi Memorial Institute for Medical Research in Accra, Ghana, and the serum was stored at -20°C until shipment to the VitMin Laboratory (Willstaett, Germany) for biomarker analysis. Serum samples were analyzed for serum ferritin (SF), serum transferrin receptor (sTfR), retinol-binding protein (RBP), C-reactive protein (CRP), and α-1-acid glycoprotein (AGP) using a sandwich enzyme-linked immunosorbent assay (ELISA) (42). SF and sTfR concentrations were adjusted for inflammation (CRP and AGP) using the regression correction approach developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project (43). Biomarker cut-offs for iron deficiency (SF and sTfR) and inflammation (CRP and AGP) were defined per the BRINDA project cut-offs for preschool-age children: inflammation-adjusted SF<12 µg/L, inflammation-adjusted sTfR>8.3 mg/L, CRP>5 mg/L, and AGP>1 g/L (43).

Stool sample collection and analysis

Caregivers were instructed to collect their child's morning stool in a sterile fecal container (Sarstedt, Germany) and store it in a shady spot until collected by the field team.

Caregivers of children under 24 months old were provided with a diaper and those with older children a clean piece of paper on which the child should defecate to prevent environmental cross-contamination. Upon collection, stool samples were placed on wet ice in a cooler box and then transported to the Noguchi Memorial Institute for Medical Research for processing. Stool samples were analyzed for soil-transmitted helminth infections (i.e., *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, *Necator americanus*) using Kato Katz methods (44). Following duplicate thick smear preparation of each stool sample, two laboratory technicians independently analyzed the slides by microscopy for helminth eggs. For enteropathogen analysis, unprocessed stool samples were aliquoted into 2mL cryovials using sterile techniques and stored at -80°C until further processing.

Microbial nucleic acid was extracted from stool samples using the QIAamp® PowerFecal® DNA Kit (Qiagen, Hilden, Germany). Following 3-5 minutes of thawing, ~250mg of stool was weighed and placed in a bead beating tube under a sterile hood. All extraction steps were then followed according to the kit protocols. In the final extraction step, 100µL of DNA was eluted into an Eppendorf™ DNA LoBind microcentrifuge tube (Eppendorf, Hamburg, Germany). Each batch of nucleic acid extractions included an extraction blank to control for laboratory contamination, which went through all the extraction steps but without any addition of stool sample. Total DNA concentration and purity were measured using a NanoDrop™ 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). DNA samples were transported to the University of Michigan (Ann Arbor, MI, USA) and stored at -80°C until further analysis.

Probe-based quantitative polymerase chain reaction (qPCR) was used to analyze DNA samples for the following enteropathogens: *Campylobacter jejuni*/*Campylobacter coli* (*C.*

jejuni/coli), enteroaggregative *Escherichia coli* (EAEC), atypical enteropathogenic *E. coli* (aEPEC), typical enteropathogenic *E. coli* (tEPEC), heat-stable enterotoxin-producing *E. coli* (ST-ETEC), heat-labile enterotoxin-producing *E. coli* (LT-ETEC), *Salmonella enterica*, Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli/Shigella* species (EIEC/*Shigella*), and *Vibrio cholerae* (*V. cholerae*). Primer and probe sequences to identify pathogens were derived from Liu et al. (45) and Taniuchi et al. (46) and validated for specificity using the NIH Nucleotide Basic Local Alignment Search Tool (BLASTn) program (**Appendix 1**).

DNA samples and extraction blanks were run single-plex for each gene target on 384-well plates using the QuantStudio™ 5 System (Applied Biosystems™, Foster City, CA, USA). DNA samples were diluted 1:10 in ddH_2O. Samples were run in quadruplicate (for *cadF*, *ipaH*, *bfpA*, *eae*, *ttr*) or triplicate (to save on reagents after determining sufficient precision between technical replicates), and each plate included a water control (water in place of DNA in the amplification well). Each amplification well contained 4.5µL 1:10-diluted DNA sample, 5.0µL TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Foster City, CA, USA), and 0.5µL of primer-probe mixture at a final concentration of 500:250nM 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). 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 min. For the *cadF* gene, annealing and extension were at 58°C for 1 min. Cycle threshold (Ct), which is inversely associated with pathogen load, was determined for each sample replicate using the Thermo Fisher Connect Platform (Thermo Fisher Scientific, Carlsbad, CA, USA). The threshold line for each gene was manually set to ensure comparability between plates for each gene target by including a repeated

positive sample on each plate. Technical replicates were inspected if the standard deviation between replicates was ≥0.5, and when warranted, individual technical replicates were manually removed if there were outliers. For each target gene, the sample Ct value was calculated as the average of amplified replicates. Samples that had amplification of ≤50% of replicates were classified as negative for that gene. None of the extraction blanks or water controls amplified. A Ct cut-off of ≤35 was applied to define positive detection of a gene. This cut-off was chosen as the lower bound at which greater variability in Ct between technical replicates and incongruent amplification between replicates was observed, indicating the technical limits of the assay's precision. We also examined gene detection by high- and low-relative quantity, which we defined as below the median Ct (high quantity) or above the median Ct (low quantity) for each gene, excluding samples with Ct>35.

PCR efficiency was calculated for each target gene by pooling at minimum four known positive samples and running eight four-fold dilutions of the pooled sample with four technical replicates at each dilution. Linear regression of the average Ct against the log10-dilution was used to estimate the slope ($\beta \pm$ 95% confidence interval) and R^2 of the standard curve. The PCR efficiency value was calculated from the slope of the standard curve. PCR efficiencies ranged from 87.3% to 112.8% (Appendix 1).

Positivity for enteric pathogens were defined as follows: tEPEC (*eae* with *bfpA*, and without *stx1* or *stx2*), aEPEC (*eae* without either *bfpA*, *stx1*, or *stx2*), STEC (*eae* with either *stx1* or *stx2*, or both, and without *bfpA*), EAEC (*aatA* and/or *aaiC*), ST-ETEC (*STh* and/or *STp*, with or without *LT*), LT-ETEC (*LT*, without either *STh* or *STp*), EIEC/*Shigella* (*ipaH*), *Salmonella* (*ttr*), *C. jejuni/coli* (*cadF*), *V. cholerae* (*hlyA*) (**Appendix 4**). We additionally assessed infections in groups by whether children were infected with enteroinvasive (EIEC/*Shigella, C. jejuni/coli*,

or *Salmonella*) or potentially enteroinvasive bacteria (EAEC or STEC), non-enteroinvasive, surface adherent bacteria (tEPEC, aEPEC, ST-ETEC, LT-ETEC), or co-infected with both enteroinvasive and non-enteroinvasive bacteria.

Diet measures and analysis

Each child's reported food consumption during the preceding day and night was defined dichotomously (any, none) for seven food groups: (i) grains, roots, and tubers; (ii) legumes and nuts; (iii) dairy products; (iv) flesh foods (meat, fish, poultry, and organ meats); (v) eggs; (vi) vitamin-A rich fruits and vegetables; and (vii) other fruits and vegetables (40). Breastmilk was included as an eighth food group for children under 24 months old per the updated 2017 World Health Organization (WHO) guidelines (47). The food groups were summed to create a continuous dietary diversity indicator, ranging from 0 to 8 for children 6-23 months old and 0 to 7 for children 24-59 months old. We created a binary variable for the minimum dietary diversity (MDD) indicator for children, defined as consuming ≥5 out of 8 food groups for children 6-23 months old and ≥4 out of 7 food groups for children 24-59 months old. MDD was calculated for children 24-59 months old using the 2010 WHO MDD indicator that does not include breastmilk as a food group. The 2010 MDD is validated for children 6-23 months old as an indicator of micronutrient adequacy (48), with validation studies for the updated 2017 MDD underway.

Statistical analysis

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

Descriptive statistics were calculated for child- and household-level characteristics and the child's health status including micronutrient deficiency, inflammation, and indications of

illness. Correlations between Hb, log-transformed SF and sTfR, and log-transformed CRP and AGP concentrations were examined to assess associations between the outcomes of interest. We assessed the overall prevalence of each pathogen in children's stool, the detection of any pathogen and the total number of pathogens detected per child. To determine whether children had symptomatic infections, we used logistic regression to model the association between enteropathogen presence and diarrhea symptoms in the past seven days, adjusting for child age and sex. We also analyzed associations of enteropathogen presence with fever, cough or cold, nausea, and vomiting in the past seven days.

Unadjusted and adjusted logistic regression were used to model the associations between the presence/absence of each enteropathogen and elevated inflammation, iron deficiency, and anemia. Anemia was defined as Hb<11.0g/dL, per the WHO recommendations (49). Adjusted models included the child's sex and age in months as covariates, chosen *a priori* as potential confounders. Malaria parasitemia and breastfeeding status were considered for inclusion as confounders, but were not included in the final models since the inclusion of these variables did not meaningfully change effect estimates and because breastfeeding was strongly correlated with child age. To examine effects by relative enteropathogen quantity, we ran adjusted models predicting each outcome by "high" and "low" gene detection. Additionally, adjusted logistic regression was used to evaluate outcomes comparing infection with one or more enteroinvasive pathogens or co-infection with enteroinvasive and non-invasive pathogens relative to infection with one or more non-invasive pathogens, excluding children with no detected infections. To examine effect modification by the children's diet quality, we used adjusted logistic regression to model the associations between dichotomous enteropathogen detection and child anemia, stratified by children who met and did not meet the MDD score. As a sensitivity analysis, we

also used adjusted linear regression models of the associations between enteropathogens, as dichotomous variables and as pathogen groupings, and the outcomes of interest as continuous variables (i.e., CRP, AGP, SF, sTfR, and Hb concentrations).

Ethical considerations

This study was approved by the University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board (protocol no. HUM00145171) and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18). At each household, after explaining the study procedures, risks, and benefits, written informed consent was provided by the index child's caregiver for their child's participation in the study and their participation in the interview with a signature or thumbprint. Informed consent was conducted in the presence of a witness if the caregiver had low literacy. Households were given a small, non-monetary gift as compensation for their participation. Children with positive malaria detection or anemia were advised to visit their nearest health clinic to confirm the test result and receive professional care.

Results

Of the 265 children's stool samples that were analyzed for enteropathogens by qPCR, one sample with missing data on age and two samples lacking iron status biomarker data were excluded, for a final analytic sample of 262 children. On average, children were 28 months old, with 47.1% of children between 6-23 months old and 52.9% between 24-59 months old (**Table 4.1**). Most households had access to an improved drinking water source, but access to adequate sanitation facilities varied, with almost one-third of households practicing open defecation, and less than 3% with a handwashing facility. Overall, 45.8% of children were anemic, of whom 46.7% had mild anemia, 50.0% had moderate anemia, and 3.3% had severe anemia (**Table 4.2**).

One quarter of children had iron deficiency, as defined by low SF concentrations. Sixteen percent of children had elevated CRP concentrations while 37.8% had elevated AGP concentrations. No children had a detectable helminth infection, but 8.4% were positive for malaria.

Biomarkers of iron status and inflammation were significantly correlated with hemoglobin concentration in the expected directions (**Table 4.3**). The correlations between the iron biomarkers, SF and sTfR, and between the inflammatory biomarkers, CRP and AGP, were positive and strong ($p<0.001$). SF showed moderate, positive correlations with CRP and AGP ($p<0.001$) before adjusting for inflammation, but not after, indicating that the BRINDA regression correction approach corrected artificially-elevated SF concentrations. sTfR was weakly associated with AGP before adjusting for inflammation ($p=0.026$), but not CRP, and was not correlated with either inflammatory biomarker after the BRINDA inflammation adjustment.

Enteropathogen detection in children

Gene target detection and associated Ct value parameters are shown in **Appendix 2**. Enteropathogens were detected in 87.0% of children's stool (**Table 4.4**). One-third of children were infected with one pathogen, one-third with two pathogens, and in one-fifth of children, three or four pathogens were detected. EAEC and aEPEC were most common, detected in 59.2% and 46.2% of children's stool, respectively. Among the other pathogens assessed, 15.3% of stool samples were positive for EIEC/*Shigella*, 11.1% for *C. jejuni/coli*, 13.4% for LT-ETEC, 7.6% for ST-ETEC, 6.5% for tEPEC, and 4.2% for STEC. *Salmonella* was rare (in only two children), and *V. cholerae* was not detected. Enteropathogens were found across all sampled age ranges (**Figure 4.1**). EAEC was more prevalent among children 6-12 months old than older children, while STEC was more prevalent among children 4-5 years old than the younger age ranges.

Associations between enteropathogen detection and indicators of morbidity and inflammation

Reported diarrhea prevalence in the preceding seven days was 6.5%, which is similar to the two-week reported diarrheal prevalence of 7.3% among children under five years old in the Greater Accra Region reported in the 2014 Ghana Demographic and Health Survey (2). Watery diarrhea was reported among 29.4% of 17 children with diarrhea, and no caregivers reported bloody diarrhea. In bivariate analysis, EAEC detection was marginally associated with diarrhea (Odds Ratio (OR): 3.44, 95% Confidence Interval (CI): 0.96, 12.29); p=0.057). After adjusting for child age and sex, only STEC infection was associated with diarrhea (OR: 5.78, 95% CI: 1.02, 32.64; p=0.047) (**Table 4.5**). No other morbidity symptoms (fever, cough or cold, nausea, or vomiting) were associated with enteropathogen detection.

C. jejuni/coli infection was associated with 3.49 times higher odds of elevated CRP concentrations (95% CI: 1.45, 8.41; p=0.005) and 4.27 times higher odds of elevated AGP concentrations (95% CI: 1.85, 9.84; p=0.001), controlling for child age and sex (**Table 4.6, Figure 4.2**). Detection of the *C. jejuni/coli cadF* gene was associated with elevated inflammation at both high and low relative quantities (**Table 4.7**). High relative quantity of the EIEC/*Shigella ipaH* gene was associated with 3.24 times higher odds of elevated CRP (95% CI: 1.18, 8.89; p=0.023) and marginally associated with higher odds of elevated AGP (OR: 2.36, 95% CI: 0.91, 6.15; p=0.079). Furthermore, detection of the EAEC virulence trait *aaiC*, but not *aatA*, was associated with 2.07 times higher odds of elevated AGP (95% CI: 1.02, 4.19; p=0.044) at high relative quantity. Infection with enteroinvasive bacteria (*C. jejuni/coli*, EIEC/*Shigella*, *Salmonella*, EAEC, or STEC) was associated with higher odds of elevated AGP (OR: 3.76, 95% CI: 1.65, 8.56; p=0.002), but not CRP, compared to infection with non-enteroinvasive bacteria

(**Table 4.8**). Detection of aEPEC in stool was associated with lower odds of elevated CRP and AGP.

Associations between enteropathogen detection and iron status and anemia

An association between enteropathogen infection and iron deficiency was observed only for EIEC/*Shigella* (**Table 4.9**). Children with EIEC/*Shigella* had 2.55 times higher odds of low SF concentrations (95% CI: 1.23, 5.29; p=0.012). Interestingly, the association with SF was observed at low relative quantity of the EIEC/*Shigella ipaH* gene (OR: 2.97, 95% CI: 1.15, 7.69; p=0.025) but not at high relative quantity (**Table 4.7**). Neither detection of EIEC/*Shigella* nor other individual enteropathogens was associated with elevated sTfR concentrations. However, infection with one or more enteroinvasive pathogens as a group was associated with higher odds of elevated sTfR concentrations (OR: 2.22, 95% CI: 1.03, 4.80, p=0.043) compared to infection with non-enteroinvasive pathogens (**Table 4.8**).

EAEC (OR: 1.69, 95% CI: 1.01, 2.84; p=0.047) and EIEC/*Shigella* (OR: 2.34, 95% CI: 1.15, 4.76; p=0.019) infections were associated with higher odds of anemia in adjusted analyses (**Table 4.9**). In sensitivity analyses, EIEC/*Shigella* was significantly associated with lower Hb concentrations (β=-0.5, 95% CI: -1.0, -0.0; p=0.038) (**Table 4.10**). An association with anemia and EIEC/*Shigella* was found only at low relative quantity of the *ipaH* gene (OR: 2.83, 95% CI: 1.08, 7.45; p=0.035) (**Table 4.7**). The enteroinvasive pathogen group was associated with lower average Hb concentrations (β=-0.9, 95% CI: -1.4, -0.4; p=0.001) compared to the non-invasive pathogen group (**Table 4.11**) as well as 2.85 times higher odds of anemia (95% CI: 1.27, 6.38); p=0.011) (**Table 4.8**) Children with co-detection of enteroinvasive and non-enteroinvasive pathogens also had higher odds of anemia than children with non-invasive pathogen infections only (OR: 2.10, 95% CI: 1.00, 4.40; p=0.049).

Effect modification by dietary quality

Two-thirds of children 24 to 59 months old and one-third of children 6 to 23 months old met the MDD requirement. Sixty-five percent of children under 24 months old had consumed breastmilk in the prior 24 hours. Children who consumed legumes or eggs had the highest dietary diversity scores, on average. Children who did not meet the MDD requirement had significantly higher odds of anemia if they were positive for EAEC or EIEC/*Shigella*, while among those who did meet the MDD requirements, associations with anemia were attenuated and non-significant (**Table 4.12**). These associations were in the same direction and of the same significance values when using the 2010 MDD indicator for all age groups.

Discussion

This cross-sectional study investigated the role of enteropathogen infections in the etiology of anemia among 6-59-month-old children in Greater Accra, Ghana. Anemia affected almost half of children, though less than one-fifth of children had iron-deficiency anemia. Almost forty percent of children had elevated CRP or AGP concentrations, indicative of inflammatory responses to infection. As has been found in other studies investigating the burden of enteropathogens among children in LMIC, most children (87.0%) in this study had at least one bacterial enteropathogen detected in their stool, with EAEC and aEPEC being the most prevalent. Infections were predominantly subclinical as there was low reported diarrhea prevalence (6.5%). Despite no apparent symptoms of infection, detection of *Campylobacter* in stool was strongly associated with systemic inflammation. Higher relative quantities of the EIEC/*Shigella* gene *ipaH* and the EAEC chromosomal gene *aaiC* were also associated with elevated systemic inflammation. EAEC infection was associated with anemia, but not iron deficiency, while EIEC/*Shigella* infection was associated with iron deficiency and anemia, and

children infected with this pathogen had Hb concentrations on average 0.5g/dL lower than children without EIEC/*Shigella*. Furthermore, detection of invasive pathogenic bacteria as a group was associated with higher inflammation and lower Hb concentrations relative to detection of non-enteroinvasive pathogenic bacteria. These findings indicate that subclinical infection with certain enteropathogens may be a risk factor for the development of anemia in young children.

The pathogens that were most associated with systemic inflammation, iron deficiency, and anemia in this study were bacteria that penetrate intestinal epithelial cells, produce tissue destruction, and cause enteric inflammation (50–53). Notably, subclinical infections of these enteropathogens – EAEC, *Campylobacter*, and EIEC/*Shigella* – are also the most deleterious for linear growth faltering (17,20). Systemic inflammation, more so than gut inflammation, appears to be a key mediator in the associations between exposure to intestinal pathogens, particularly invasive bacterial pathogens, and child growth (17,54). This mechanism is likely also responsible for iron dysregulation and anemia among children infected with enteropathogens, as systemic inflammation inhibits absorption of hematopoietic nutrients and induces iron sequestration via upregulation of hepcidin. Indeed, systemic inflammation, but not gut inflammation, has been shown to predict hepcidin concentrations in African infants (30,55). Prentice et al. (2019) found that almost half of Gambian children 6 to 27 months old were physiologically blocking iron absorption, and that CRP concentrations, even at low levels, were correlated with hepcidin concentrations (30). Thus, it is plausible that in our study, the systemic inflammation associated with enteroinvasive pathogen infections produced anemia via elevated hepcidin, though future studies are needed to confirm this as we did not measure hepcidin concentrations in children.

Nevertheless, other studies have identified associations of gut permeability and gut inflammation with iron status and anemia, suggesting a potential alternative pathway via the

chronic inflammatory state characteristic of EED. Lauer et al. (2020) found inverse associations between markers of an immune response to microbial translocation (anti-flagellin IgA, anti-flagellin IgG, and anti-LPS IgA) and hemoglobin concentrations in a cross-sectional study of 6-month-old Ugandan infants (28). In an analysis of Bangladeshi children from the MAL-ED birth cohort, Fahim et al. (2018) reported an association between higher levels of α-1-antitrypsin (AAT) (a marker of intestinal permeability and protein loss) and decreased ferritin values at two years of age, but no associations with myeloperoxidase (MPO) or neopterin (NEO) (markers of intestinal inflammation) (27). McCormick et al. (2019), in a longitudinal analysis of 9- to 15-month-old infants from the MAL-ED birth cohort at all study sites, found a higher risk of low ferritin concentrations with higher concentrations of AAT and NEO, as well as a greater risk of anemia with higher MPO concentrations and a higher lactulose z-score to mannitol z-score ratio (indicative of greater gut dysfunction). Though causal links between EED and anemia have not been elucidated (33), and we did not measure EED biomarkers, it is likely that gut dysfunction and inflammation in response to repeated enteric infections, combined with the systemic inflammatory response to acute infection may mediate associations between enteropathogen infection and anemia. Our findings further suggest that mechanisms may differ by enteropathogen.

Among the invasive pathogens, only EIEC/*Shigella* infection was associated with both systemic inflammation and alterations in iron status and anemia. EIEC/*Shigella* infection at higher relative quantities was associated with elevated inflammation and overall detection of this pathogen was associated with higher odds of low ferritin concentrations and anemia. Given the short duration of *Shigella* incubation (1-4 days) and infection (2-3 days) (56), it is plausible that the association observed between detection of the *Shigella ipaH* gene and CRP at higher relative

quantities, but not at lower relative quantities, reflects an acute systemic inflammatory response. Further, associations with low SF and anemia at low relative quantities may reflect the development of iron deficiency and anemia post-infection. *Shigella* infection in young children, more so than other enteric pathogens, is significantly implicated in long-term growth faltering (20,57). Rogawski et al. (2020) found a dose-response relationship between *Shigella* quantity and AGP concentrations, though they did not measure CRP (57). Given that *Shigella* infection is associated with both iron deficiency and anemia, and evidence that subclinical *Shigella* infection is associated with intestinal inflammation (57), *Shigella* infection may reduce iron availability in the body for hematopoiesis via both reduced micronutrient absorption and systemic inflammation. Alternatively, it may be that overt blood loss from dysentery caused by *Shigella* infection leads to anemia. Though no children in our study were reported to have had bloody stool in the previous seven days, dysentery before the recall period or continued microscopic blood loss may have contributed to the development of anemia in children infected with *Shigella*.

Detection of EAEC in stool was associated with anemia, but not with iron deficiency nor with elevated systemic inflammation. Given that EAEC is one of the most pervasive and persistent enteric infections detected in young children in LMIC (16,19,20), it is possible that associations observed with anemia in this study were due to repeated exposure to EAEC. Analysis from the MAL-ED birth cohort found that frequent EAEC detection was associated with long-term growth faltering, but monthly detection was not associated with short-term growth velocity in weight or length (19). As in our study, EAEC detection among the MAL-ED birth cohort was not associated with elevated AGP (19), though there is evidence that subclinical EAEC detection is associated with intestinal inflammation (19,58) and that its inflammatory effects may depend on interactions with co-infections (59). Our study did not evaluate repeat

infections, making further research important to understanding whether frequent EAEC detection in early life contributes to anemia in children.

A strong association between *Campylobacter jejuni/coli* detection and systemic inflammation has been shown previously for AGP (18) and is consistent with *Campylobacter's* induction of a robust immune response following epithelial invasion (51,52). However, neither associations with anemia nor iron deficiency were observed, which suggests that the inflammatory response induced by *Campylobacter* was insufficient to alter iron homeostasis in this sample. Interestingly, the detection of aEPEC was associated with lower odds of systemic inflammation in this study. Such reduced inflammatory response may reflect the pathogenic mechanism by which EPEC persists in the intestine – evading the mucosal immune system by inhibiting cell cytokine secretion and NF-κB signaling (which is upstream of CRP and AGP production) (60). Furthermore, co-detection of non-enteroinvasive bacteria (EPEC or ETEC) and enteroinvasive bacteria was not associated with a higher inflammatory response compared to non-enteroinvasive detection only, suggesting a possible counteracting of the inflammatory response seen in children infected with only enteroinvasive bacteria. Further epidemiologic research is needed to confirm these findings and to investigate whether co-infections of EPEC and invasive pathogens interact to alter systemic inflammation.

There was evidence that diet quality may modify the association between enteropathogen detection and anemia, such that children with poor-quality diets (i.e., not meeting the MDD requirement) experienced exacerbated infections. Though we could not determine whether this interaction was due to differences in gut function or the immune system, other evidence indicates that nutrient intake density from complementary foods is negatively associated with EED biomarkers (26), suggesting a role for nutrient intake in gut function integrity. However, given

our study design, we cannot rule out that the observed associations are due to reverse causality if children with more severe infections have lower appetites and hence poorer dietary diversity scores in the preceding 24 hours.

This study is the first to assess childhood associations between individual bacterial enteropathogen infections, using molecular diagnostics, and iron deficiency and anemia, using multiple biomarkers for systemic inflammation and iron status. However, this study had several limitations. First, we could not ascertain temporal relationships between enteropathogen detection and the outcomes of interest given the cross-sectional study design. Since inflammation may occur days after infection while iron deficiency and anemia can take several weeks to develop, concurrent collection of stool and blood samples precluded longitudinal assessment of these relationships. Thus, observed associations with anemia may reflect either prior infections or persistent infections, which we did not measure. Furthermore, the study sample size was smaller than that of many multi-site studies, which may have lowered our power to detect associations, especially among pathogens with low prevalences, though our sample size was within the range of sample sizes achieved per site in the MAL-ED birth cohort (26). Since data were collected in only one region of Ghana, prevalence estimates cannot be generalized to other regions of Ghana. Finally, the PCR diagnostics were limited by a lack of internal control, control for PCR inhibition, and pathogen load quantification. Although we examined relative quantification of high and low pathogen burden, these assessments are not based on clinically-relevant cut-offs, thus limiting the interpretability of the results.

Conclusion

Our findings suggest that subclinical detections of certain enteropathogens, particularly EAEC and EIEC/*Shigella*, are associated with anemia in young children. Results supports the

hypothesis that enteropathogen exposure due to poor water, sanitation, and hygiene contributes to anemia among young children living in LMIC (61). Addressing subclinical enteric infections through improved water, sanitation, and hygiene infrastructure and other approaches such as vaccines may help reduce the anemia burden among young children in Ghana and potentially other similar settings. Additional studies are needed to confirm our findings and assess the relative contribution of enteropathogen infections to the anemia burden compared to other infectious diseases and micronutrient deficiencies. Furthermore, research linking longitudinal assessment of individual enteropathogen infections and iron deficiency and anemia, alongside EED markers, hepcidin, and systemic inflammatory biomarkers, would improve our understanding of how bacterial enteropathogens may contribute to anemia among young children and to identify potential therapeutic targets.

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Ethical Statement

This study was approved by the University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board (protocol no. HUM00145171) and the Noguchi Memorial Institute for Medical Research Institutional Review Board (protocol no. 098/17-18).

References

1. World Health Organization. Global Health Observatory Data Repository: Anaemia in Children <5 Years by Region. [Internet]. 2017 [cited 2020 Apr 20]. Available from: https://apps.who.int/gho/data/view.main.ANEMIACHILDRENv
2. Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF International. Ghana Demographic and Health Survey 2014. Rockville, Maryland, USA; 2015.
3. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28:S560-71.
4. McCann JC, Ames BN. An overview of evidence for a causal relationship between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931–45.
5. Scott SP, Chen-Edinboro LP, Caulfield LE, Murray-Kolb LE. The impact of anemia on child mortality: An updated review. Nutrients. 2014;6:5915–32.
6. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. Lancet. 2011;378:2123–35.
7. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019;1450:15–31.
8. Kassebaum NJ. The global burden of anemia. Hematol Oncol Clin North Am. 2016;30:247–308.
9. Engle-Stone R, Aaron GJ, Huang J, Wirth JP, Namaste SM, Williams AM, Peerson JM, Rohner F, Varadhan R, Addo OY, et al. Predictors of anemia in preschool children: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106(Suppl):402S–15S.
10. Wegmüller R, Bentil H, Wirth JP, Petry N, Tanumihardjo SA, Allen L, Williams TN, Selenje L, Mahama A, Amoaful E, et al. Anemia, micronutrient deficiencies, malaria, hemoglobinopathies and malnutrition in young children and non-pregnant women in Ghana: Findings from a national survey. PLoS One. 2020;15:1–19.
11. Petry N, Wirth JP, Adu-Afarwuah S, Wegmuller R, Woodruff BA, Tanumihardjo SA, Bentil H, Donkor WES, Williams TN, Shahab-Ferdows S, et al. Risk factors for anaemia among Ghanaian women and children vary by population group and climate zone. Matern Child Nutr. 2020;1–10.
12. SPRING GHS. Ghana: Landscape Analysis of Anemia and Anemia Programming. Arlington, VA; 2016.
13. Kothari MT, Coile A, Huestis A, Pullum T, Garrett D, Engmann C. Exploring associations between water, sanitation, and anemia through 47 nationally representative demographic and health surveys. Ann N Y Acad Sci. 2019;1450:nyas.14109.
14. Yu EX, Addo OY, Williams AM, Engle-Stone R, Ou J, Huang W, Guo J, Suchdev PS, Young MF. Association between anemia and household water source or sanitation in preschool children: the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2020;112:488S-497S.
15. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet. 2013;382:209–22.
16. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJJ, McGrath M, Olortegui MP, Samie A, et al. Pathogen-specific burdens of community

diarrhoea in developing countries: A multisite birth cohort study (MAL-ED). Lancet Glob Heal. 2015;3:e564–75.
17. Kosek MN, Ahmed T, Bhutta ZA, Caulfield L, Guerrant RL, Houpt E, Kang G, Lee G, Lima AAM, McCormick BJJ, et al. Causal pathways from enteropathogens to environmental enteropathy: findings from the MAL-ED birth cohort study. EBioMedicine. 2017;18:109–17.
18. Amour C, Gratz J, Mduma ER, Svensen E, Rogawski ET, McGrath M, Seidman JC, McCormick BJJ, Shrestha S, Samie A, et al. Epidemiology and impact of *Campylobacter* infection in children in 8 low-resource settings: Results from the MAL-ED study. Clin Infect Dis. 2016;63:1171–9.
19. Rogawski ET, Guerrant RL, Havt A, Lima IFN, Medeiros PHQS, Seidman JC, McCormick BJJ, Babji S, Hariraju D, Bodhidatta L, et al. Epidemiology of enteroaggregative *Escherichia coli* infections and associated outcomes in the MAL-ED birth cohort. PLoS Negl Trop Dis. 2017;11:1–17.
20. Rogawski ET, Liu J, Platts-Mills JA, Kabir F, Lertsethtakarn P, Siguas M, Khan SS, Praharaj I, Murei A, Nshama R, et al. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. Lancet Glob Heal. 2018;6:e1319–28.
21. Berendes D, Capone D, Knee J, Holcomb D, Sultana S, Pickering AJ, Brown J. Associations between enteric pathogen carriage and height-for-age, weight-for-age and weight-for-height in children under 5 years old in urban Dhaka, Bangladesh. Epidemiol Infect. 2020;148:e39.
22. Haque MA, Platts-Mills JA, Mduma E, Bodhidatta L, Bessong P, Shakoor S, Kang G, Kosek MN, Lima AAM, Shrestha SK, et al. Determinants of *Campylobacter* infection and association with growth and enteric inflammation in children under 2 years of age in low-resource settings. Sci Rep. 2019;9:1–8.
23. Watanabe K, Petri WA. Environmental enteropathy: elusive but significant subclinical abnormalities in developing countries. EBioMedicine. 2016;10:25–32.
24. Syed S, Ali A, Duggan C. Environmental enteric dysfunction in children. J Pediatr Gastroenterol Nutr. 2016;63:6–14.
25. Murray-Kolb LE, Acosta AM, De Burga RR, Chavez CB, Flores JT, Olotegui MP, Pinedo SR, Salas MS, Trigoso DR, Vasquez AO, et al. Early childhood cognitive development is affected by interactions among illness, diet, enteropathogens and the home environment: findings from the MAL-ED birth cohort study. BMJ Glob Heal. 2018;3:e000752.
26. McCormick BJJ, Murray-Kolb LE, Lee GO, Schulze KJ, Ross AC, Bauck A, Lima AAM, Maciel BLL, Kosek MN, Seidman JC, et al. Intestinal permeability and inflammation mediate the association between nutrient density of complementary foods and biochemical measures of micronutrient status in young children: results from the MAL-ED study. Am J Clin Nutr. 2019;110:1015–25.
27. Fahim SM, Das S, Sanin KI, Gazi MA, Mahfuz M, Munirul Islam M, Ahmed T. Association of fecal markers of environmental enteric dysfunction with zinc and iron status among children at first two years of life in Bangladesh. Am J Trop Med Hyg. 2018;99:489–94.
28. Lauer JM, Ghosh S, Ausman LM, Webb P, Bashaasha B, Agaba E, Turyashemererwa FM, Tran HQ, Gewirtz AT, Erhardt J, et al. Markers of environmental enteric dysfunction are

associated with poor growth and iron status in rural Ugandan infants. J Nutr. 2020;150:2175–82.
29. Goto K, Chew F, Torún B, Peerson JM, Brown KH. Epidemiology of altered intestinal permeability to lactulose and mannitol in Guatemalan infants. J Pediatr Gastroenterol Nutr. 1999;282–90.
30. Prentice AM, Bah A, Jallow MW, Jallow AT, Sanyang S, Sise EA, Ceesay K, Danso E, Armitage AE, Pasricha SR, et al. Respiratory infections drive hepcidin-mediated blockade of iron absorption leading to iron deficiency anemia in African children. Sci Adv. 2019;5.
31. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. Science. 2012;338:768–72.
32. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. Nat Rev Immunol. 2015;15:500–10.
33. Pasricha S-R, Armitage AE, Prentice AM, Drakesmith H. Reducing anaemia in low income countries: control of infection is essential. BMJ. 2018;3165:k3165.
34. Bourke CD, Berkley JA, Prendergast AJ. Immune dysfunction as a cause and consequence of malnutrition. Trends Immunol. 2016;37:386–98.
35. Farré R, Fiorani M, Abdu Rahiman S, Matteoli G. Intestinal permeability, inflammation and the role of nutrients. Nutrients. 2020;12:1185.
36. Khoshbin K, Camilleri M. Effects of dietary components on intestinal permeability in health and disease. Am J Physiol Liver Physiol. 2020;319:G589–608.
37. Jaeggi T, Kortman GAM, Moretti D, Chassard C, Holding P, Dostal A, Boekhorst J, Timmerman HM, Swinkels DW, Tjalsma H, et al. Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. Gut. 2015;64:731–42.
38. Zimmermann MB, Chassard C, Rohner F, Goran KNE, Nindjin C, Dostal A, Utzinger J, Ghattas H, Lacroix C, Hurrell RF. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. Am J Clin Nutr. 2010;92:1406–15.
39. Jonker FAM, te Poel E, Bates I, Boele van Hensbroek M. Anaemia, iron deficiency and susceptibility to infection in children in sub-Saharan Africa, guideline dilemmas. Br J Haematol. 2017;177:878–83.
40. World Health Organization. Indicators for assessing infant and young child feeding practices: Part 2 Measurement. Geneva, Switzerland; 2010.
41. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. Geneva, Switzerland; 2010.
42. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich Enzyme-Linked Immunosorbent Assay Technique. J Nutr. 2004;134:3127–32.
43. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS. Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106:333S-347S.
44. World Health Organization. Bench aids for the diagnosis of intestinal parasites. 1994.
45. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, Mduma E, Platts-Mills J, Boisen N, Nataro J, et al. Optimization of quantitative PCR Methods for enteropathogen detection. PLoS One. 2016;11:e0158199.

46. Taniuchi M, Walters CC, Gratz J, Maro A, Kumburu H, Serichantalergs O, Sethabutr O, Bodhidatta L, Kibiki G, Toney DM, et al. Development of a multiplex polymerase chain reaction assay for diarrheagenic *Escherichia coli* and *Shigella spp*. and its evaluation on colonies, culture broths, and stool. Diagn Microbiol Infect Dis. 2012;73:121–8.
47. WHO. Global Nutrition Monitoring Framework: Operational guidance for tracking progress in meeting targets for 2025. World Health Organization. Geneva; 2017.
48. Working Group on Infant and Young Child Feeding Indicators. Developing and Validating Simple Indicators of Dietary Quality and Energy Intake of Infants and Young Children in Developing Countries: Summary of findings from analysis of 10 data sets. Washington, D.C.; 2006.
49. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. Vitamin and Mineral Nutrition Information System. Geneva; 2011. Available from: http://www.who.int/vmnis/indicators/haemoglobin.pdf
50. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev. 2013;26:822–80.
51. Young KT, Davis LM, DiRita VJ. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol. 2007;5:665–79.
52. Schnee AE, Petri WA. *Campylobacter jejuni* and associated immune mechanisms: short-term effects and long-term implications for infants in low-income countries. Curr Opin Infect Dis. 2017;30:322–8.
53. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. *Shigellosis*. Lancet. 2018;391:801–12.
54. Harper KM, Mutasa M, Prendergast AJ, Humphrey J, Manges AR. Environmental enteric dysfunction pathways and child stunting: a systematic review. PLoS Negl Trop Dis. 2018;12:e0006205.
55. Jaeggi T, Moretti D, Kvalsvig J, Holding PA, Tjalsma H, Kortman GAM, Joosten I, Mwangi A, Zimmermann MB. Iron status and systemic inflammation, but not gut inflammation, strongly predict gender-specific concentrations of serum hepcidin in infants in rural Kenya. PLoS ONE. 2013;8:1–8.
56. Goering R V., Dockrell HM, Zuckerman M, Chiodini PL. Gastrointestinal tract infections. MIMS' Medical Microbiology and Immunology. Sixth Ed. Elsevier; 2019. p. 265–77.
57. Rogawski Mcquade ET, Shaheen F, Kabir F, Rizvi A, Platts-Mills JA, Aziz F, Kalam A, Qureshi S, Elwood S, Liu J, et al. Epidemiology of *Shigella* infections and diarrhea in the first two years of life using culture-independent diagnostics in 8 low-resource settings. PLoS Negl Trop Dis. 2020;14:1–17.
58. Opintan JA, Newman MJ, Ayeh-Kumi PF, Affrim R, Gepi-Attee R, Sevilleja JEAD, Roche JK, Nataro JP, Warren CA, Guerrant RL. Pediatric diarrhea in Southern Ghana: etiology and association with intestinal inflammation and malnutrition. Am J Trop Med Hyg. 2010;83:936–43.
59. Lima AAM, Soares AM, Filho JQS, Havt A, Lima IFN, Lima NL, Abreu CB, Francisco S, Mota RMS, Pan WKY, et al. Enteroaggregative *Escherichia coli* subclinical infection and coinfections and impaired child growth in the MAL-ED cohort study. J Pediatr Gastroenterol Nutr. 2018;66:325–33.
60. Pearson JS, Giogha C, Wong Fok Lung T, Hartland EL. The genetics of enteropathogenic *Escherichia coli* virulence. Annu Rev Genet. 2016;50:493–513.

61. Baldi AJ, Clucas D, Pasricha SR. Anemia and water, sanitation, and hygiene (WASH)-is there really a link? Am J Clin Nutr. Oxford University Press; 2020;112:1145–6.

Figures and Tables

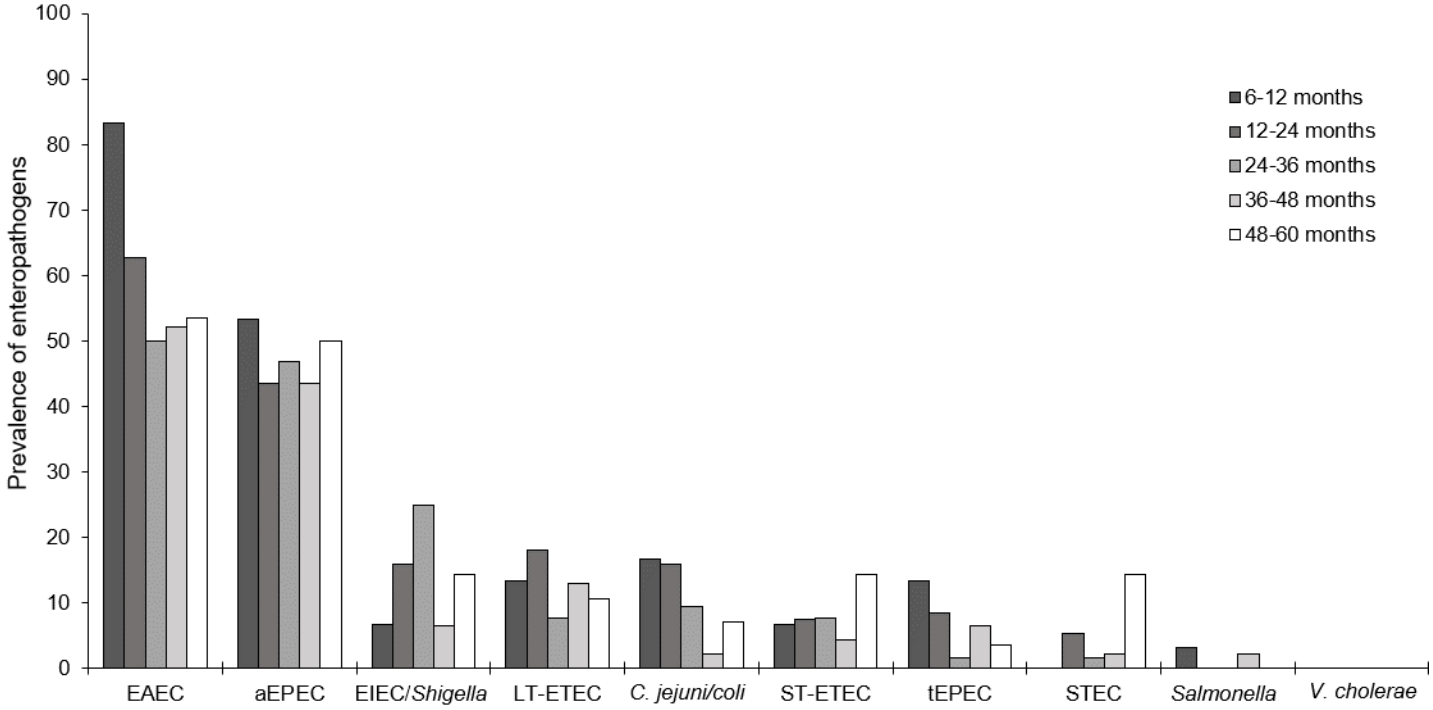

Figure 4.1 Prevalence of enteropathogens detected in children's stool by age (months) in the Greater Accra Region, Ghana, October-November 2018 (n=262). Age categories: 6-12 months (n=30), 12-24 months (n=94), 24-36 months (n=64), 36-48 months (n=46), 48-60 months (n=28). Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

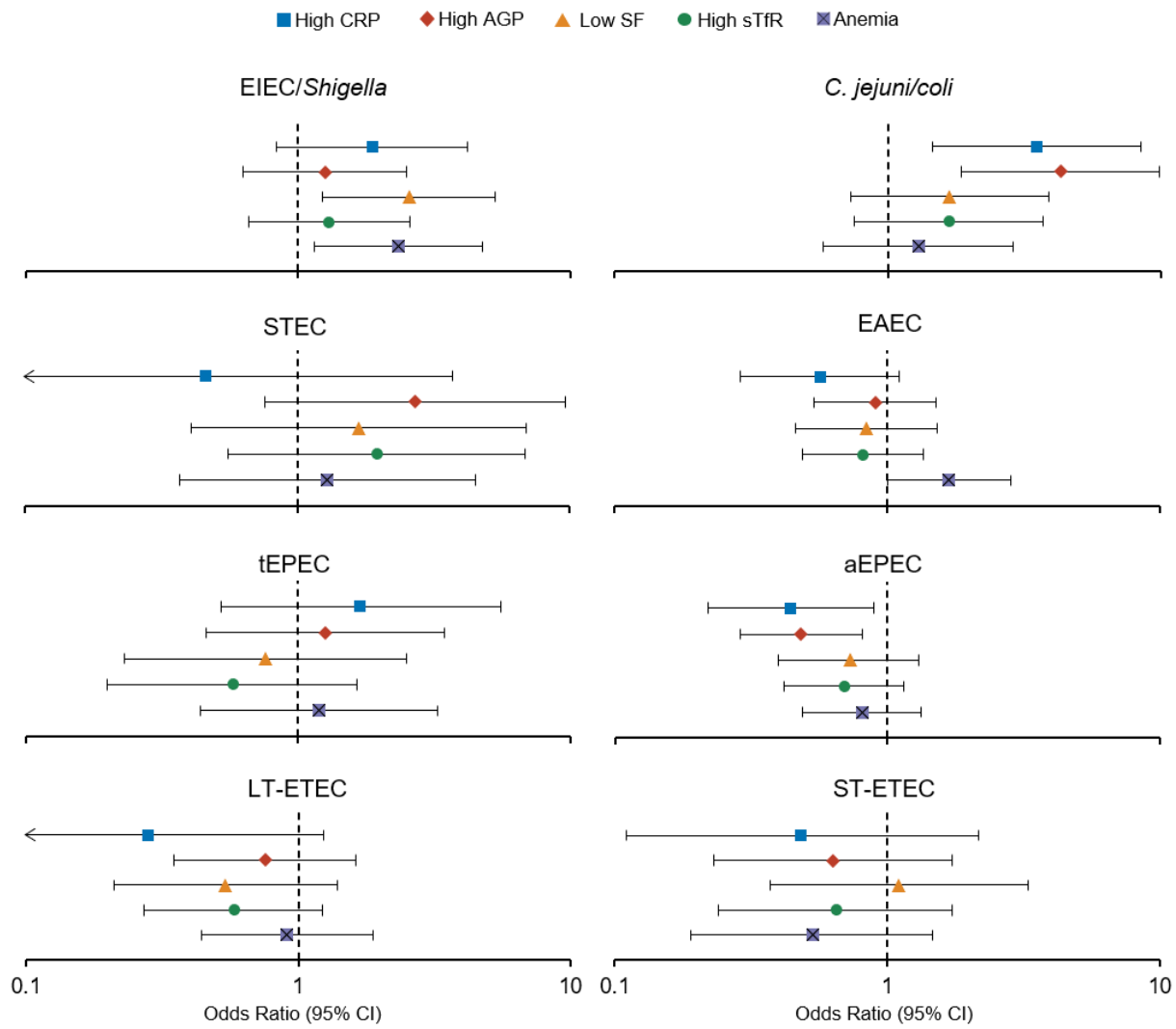

Figure 4.2 Odds Ratios (OR) and 95% Confidence Intervals (CI) of the associations between enteropathogen infections and inflammation, iron deficiency, and anemia among children 6-59 months old in Greater Accra Region, Ghana. OR and 95% CI derived from adjusted logistic regression controlling for child age and sex (see Tables 4.6 and 4.9). Sample size: n=262. Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.1 Study sample characteristics of children 6-59 months old from two districts of Greater Accra Region, Ghana (n=262)[1]

Characteristics	Values
Child characteristics	
Sex, female	127 (48.5)
Age, months	27.7 ± 14.0
Currently breastfeeding	82 (31.3)
Met MDD[2]	133 (50.8)
Household characteristics	
Number of children under 5 years	1.3 ± 0.6
Head of household sex, female	55 (21.0)
Maternal education[3]	
None, nursery, or primary	120 (46.3)
Junior or higher	139 (53.7)
Improved drinking water source[4]	255 (97.3)
Sanitation facility	
Flush or pour-flush	28 (10.7)
Pit latrine	154 (58.8)
No facility/open defecation	80 (30.5)
Presence of handwashing facility	7 (2.7)
Ownership of any livestock	162 (61.8)
District	
Ga East	91 (34.7)
Shai Osudoku	171 (65.3)

[1]Values are n (%) or mean ± SD.
[2]Meeting the MDD (minimum dietary diversity) criteria is defined as consuming ≥5 out of 8 food groups for children 6-23 months old and ≥4 out of 7 food groups for children 24-59 months old.
[3]n=259
[4]Includes piped water, public pipe/standpipe, tube well or borehole, protected dug well, protected spring, bottled water/sachet water, cart with tank, and rain water.

Table 4.2 Micronutrient status, inflammation, and illness in children 6-59 months old from Greater Accra Region, Ghana (n=262)

Indicator	Value
Micronutrient status biomarkers[1]	
Hb (g/dL); mean (SD)	10.9 (1.5)
Anemia, n (%)	
None (Hb ≥ 11.0g/dL)	142 (54.2)
Mild (Hb 10.0-10.9g/dL)	56 (21.4)
Moderate (Hb 7.0-9.9g/dL)	60 (22.9)
Severe (Hb < 7.0g/dL)	4 (1.5)
Iron deficiency anemia; n (%)[2]	47 (17.9)
SF (µg/L); median (IQR)	22.9 (12.2, 42.9)
Iron deficiency (SF<12µg/L); n (%)	64 (24.4)
sTfR (mg/L); median (IQR)	8.0 (6.6, 10.4)
Iron deficiency (sTfR>8.3mg/L); n (%)	116 (44.3)
RBP (µmol/L); mean (SD)	0.95 (0.27)
Inflammation biomarkers	
CRP (mg/L); median (IQR)	0.52 (0.16, 2.24)
Inflammation (CRP>5mg/L); n (%)	43 (16.4)
AGP (g/L); median (IQR)	0.83 (0.58, 1.29)
Inflammation (AGP>1g/L); n (%)	99 (37.8)
Infection and illness symptoms	
Malaria; n (%)	22 (8.4)
Helminthiasis; n (%)[3]	0 (0.0)
Fever; n (%)[4]	66 (25.2)
Diarrhea; n (%)	17 (6.5)
Cough/cold; n (%)	65 (24.8)
Nausea; n (%)	15 (5.7)
Vomiting; n (%)	16 (6.1)

[1]SF and sTfR are inflammation-adjusted values. Unadjusted SF values: 30.8µg/L (15.7, 56.1). Unadjusted sTfR values: 10.1 ± 5.6mg/L.
[2]Iron deficiency anemia is defined as concomitant anemia and iron deficiency (SF<12µg/L).
[3]N=257
[4]13.6% of fever associated with malaria.
Abbreviations: Hb, hemoglobin; SF, serum ferritin; sTfR, serum transferrin receptor; RBP, retinol-binding protein; CRP, C-reactive protein; AGP, α-1-acid glycoprotein.

Table 4.3 Correlation matrix between hemoglobin, iron status biomarkers, and inflammatory biomarkers among children 6-59 months old[1]

	Hb	SF	sTfR	CRP
Hb	-			
SF	**0.363******	-		
sTfR	**-0.562******	**-0.530******	-	
CRP	**-0.226*****	0.041	0.012	-
AGP	**-0.187****	0.072	0.018	**0.731******

[1]Values are Pearson correlation coefficients. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. SF, sTfR, CRP, and AGP are natural log-transformed. SF and sTfR are inflammation-adjusted values. Abbreviations: Hb, hemoglobin; SF, serum ferritin; sTfR, serum transferrin receptor; CRP, C-reactive protein; AGP, α-1-acid glycoprotein.

Table 4.4 Prevalence of enteropathogens detected in stool of children 6-59 months old from Greater Accra Region, Ghana, October-November 2018 (n=262)[1]

Pathogen	N (%)
EAEC	155 (59.2)
aEPEC	121 (46.2)
EIEC/*Shigella*	40 (15.3)
LT-ETEC	35 (13.4)
C. jejuni/coli	29 (11.1)
ST-ETEC	20 (7.6)
tEPEC	17 (6.5)
STEC	11 (4.2)
Salmonella	2 (0.8)
V. cholerae	0 (0.0)
Any pathogen	
Yes	228 (87.0)
No	34 (13.0)
Number of detected pathogens per sample[2]	
0	34 (13.0)
1	92 (35.1)
2	82 (31.3)
3	42 (16.0)
4	12 (4.6)
≥5	0 (0.0)

[1]Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*
[2]Sum ranges from 0 to 10.

Table 4.5 Adjusted associations between enteropathogen infection and symptoms of illness among children 6-59 months old[1]

Pathogen	Diarrhea	Fever	Cough/Cold	Nausea	Vomiting
EAEC	2.87 (0.79, 10.45)	0.83 (0.47, 1.48)	0.74 (0.42, 1.32)	1.92 (0.59, 6.31)	1.04 (0.36, 3.01)
aEPEC	0.60 (0.21, 1.69)	0.89 (0.51, 1.56)	0.92 (0.52, 1.61)	1.03 (0.36, 2.92)	1.18 (0.43, 3.25)
LT-ETEC	1.97 (0.59, 6.52)	1.67 (0.78, 3.60)	0.73 (0.30, 1.76)	0.43 (0.05, 3.42)	0.87 (0.19, 4.03)
EIEC/*Shigella*	1.27 (0.34, 4.72)	1.02 (0.47, 2.22)	1.35 (0.64, 2.85)	2.23 (0.67, 7.45)	0.80 (0.17, 3.67)
C. jejuni/coli	-	1.21 (0.50, 2.93)	1.41 (0.60, 3.31)	0.56 (0.07, 4.55)	0.45 (0.06, 3.62)
ST-ETEC	3.42 (0.84, 13.90)	0.15 (0.02, 1.15)	0.98 (0.34, 2.85)	0.97 (0.12, 7.99)	0.89 (0.11, 7.24)
tEPEC	1.68 (0.34, 8.27)	1.77 (0.62, 5.04)	0.62 (0.17, 2.24)	2.46 (0.50, 12.10)	2.05 (0.42, 10.00)
STEC	**5.78* (1.02, 32.64)**	0.61 (0.13, 2.95)	0.29 (0.04, 2.36)	-	-

[1]Values are Odds Ratio (95% Confidence Interval) using logistic regression models, adjusting for child sex and age in months. **p<0.05, **p<0.01.* Dash (-) indicates that the logistic model could not run due to complete separation. Sample size: n=262

Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.6 Unadjusted and adjusted associations between enteropathogen infection and systemic inflammation among children 6-59 months old[1]

Pathogen	CRP>5mg/L		AGP>1g/L	
	Unadjusted OR (95% CI)	**Adjusted OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
EAEC	0.54 (0.28, 1.05)	0.57 (0.29, 1.11)	0.84 (0.51, 1.40)	0.91 (0.54, 1.52)
aEPEC	**0.45* (0.22, 0.90)**	**0.44* (0.22, 0.89)**	**0.49** (0.29, 0.82)**	**0.48** (0.29, 0.81)**
LT-ETEC	0.27 (0.06, 1.19)	0.28 (0.07, 1.24)	0.72 (0.34, 1.55)	0.76 (0.35, 1.63)
EIEC/*Shigella*	1.91 (0.85, 4.27)	1.87 (0.83, 4.20)	1.26 (0.64, 2.50)	1.26 (0.63, 2.50)
C. jejuni/coli	**3.19** (1.36, 7.46)**	**3.49** (1.45, 8.41)**	**3.63** (1.61, 8.19)**	**4.27** (1.85, 9.84)**
ST-ETEC	0.54 (0.12, 2.44)	0.48 (0.11, 2.16)	0.69 (0.25, 1.85)	0.63 (0.23, 1.72)
tEPEC	1.63 (0.50, 5.25)	1.69 (0.52, 5.53)	1.16 (0.43, 3.16)	1.26 (0.46, 3.46)
STEC	0.50 (0.06, 3.99)	0.46 (0.06, 3.74)	3.02 (0.86, 10.61)	2.72 (0.76, 9.67)

[1]Values are Odds Ratio (95% Confidence Interval) using logistic regression models. Adjusted models control for child sex and age in months. **p<0.05, **p<0.01.* Sample size: n=262
Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.7 Adjusted associations of high and low relative gene target quantity and inflammation, iron deficiency, and anemia among children 6-59 months old[1]

Pathogen	Gene Target	Relative Quantity	N	CRP>5mg/L	AGP>1g/L	SF<12µg/L	sTfR>8.3mg/L	Hb<11.0g/dL
EIEC/*Shigella*	*ipaH*	No detection	222	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥29.8)	21	0.94 (0.26, 3.40)	0.68 (0.25, 1.82)	**2.97* (1.15, 7.69)**	2.18 (0.86, 5.52)	**2.83* (1.08, 7.45)**
		High (Ct<29.8)	19	**3.24* (1.18, 8.89)**	2.36 (0.91, 6.15)	2.14 (0.78, 5.90)	0.73 (0.27, 1.94)	1.91 (0.73, 5.05)
C. jejuni/coli	*cadF*	No detection	233	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥28.4)	14	**3.74* (1.16, 12.10)**	**3.92* (1.25, 12.27)**	1.20 (0.36, 4.06)	1.68 (0.56, 5.03)	1.44 (0.48, 4.38)
		High (Ct<28.4)	15	**3.27* (1.02, 10.55)**	**4.64** (1.49, 14.43)**	2.24 (0.75, 6.68)	1.66 (0.56, 4.91)	1.15 (0.39, 3.40)
EAEC	*aatA*	No detection	122	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥28.3)	70	0.49 (0.21, 1.17)	0.74 (0.40, 1.38)	0.82 (0.39, 1.73)	0.84 (0.46, 1.54)	1.44 (0.79, 2.64)
		High (Ct<28.3)	70	0.59 (0.25, 1.38)	1.09 (0.58, 2.05)	1.14 (0.57, 2.30)	0.77 (0.41, 1.43)	**1.88* (1.00, 3.51)**
EAEC	*aaiC*	No detection	184	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥26.3)	39	0.98 (0.37, 2.57)	1.11 (0.54, 2.31)	0.82 (0.35, 1.89)	1.39 (0.68, 2.80)	1.71 (0.83, 3.49)
		High (Ct<26.3)	39	1.18 (0.47, 2.95)	**2.07* (1.02, 4.19)**	1.14 (0.51, 2.53)	0.76 (0.37, 1.57)	1.04 (0.51, 2.11)
tEPEC/ aEPEC/ STEC	*eae*	No detection	99	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥29.0)	80	**0.38* (0.17, 0.88)**	0.60 (0.32, 1.11)	0.66 (0.32, 1.36)	0.82 (0.45, 1.49)	1.00 (0.54, 1.83)
		High (Ct<29.0)	83	**0.43* (0.19, 0.97)**	0.77 (0.42, 1.41)	0.83 (0.42, 1.64)	0.78 (0.43, 1.42)	0.70 (0.38, 1.28)
tEPEC	*bfpA*	No detection	240	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥25.8)	11	1.26 (0.26, 6.14)	1.05 (0.30, 3.72)	2.35 (0.66, 8.35)	1.37 (0.40, 4.74)	1.28 (0.37, 4.38)
		High (Ct<25.8)	11	2.14 (0.53, 8.67)	2.39 (0.69, 8.21)	0.21 (0.03, 1.74)	0.36 (0.09, 1.42)	0.77 (0.22, 2.67)
STEC	*stx1*	No detection	245	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥32.4)	9	0.58 (0.07, 4.81)	3.39 (0.82, 14.05)	3.14 (0.76, 13.02)	0.67 (0.16, 2.80)	0.35 (0.07, 1.76)
		High (Ct<32.4)	8	-	1.97 (0.48, 8.13)	0.92 (0.18, 4.72)	1.92 (0.44, 8.31)	1.61 (0.37, 6.95)
STEC	*stx2*	No detection	249	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥32.1)	6	2.29 (0.40, 13.19)	8.17 (0.93, 71.65)	4.78 (0.83, 27.46)	8.10 (0.89, 73.77)	8.87 (0.96, 81.68)
		High (Ct<32.1)	7	-	4.24 (0.80, 22.51)	1.58 (0.28, 9.05)	0.23 (0.03, 1.95)	0.55 (0.10, 3.00)
LT-ETEC/ ST-ETEC	*LT*	No detection	215	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥29.2)	23	0.19 (0.03, 1.48)	0.85 (0.34, 2.11)	0.69 (0.24, 2.00)	0.45 (0.18, 1.17)	0.96 (0.40, 2.32)
		High (Ct<29.2)	24	0.19 (0.02, 1.43)	0.53 (0.20, 1.40)	0.35 (0.10, 1.25)	0.63 (0.26, 1.54)	0.63 (0.26, 1.52)
ST-ETEC	*STh*	No detection	253	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥21.8)	4	-	0.44 (0.04, 4.40)	-	1.34 (0.17, 10.43)	0.49 (0.05, 5.21)
		High (Ct<21.8)	5	3.41 (0.54, 21.39)	0.41 (0.04, 3.73)	4.65 (0.73, 29.43)	0.28 (0.03, 2.60)	0.27 (0.03, 2.47)
ST-ETEC	*STp*	No detection	250	Ref	Ref	Ref	Ref	Ref
		Low (Ct≥30.2)	6	-	0.74 (0.13, 4.18)	-	0.63 (0.11, 3.60)	0.27 (0.03, 2.43)
		High (Ct<30.2)	6	-	1.52 (0.30, 7.79)	1.84 (0.31, 11.01)	0.66 (0.12, 3.74)	1.39 (0.26, 7.35)

[1]Values are Odds Ratio (95% Confidence Interval) using logistic regression models, adjusting for child sex and age in months. High- and low-relative quantity are defined as below and above the median Ct for each gene target, excluding detection of Ct>35. **p<0.05, **p<0.01.* SF and sTfR cut-offs use inflammation-adjusted values. Sample size: n=262

Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.8 Adjusted odds of inflammation, iron deficiency, and anemia comparing enteroinvasive and enteroinvasive/non-enteroinvasive pathogen co-infection in reference to non-enteroinvasive pathogen infection[1]

Pathogen group	N	CRP>5mg/L	AGP>1g/L	SF<12µg/L	sTfR>8.3 mg/L	Hb<11.0 g/dL
Non-enteroinvasive only	49	Ref	Ref	Ref	Ref	Ref
Enteroinvasive and non-enteroinvasive co-infection	113	1.75 (0.54, 5.68)	1.57 (0.73, 3.40)	0.96 (0.41, 2.25)	1.03 (0.51, 2.10)	**2.10* (1.00, 4.40)**
Enteroinvasive only	66	3.02 (0.91, 10.02)	**3.76** (1.65, 8.56)**	2.05 (0.84, 4.97)	**2.22* (1.03, 4.80)**	**2.85* (1.27, 6.38)**

[1] Values are Odds Ratio (95% Confidence Interval) using adjusted logistic regression models, controlling for child sex and age in months. **p<0.05, **p<0.01.* SF and sTfR cut-offs use inflammation-adjusted values. Sample size: n=228

[2]The enteroinvasive only pathogen group includes children with detection of one or more enteropathogens that invade (*Campylobacter jejuni/coli*, enteroinvasive *E. coli/Shigella*, *Salmonella*) or potentially invade intestinal epithelial cells (enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC)). The non-enteroinvasive pathogen only group includes children with detection of one or more enteropathogens that are not known to invade intestinal epithelial cells (enteropathogenic *E. coli* (EPEC), enterotoxin-producing *E. coli* (ETEC)). The enteroinvasive and non-enteroinvasive pathogen group includes children with detection of both invasive and non-invasive enteropathogens.

Table 4.9 Unadjusted and adjusted associations between enteropathogen infection and iron status and anemia among children 6-59 months old[1]

Pathogen	Iron Deficiency (SF<12 ug/L)		Iron Deficiency (sTfR>8.3 mg/L)		Anemia (Hb<11.0 g/dL)	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
EAEC	1.01 (0.57, 1.80)	0.84 (0.46, 1.53)	0.90 (0.55, 1.48)	0.82 (0.49, 1.36)	**1.91* (1.15, 3.16)**	**1.69* (1.01, 2.84)**
aEPEC	0.74 (0.42, 1.31)	0.73 (0.40, 1.30)	0.71 (0.43, 1.15)	0.70 (0.42, 1.15)	0.81 (0.50, 1.32)	0.81 (0.49, 1.33)
LT-ETEC	0.60 (0.24, 1.53)	0.54 (0.21, 1.39)	0.62 (0.29, 1.30)	0.58 (0.27, 1.23)	1.00 (0.49, 2.03)	0.91 (0.44, 1.89)
EIEC/*Shigella*	**2.42* (1.19, 4.91)**	**2.55* (1.23, 5.29)**	1.31 (0.67, 2.58)	1.30 (0.66, 2.57)	**2.23* (1.11, 4.46)**	**2.34* (1.15, 4.76)**
C. jejuni/coli	2.08 (0.92, 4.67)	1.68 (0.73, 3.86)	1.92 (0.88, 4.20)	1.67 (0.75, 3.70)	1.53 (0.70, 3.32)	1.29 (0.58, 2.86)
ST-ETEC	1.03 (0.36, 2.97)	1.10 (0.37, 3.28)	0.66 (0.25, 1.70)	0.65 (0.24, 1.72)	0.48 (0.18, 1.29)	0.53 (0.19, 1.47)
tEPEC	0.95 (0.30, 3.02)	0.76 (0.23, 2.51)	0.67 (0.24, 1.87)	0.58 (0.20, 1.64)	1.36 (0.51, 3.64)	1.19 (0.44, 3.25)
STEC	1.17 (0.30, 4.54)	1.68 (0.41, 6.97)	1.54 (0.46, 5.17)	1.96 (0.56, 6.87)	0.99 (0.29, 3.31)	1.29 (0.37, 4.54)

[1]Values are Odds Ratio (95% Confidence Interval) using logistic regression models. Adjusted models control for child sex and age in months. **p<0.05.* SF and sTfR cut-offs use inflammation-adjusted values. Sample size: n=262

Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.10 Adjusted associations between enteropathogen infection and concentrations of inflammatory biomarkers, iron status biomarkers, and hemoglobin among children 6-59 months old[1]

Pathogen	CRP (mg/L)	AGP (g/L)	SF (µg/L)	sTfR (mg/L)	Hb (g/dL)
EAEC	-1.39 (-4.27, 1.49)	-0.09 (-0.23, 0.05)	-2.78 (-8.59, 3.03)	0.13 (-1.17, 1.43)	-0.2 (-0.6, 0.1)
aEPEC	-1.79 (-4.58, 1.00)	**-0.23** (-0.36, -0.10)**	-1.61 (-7.25, 4.02)	-0.89 (-2.15, 0.36)	0.3 (-0.1, 0.6)
LT-ETEC	-3.23 (-7.32, 0.87)	-0.16 (-0.36, 0.04)	0.25 (-8.04, 8.53)	-0.39 (-2.24, 1.46)	0.2 (-0.3, 0.7)
EIEC/*Shigella*	0.86 (-3.02, 4.74)	0.04 (-0.15, 0.23)	-5.52 (-13.32, 2.28)	1.36 (-0.38, 3.10)	**-0.5* (-1.0, -0.03)**
C. jejuni/coli	3.26 (-1.23, 7.74)	**0.34** (0.12, 0.55)**	-0.78 (-9.85, 8.29)	1.04 (-0.98, 3.06)	-0.3 (-0.9, 0.3)
ST-ETEC	-3.37 (-8.66, 1.92)	-0.20 (-0.45, 0.06)	1.55 (-9.14, 12.24)	-1.72 (-4.10, 0.65)	0.5 (-0.2, 1.2)
tEPEC	**7.50** (1.89, 13.12)**	0.19 (-0.09, 0.47)	1.34 (-10.12, 12.81)	-1.65 (-4.20, 0.90)	-0.2 (-0.9, 0.6)
STEC	-2.86 (-9.87, 4.15)	0.09 (-0.25, 0.43)	-10.99 (-25.07, 3.09)	2.67 (-0.47, 5.81)	0.04 (-0.8, 0.9)

[1]Values are β coefficient (95% Confidence Interval) from linear regression models, adjusting for child sex and age in months. SF and sTfR are inflammation-adjusted values. **p<0.05, **p<0.01* Sample size: n=262

Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

Table 4.11 Estimated difference in concentrations of inflammatory biomarkers, iron status biomarkers, and hemoglobin comparing enteroinvasive and enteroinvasive/non-enteroinvasive pathogen co-infection in reference to non-enteroinvasive pathogen infection using adjusted linear regression[1]

Pathogen group	N	CRP (mg/L)	AGP (g/L)	SF (µg/L)	sTfR (mg/L)	Hb (g/dL)
Non-enteroinvasive only	49	Ref	Ref	Ref	Ref	Ref
Enteroinvasive and non-enteroinvasive co-infection	113	-1.60 (-5.56, 2.35)	0.04 (-0.14, 0.21)	-6.50 (-14.26, 1.27)	0.32 (-1.40, 2.04)	-0.3 (-0.8, 0.2)
Enteroinvasive only	66	0.04 (-4.31, 4.38)	**0.29** (0.09, 0.48)**	-6.53 (-15.07, 2.00)	**2.08* (0.18, 3.97)**	**-0.9** (-1.4, -0.4)**

[1]Values are β coefficients (95% Confidence Interval) using adjusted linear regression models, controlling for child sex and age in months. **p<0.05, **p<0.01.* SF and sTfR are inflammation-adjusted values. Sample size: n=228

[2]The enteroinvasive only pathogen group includes children with detection of one or more enteropathogens that invade (*Campylobacter jejuni/coli*, enteroinvasive *E. coli/Shigella*, *Salmonella*) or potentially invade intestinal epithelial cells (enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC)). The non-enteroinvasive pathogen only group includes children with detection of one or more enteropathogens that are not known to invade intestinal epithelial cells (enteropathogenic *E. coli* (EPEC), enterotoxin-producing *E. coli* (ETEC)). The enteroinvasive and non-enteroinvasive pathogen group includes children with detection of both invasive and non-invasive enteropathogens.

Table 4.12 Associations between enteropathogen infection and anemia among children, stratified by meeting the minimum dietary diversity (MDD) score (n=262)[1]

Pathogen	Did not meet MDD[2] (n=129)	Met MDD (n=133)
EAEC	**2.40* (1.12, 5.12)**	1.25 (0.60, 2.60)
aEPEC	0.61 (0.30, 1.26)	0.98 (0.48, 2.03)
LT-ETEC	1.07 (0.34, 3.37)	0.88 (0.32, 2.38)
EIEC/*Shigella*	**4.51** (1.48, 13.80)**	1.24 (0.45, 3.38)
C. jejuni/coli	1.62 (0.53, 4.95)	0.83 (0.25, 2.78)
ST-ETEC	2.15 (0.52, 8.92)	**0.11* (0.01, 0.88)**
tEPEC	0.93 (0.24, 3.56)	1.62 (0.32, 8.13)
STEC	1.79 (0.35, 9.28)	0.63 (0.06, 6.86)

[1]Values are Odds Ratio (95% Confidence Intervals) using logistic regression models, adjusting for child sex and age in months. **p<0.05, **p<0.01.* Sample size: n=262

[2]MDD is defined as dietary diversity score (DDS)≥4 among children 24-59 months old and DDS≥5 (including breastmilk as a food group in the DDS) among children 6-23 months old.

Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli (E. coli)*; C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli;* ST-ETEC, heat-stable enterotoxin-producing *E. coli;* tEPEC, typical enteropathogenic *E. coli.*

CHAPTER 5 Conclusion

Summary of dissertation findings

Anemia among young children in low- and middle-income countries (LMICs) is a leading public health concern, and remains challenging to address given its many underlying causes, including micronutrient deficiencies, infections, and genetic disorders, among others (1,2). As this dissertation has discussed, small-scale livestock production may help to alleviate child anemia associated with micronutrient deficiencies by providing a direct source of nutrient-dense animal-source foods (ASFs) (3–8). Yet livestock fecal contamination may also expose children to enteric pathogens that can increase children's risk of anemia by way of increased inflammation and impaired nutrient absorption (9–11). The overarching objective of this dissertation was to examine these potentially contradictory pathways by assessing the association between livestock ownership and child anemia, and investigate the nutritional and infectious disease pathways underlying this association. Using a cross-sectional design involving children 6 to 59 months old in two districts of Greater Accra, Ghana, we collected individual- and household-level survey data, blood and stool samples from children, and stool samples from chickens. The biological samples were tested for infections and indicators of inflammation and anemia, and then analyzed for associations between indicators of household livestock ownership and management, children's iron and inflammatory status, anemia, malaria parasitemia, helminth infections, and bacterial enteropathogen infections.

In Chapter 2, the overall association between household livestock ownership and child anemia was assessed, including whether relationships were mediated by child ASF consumption or by child morbidity and inflammation. Nearly half (48%) of children were anemic. Anemia was associated with iron deficiency, vitamin A deficiency, systemic inflammation, and malaria, and was more common among children 6 to 23 months old. We categorized household livestock ownership into five categories based on the types and number of livestock owned. Slightly more than half of households owned no livestock (Type 1), 15% owned less than 12 poultry (Type 2), 13% owned 12 or more poultry (Type 3), 13% owned small livestock (goats, sheep, or pigs) with or without poultry (Type 4), and 3% owned cattle with or without small livestock or poultry (Type 5). We found that children in Type 5 households, but not those in other livestock-owning typologies, had lower odds of anemia compared to children from households with no livestock. Owning more cattle or goats, sheep, and pigs was also associated with lower odds of anemia. However, no associations with iron deficiency were observed. Importantly, contrary to our hypothesis, poultry ownership was not associated with higher odds of anemia, nor did children from livestock-owning households have greater risk of systemic inflammation or symptoms of illness (diarrhea, fever, cough or cold) compared to children in non-livestock-owning households.

We thus investigated whether ASF consumption mediated the association between livestock ownership and lower odds of anemia. Chicken meat and eggs were the most commonly consumed livestock-derived ASF in the three months prior to the survey, and children in Type 3 households were more likely to have consumed chicken meat than children in Type 1 households. Almost half of children in Type 5 had consumed fresh cow's milk in the prior three months compared to 11-19% in the other livestock types and 6% in non-livestock owning

households. However, there was no evidence that chicken meat or cow milk consumption mediated the association between livestock ownership and lower odds of anemia. Rather, we found that overall own-consumption of livestock and livestock products was uncommon. Only one-third of poultry-owning households consumed meat or eggs from their own livestock holdings in the prior three months, and less than one-fifth of small livestock owning households had used their animals for household consumption in the prior year. As qualitative research conducted in Ghana has shown (12), households typically keep larger livestock for financial purposes, and poultry for finances and for food. Overall, the results from this aim indicate that ownership of cattle and small livestock, may lower children's risk of anemia, though this does not appear to be via a dietary pathway.

To explore potential negative effects of animal infections on child anemia, analyses in Chapter 3 assessed whether livestock ownership, exposure to animal feces, and consumption of animal-source foods, were associated with children's bacterial zoonotic enteropathogen infections. In a subset of children from the study sample used in Chapter 2, we measured bacterial enteropathogens in children's stool and found that 46% of children were positive for atypical enteropathogenic *Escherichia coli (E. coli)* (aEPEC), 12% for *Campylobacter jejuni/coli* (*C. jejuni/coli*), 4% for Shiga-toxin producing *E. coli* (STEC), and <1% for *Salmonella*. No livestock-related risk factors were associated with aEPEC or *C. jejuni/coli* infection in children, yet 39% of chicken fecal samples contained aEPEC and 45% had *C. jejuni/coli*. Associations with *Salmonella* were not examined given the low prevalence of infection in the study sample. STEC was only detected among children living in the Shai Osudoku district, and infection was associated with household ownership of goats or sheep, consumption of fresh cow's milk at least once per week, and STEC detection in household chicken feces. Thus, contrary to our

hypothesis, we did not find associations between poultry ownership and *Campylobacter* infection in children. However, most households' chickens, goats, and sheep roamed freely in the household yard or compound during the day, suggesting that community-wide contamination with livestock feces is likely. Indeed, we observed widespread fecal contamination from livestock in the yards of both livestock-owning and non-livestock-owning households. Additionally, humans in almost one-third of households practiced open defecation, which was associated with higher odds of aEPEC and STEC infection in children, suggesting that human fecal contamination serves as a transmission route for infection with these pathogens. Overall results from this aim showed that ruminants in southern Ghana may expose children to STEC infection through fecal contamination and foodborne routes. Further, livestock ownership was not associated with aEPEC and *C. jejuni/coli* in children at the household level, despite high prevalences in both children and chickens.

In Chapter 4, we investigated whether bacterial enteropathogen infections in children were associated with indicators of inflammation, iron deficiency, and anemia. Enteropathogens were detected in 87% of children's stool, yet infections were predominantly subclinical. Enteroaggregative *E. coli* (EAEC) and aEPEC infections were most prevalent, being detected in 59% and 46% of children's stool, respectively. We evaluated relationships by individual enteropathogens and by pathogen groupings: infection with enteroinvasive pathogens only (*C. jejuni/coli*, EAEC, enteroinvasive *E. coli* (EIEC)/*Shigella*, *Salmonella*, STEC), non-invasive pathogens only (typical EPEC (tEPEC), aEPEC, heat-stable (ST)/heat-labile (LT) enterotoxin producing *E. coli* (ST-ETEC or LT-ETEC)), or co-infection with invasive and non-invasive enteropathogens. *C. jejuni/coli* infection was present in 11% of children, and was associated with higher odds of elevated C-reactive protein (CRP) and α-1-acid glycoprotein concentrations

(AGP), while no other individual enteropathogens were associated with higher odds of systemic inflammation. However, children infected with one or more invasive pathogens had higher odds of elevated AGP compared to those infected with only non-invasive pathogens, while co-infection with invasive and non-invasive pathogens was not associated with inflammation. Interestingly, aEPEC infection was associated with lower odds of inflammation, which may reflect EPEC's evasion of the mucosal immune system via downregulation of inflammatory signaling (13). Infection with invasive pathogens was associated with higher odds of anemia and lower hemoglobin concentrations in comparison to infection with non-invasive pathogens, and associated with elevated serum transferrin receptor (indicative of low circulating iron). In analyses by individual enteropathogens, only EIEC/*Shigella* and EAEC were associated with higher odds of anemia. Infection with EIEC/*Shigella* was also the only pathogen associated with higher odds of low serum ferritin concentrations (indicative of low iron storage). In stratified analysis, children who did not meet the minimum dietary diversity (MDD) indicator had higher odds of anemia if infected with EAEC or EIEC/*Shigella*, while these associations were attenuated and no longer significant among children who met the MDD. Thus, in line with our hypothesis, enteropathogen infection was associated with inflammation, iron deficiency, and anemia; however, these relationships were not true for all enteropathogens examined. Rather, infections with enteroinvasive pathogens, but not non-invasive pathogens, appeared to contribute to anemia, possibly through mechanisms involving systemic inflammation. Importantly, this aim provides evidence that certain subclinical enteropathogen infections may contribute to the anemia burden among young Ghanaian children.

Untangling the relationship between livestock ownership and anemia

Taken together, the results presented in this dissertation demonstrate a complex picture of the potential benefits and drawbacks of livestock ownership on child anemia. On one hand, ownership of cattle, alongside small livestock and poultry, was negatively associated with anemia, indicating a benefit of owning large livestock. We hypothesize this beneficial association may be through greater purchasing power via livestock and livestock product sales, as livestock were predominantly kept for income and not household meat, egg, or milk consumption. Nevertheless, there were clear infectious disease risks associated with livestock rearing. Firstly, livestock contributed to fecal contamination of household environments, even among non-livestock owning households. Secondly, household chickens carried several pathogenic bacteria in their feces, including *C. jejuni/coli*, aEPEC, and STEC. Thirdly, ownership of goats and sheep, exposure to STEC in chicken feces, and consumption of fresh cow's milk was associated with STEC infection in children, particularly in cattle-rearing communities. Despite these risks, we found no associations between livestock ownership and inflammation or illness symptoms in children, nor associations with iron deficiency or anemia. Results from Chapter 4 may help explain this seemingly contradictory result. No zoonotic enteropathogens were individually associated with iron deficiency or anemia. Rather, only EAEC and EIEC/*Shigella* infections were associated with anemia, and both of these enteric pathogens are transmitted via human fecal-oral contamination of food and water, and have no known livestock reservoirs (14). Further, although *C. jejuni/coli* was associated with inflammation in children in this study sample, it was not associated with livestock ownership.

Contrary to our findings, other cross-sectional studies conducted in Ghana have identified positive associations between ownership of individual livestock species and anemia in young

children, particularly of poultry (15) and sheep and goats (16). An analysis of Demographic Health Surveys in 28 sub-Saharan African countries also found that childhood anemia was greater in households that owned cattle, sheep, or goats (17). These studies hypothesized that livestock may expose children to enteric pathogens that lead to anemia. In the context of other work that has found associations between poultry ownership and *Campylobacter* infection in children (18–21) and based on our work that showed *Campylobacter* infection is significantly associated with systemic inflammation, perhaps *Campylobacter* infection underlies these positive associations between livestock ownership and anemia. Contrary results in our study compared to prior studies may reflect different livestock rearing contexts. Our study was conducted in the wealthiest region of Ghana using a sample of peri-urban and semi-rural communities where very few households kept their livestock indoors at night. While the studies just cited did not include data on animal management practices, research from other reports on livestock ownership suggest that higher contact and exposure to livestock, for example by keeping animals inside at night and having frequent physical interactions with animals, increases risk of pathogen infection (21–23). Thus, associations between livestock ownership and anemia are context-specific, and depend on the ways in which households, and livestock-rearing communities at large, use and manage their livestock. More extensive research is needed to further understand context-specific associations between livestock ownership and use, disease exposure, and anemia.

Dissertation strengths and limitations

This dissertation adds to the literature in several important ways. First, we gathered detailed data on livestock ownership that included not just the types and quantities of livestock owned, but also livestock housing management, the sale of and income from livestock and livestock production, and consumption of own-produced livestock and livestock products.

Coupled with observations of free-roaming livestock and livestock feces in the yard, these data allowed us to observe several dimensions of how households use and manage their livestock for deeper interpretation of our results. Second, we collected detailed data on children's consumption of many types of ASFs allowing for disaggregated analysis of associations by livestock species, and we used a recall period of three months which allowed for the assessment of infrequently consumed foods. Furthermore, we captured children's frequency of consumed foods, how foods were consumed (e.g., boiled versus raw milk), and whether foods were from own livestock or were purchased. As with the livestock data, this allowed for a more complete and detailed understanding of children's ASF consumption. Third, we assessed iron and inflammatory biomarkers that allowed for assessment of potential mechanisms linking livestock and enteropathogen infections to anemia. Inflammatory measures, in particular, build on existing literature by quantifying the body's biologic response to infection. Combined with molecular measurement of several enteric pathogens, these data provided more sensitive and precise measures of infection rather than the use of proxy measures of infection such as diarrhea that a) do not capture subclinical infections and b) are subject to recall bias. Iron biomarkers allowed for assessment of children's iron status, which provided insights into the underlying etiology of anemia among our study sample.

However, the findings from this dissertation should be interpreted in light of several limitations. First, as a cross-sectional study, we cannot determine causality between the relationships investigated. Our study is subject to issues of residual confounding and temporality. Though we attempted to control for potential confounding factors in our models, there remains the possibility that households with livestock differed in some way from those without livestock which influenced children's anemia status. For example, livestock ownership may be correlated

with household wealth (24,25). Although we controlled for socioeconomic status using an asset-based wealth index, durable assets may not always accurately represent the multi-faceted dimensions of household socioeconomic status that could influence the health and well-being of household members. In addition, relationships along the hypothesized causal pathway between livestock ownership, infection, inflammation, and anemia and between livestock, ASF consumption, micronutrient status, and anemia are temporal. Exposure to pathogens from livestock, for example, may take days to manifest as infections, and then days to weeks to produce changes in inflammation, iron status, and anemia. We may thus have missed important associations by collecting data at only one time point. Second, we did not assess the quantity of ASFs consumed by children, which may have prevented us from seeing any associations in mediation analyses. There may be a threshold for ASF consumption quantity that is necessary to impact children's micronutrient status. Third, we did not measure how much livestock-derived income contributed to household earnings relative to total income, nor how livestock-derived income was used for purchases. This limited us from assessing whether income from livestock mediated the association between ownership and child anemia.

Future research

The findings presented in this dissertation suggest several areas for future research that should further improve understanding of the relationships between livestock ownership and anemia. First, our study and others (12,15,26) have shown that household livestock are not prioritized for food. However, we were unable to investigate whether livestock-derived income acts as a mediator of associations between livestock ownership and lower odds of child anemia. Future research examining how livestock-derived income contributes to overall household income, which household member controls this income, and decision-making around use of that

income, should help to elucidate whether livestock-derived income contributes to purchases that improve children's health. Given that women and children can own poultry, but men often control larger livestock assets (6), future studies should disaggregate livestock-derived income by livestock species and by the quantity of livestock owned. Such investigations could help inform how livestock-based interventions can leverage livestock-derived income for improved child nutrition outcomes, such as encouraging caregivers to purchase small quantities of ASF and other nutrient-rich fruits and vegetables.

Second, our perplexing finding that household-level livestock ownership was not associated with *Campylobacter* or aEPEC infection, despite high prevalences of these pathogens in both children and chickens, warrants further investigation. Because livestock are often free-roaming in communities, thus depositing feces near non-livestock-owning households, more detailed analysis could help explain whether neighborhood livestock elevate fecal pathogen exposure risk to all children in a community. Thereby, childhood exposure may depend on frequency of contact and exposure to animals and animal feces, independent of ownership. More extensive observation of children's interactions with livestock is warranted, such as that of Ngure et al. and Reid et al. (27,28), to quantify whether exposure modifies the risks of zoonotic transmission.

Third, our findings highlight potentially important relationships between bacterial enteric pathogen infection and anemia, though much work remains to be done to elucidate the underlying mechanisms. Our study findings need to be confirmed using longitudinal cohort data. Furthermore, to better understand how bacterial enteropathogens may lead to altered iron status and anemia, more extensive inquiry is required that assesses markers of environmental enteric

dysfunction and hepcidin, in conjunction with inflammatory biomarkers, iron status biomarkers, and hemoglobin to model pathways between enteric pathogen infection and anemia.

Public and planetary health implications

It is widely recognized that the burden of anemia among young children in LMICs, including in Ghana, has complex and multifactorial origins and mechanisms. Consistent with findings of others who analyzed the 2017 Ghana Micronutrient Survey data (29,30), we demonstrated that anemia among children 6 to 59 months old in our sample was associated with a multitude of nutritional and infectious disease factors (including iron deficiency, vitamin A deficiency, inflammation, and malaria), highlighting the complexity of tackling this condition. Importantly, we also found that subclinical infection with certain bacterial enteropathogens may contribute to anemia among these young children. Enteric infections may be transmitted to children via poor water, sanitation, and hygiene (WASH) infrastructure and practices, and poor WASH is associated with anemia in children (31,32). Thus, in contexts where child anemia is primarily due to infectious diseases, mitigating anemia must include reducing exposure to enteropathogens, which could include efforts to end open defecation and improve latrines, provide universal access to clean drinking water, and improve hygiene practices, as well as other medical advancements such as vaccines. Preventing transmission of enteric pathogens to humans through improved WASH, however, will be insufficient to reduce the overall enteropathogen burden among young children. There is a renewed call for "transformative WASH," (33), which includes addressing fecal contamination from animals (34). Recognizing the importance of livestock to the livelihoods of millions of people, such "transformation" does not preclude livestock rearing, but rather would require strategies that severely limit transmission of enteric pathogens from animals to humans. Livestock provide many essential benefits to households,

and understanding ways to reduce livestock-associated infections while enhancing livestock-based nutrition is essential for improving child health and development.

There is also need to understand how to harness the benefits of livestock in a manner that is context-appropriate. In many settings, as was found in this dissertation research, livestock are primarily reared for financial purposes (12,35,36), yet livestock-based interventions have focused on shifting behavior towards consuming own-derived ASFs. If this is not a cultural norm, a shift in research and program design is needed that recognizes and incorporates decision-making around the allocation of livestock-derived income, and how supplemental income is used to improve child nutrition and health. By highlighting infectious disease contributors to child anemia, findings presented in this dissertation should not be seen as suggesting that nutritional determinants of anemia are irrelevant or unimportant. Rather, multi-faceted approaches that target nutrient deficiencies combined with infectious disease control will be necessary to meaningfully reduce the anemia burden among young children. Therefore, consuming ASFs, even in small quantities, along with a diversity of vitamin-rich fruits and vegetables, is still essential to reducing childhood anemia.

Many questions remain about the role of livestock in the global food system. In addition to associated infectious disease risks, livestock are major contributors to anthropogenic greenhouse gas emissions, land and water use, deforestation, and environmental pollution (3,37). In light of global climate change and an urgent need to reduce humanity's environmental footprint, there have been calls to reduce ASF consumption globally (38). Yet, ASF are not equitably consumed globally, and, as discussed throughout this dissertation, ASF consumption may be especially important for young children and other vulnerable populations living in LMICs (37,39). Finding an equitable balance between the risks and benefits of livestock for

global nutrition in the next decade, particularly in an increasingly vulnerable climate, will require innovative strategies to reduce malnutrition while also preventing further contributions to climate change.

References

1. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. Lancet. 2011;378:2123–35.
2. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019;1450:15–31.
3. Herrero M, Grace D, Njuki J, Johnson N, Enahoro D, Silvestri S, Rufino MC. The roles of livestock in developing countries. Animal. 2013;7:3–18.
4. Ruel MT, Quisumbing AR, Balagamwala M. Nutrition-sensitive agriculture: What have we learned so far? Glob Food Sec. 2018;17:128–53.
5. Alders RG, Dumas SE, Rukambile E, Magoke G, Maulaga W, Jong J, Costa R. Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. Matern Child Nutr. 2018;14:1–14.
6. Wong JT, de Bruyn J, Bagnol B, Grieve H, Li M, Pym R, Alders RG. Small-scale poultry and food security in resource-poor settings: A review. Glob Food Sec. 2017;15:43–52.
7. Iannotti LL, Lutter CK, Bunn DA, Stewart CP. Eggs: The uncracked potential for improving maternal and young child nutrition among the world's poor. Nutr Rev. 2014;72:355–68.
8. Neumann C, Harris DM, Rogers LM. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutr Res. 2002;22:193–220.
9. Penakalapati G, Swarthout J, Delahoy MJ, McAliley L, Wodnik B, Levy K, Freeman MC. Exposure to animal feces and human health: a systematic review and proposed research priorities. Environ Sci Technol. 2017;51:11537–52.
10. Delahoy MJ, Wodnik B, McAliley L, Penakalapati G, Swarthout J, Freeman MC, Levy K. Pathogens transmitted in animal feces in low- and middle-income countries. Int J Hyg Environ Health. 2018;221:661–76.
11. Jonker FAM, te Poel E, Bates I, Boele van Hensbroek M. Anaemia, iron deficiency and susceptibility to infection in children in sub-Saharan Africa, guideline dilemmas. Br J Haematol. 2017;177:878–83.
12. Nyantakyi-Frimpong H, Colecraft EK, Awuah RB, Adjorlolo LK, Wilson ML, Jones AD. Leveraging smallholder livestock production to reduce anemia: A qualitative study of three agroecological zones in Ghana. Soc Sci Med. 2018;212:191–202.
13. Pearson JS, Giogha C, Wong Fok Lung T, Hartland EL. The genetics of enteropathogenic *Escherichia coli* virulence. Annu Rev Genet. 2016;50:493–513.
14. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev. 2013;26:822–80.
15. Jones AD, Colecraft EK, Awuah RB, Boatemaa S, Lambrecht NJ, Adjorlolo LK, Wilson ML. Livestock ownership is associated with higher odds of anaemia among preschool-aged children, but not women of reproductive age in Ghana. Matern Child Nutr. 2018;14:1–13.
16. Christian AK, Wilson ML, Aryeetey RNO, Jones AD. Livestock ownership, household food security and childhood anaemia in rural Ghana. PLoS One. 2019;14:1–14.
17. Acharya Y, Yang D, Jones AD. Livestock ownership and anaemia in Sub-Saharan African households. Public Health Nutr. 2020;1–12.
18. Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated

with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2014;108:313–25.
19. El-Tras WF, Holt HR, Tayel AA, El-Kady NN. *Campylobacter* infections in children exposed to infected backyard poultry in Egypt. Epidemiol Infect. 2015;143:308–15.
20. Vasco K, Graham JP, Trueba G. Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in Ecuador. Appl Environ Microbiol. 2016;82:4218–24.
21. Budge S, Barnett M, Hutchings P, Parker A, Tyrrel S, Hassard F, Garbutt C, Moges M, Woldemedhin F, Jemal M. Risk factors and transmission pathways associated with infant *Campylobacter spp*. prevalence and malnutrition: A formative study in rural Ethiopia. PLoS One. 2020;15:e0232541.
22. Lowenstein C, Vasco K, Sarzosa S, Salinas L, Torres A, Perry MJ, Simmens SJ, Trueba G, Eisenberg JNS, Graham JP. Determinants of childhood zoonotic enteric infections in a semirural community of Quito, Ecuador. Am J Trop Med Hyg. 2020;102:1269–78.
23. Headey D, Hirvonen K. Is exposure to poultry harmful to child nutrition? An observational analysis for rural Ethiopia. PLoS One. 2016;11:e0160590.
24. de Bruyn J, Thomson PC, Darnton-Hill I, Bagnol B, Maulaga W, Alders RG. Does village chicken-keeping contribute to young children's diets and growth? A longitudinal observational study in rural Tanzania. Nutrients. 2018;10:1799.
25. Pica-Ciamarra U, Tasciotti L, Otte J, Zezza A. Livestock in the household economy: Cross-country evidence from microeconomic data. Dev Policy Rev. 2015;33:61–81.
26. Colecraft E, Marquis GS, Aryeetey R, Sakyi-Dawson O, Lartey A, Ahunu B, Canacoo E, Butler LM, Reddy MB, Jensen HH, et al. Constraints on the use of animal source foods for young children in Ghana: A participatory rapid appraisal approach. Ecol Food Nutr. 2006;45:351–77.
27. Ngure F, Gelli A, Becquey E, Ganaba R, Headey D, Huybregts L, Pedehombga A, Sanou A, Traore A, Zongo F, et al. Exposure to livestock feces and water quality, sanitation, and hygiene (WASH) conditions among caregivers and young children: formative research in rural Burkina Faso. Am J Trop Med Hyg. 2019;100:998–1004.
28. Reid B, Orgle J, Roy K, Pongolani C, Chileshe M, Stoltzfus R. Characterizing potential risks of fecal–oral microbial transmission for infants and young children in Rural Zambia. Am J Trop Med Hyg. 2018;98:816–23.
29. Wegmüller R, Bentil H, Wirth JP, Petry N, Tanumihardjo SA, Allen L, Williams TN, Selenje L, Mahama A, Amoaful E, et al. Anemia, micronutrient deficiencies, malaria, hemoglobinopathies and malnutrition in young children and non-pregnant women in Ghana: Findings from a national survey. PLoS One. 2020;15:1–19.
30. Petry N, Wirth JP, Adu-Afarwuah S, Wegmuller R, Woodruff BA, Tanumihardjo SA, Bentil H, Donkor WES, Williams TN, Shahab-Ferdows S, et al. Risk factors for anaemia among Ghanaian women and children vary by population group and climate zone. Matern Child Nutr. 2020;1–10.
31. Kothari MT, Coile A, Huestis A, Pullum T, Garrett D, Engmann C. Exploring associations between water, sanitation, and anemia through 47 nationally representative demographic and health surveys. Ann N Y Acad Sci. 2019;1450:nyas.14109.
32. Yu EX, Addo OY, Williams AM, Engle-Stone R, Ou J, Huang W, Guo J, Suchdev PS, Young MF. Association between anemia and household water source or sanitation in preschool children: the Biomarkers Reflecting Inflammation and Nutritional Determinants

of Anemia (BRINDA) project. Am J Clin Nutr. 2020;112:488S-497S.
33. Pickering AJ, Null C, Winch PJ, Mangwadu G, Arnold BF, Prendergast AJ, Njenga SM, Rahman M, Ntozini R, Benjamin-Chung J, et al. The WASH Benefits and SHINE trials: interpretation of WASH intervention effects on linear growth and diarrhoea. Lancet Glob Heal. 2019;7:e1139–46.
34. Prendergast AJ, Gharpure R, Mor S, Viney M, Dube K, Lello J, Berger C, Siwila J, Joyeux M, Hodobo T, et al. Putting the "A" into WaSH: a call for integrated management of water, animals, sanitation, and hygiene. Lancet Planet Heal. 2019;3:e336–7.
35. Bundala N, Kinabo J, Jumbe T, Rybak C, Sieber S. Does homestead livestock production and ownership contribute to consumption of animal source foods? A pre-intervention assessment of rural farming communities in Tanzania. Sci African. 2020;7.
36. Haileselassie M, Redae G, Berhe G, Henry CJ, Nickerson MT, Tyler B, Mulugeta A. Why are animal source foods rarely consumed by 6-23 months old children in rural communities of Northern Ethiopia? A qualitative study. PLoS One. 2020;15:e0225707.
37. Fanzo J, Davis C, McLaren R, Choufani J. The effect of climate change across food systems: Implications for nutrition outcomes. Glob Food Sec. 2018;18:12–9.
38. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 2019;393:447–92.
39. Adesogan AT, Havelaar AH, McKune SL, Eilittä M, Dahl GE. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. Glob Food Sec. 2020;25.

Appendices

Appendix A: Supplemental Tables

Table A.1 Primer and probe sequences for qPCR detection of pathogen gene targets and assay performance characteristics[1]

Pathogen	Target Gene	Primer and probe sequences[2] (F: Forward primer, R: Reverse primer, P: Probe)	Ref	PCR efficiency, % (95% CI)	PCR R2
C. jejuni/C. coli	*cadF*	F: CTGCTAAACCATAGAAATAAAATTTCTCAC R: CTTTGAAGGTAATTTAGATATGGATAATCG P: CATTTTGACGATTTTTGGCTTGA	(1)	88.8 (88.1, 89.5)	1.000
EPEC	*eae*	F: CATTGATCAGGATTTTTCTGGTGATA R: CTCATGCGGAAATAGCCGTTA P: CGAATACTGGCGAGACTATTTCAA	Modified from (1)[3]	87.3 (77.3, 100.1)	0.982
EPEC	*bfpA*	F: TGGTGCTTGCGCTTGCT R: CGTTGCGCTCATTACTTCTG P: CAGTCTGCGTCTGATTCCAA	(1)	107.9 (102.2, 114.3)	0.997
STEC	*stx1*	F: ACTTCTCGACTGCAAAGACGTATG R: ACAAATTATCCCCTGWGCCACTATC P: CTCTGCAATAGGTACTCCA	(1)	105.2 (96.7, 115.2)	0.990
STEC	*stx2*	F: CCACATCGGTGTCTGTTATTAACC R: GGTCAAAACGCGCCTGATAG P: TTGCTGTGGATATACGAGG	(1)	112.8 (110.6, 115.0)	1.000
EAEC	*aatA*	F: CTGGCGAAAGACTGTATCAT R: TTTTGCTTCATAAGCCGATAGA P: TGGTTCTCATCTATTACAGACAGC	(1)	87.6 (85.9, 89.3)	0.999
EAEC	*aaiC*	F: ATTGTCCTCAGGCATTTCAC R: ACGACACCCCTGATAAACAA P: TAGTGCATACTCATCATTTAAG	(1)	97.3 (96.2, 98.4)	1.000
LT-ETEC	*LT*	F: TTCCCACCGGATCACCAA R: CAACCTTGTGGTGCATGATGA	(1)	105.7 (103.7, 107.8)	0.999

		P: CTTGGAGAGAAGAACCCT			
ST-ETEC	*STh*	F: TTCACCTTTCGCTCAGGATG R: AGCACCCGGTACAAGCAG P: ATTACTGCTGTGAATTGTG	(2)	95.6 (94.8, 96.4)	1.000
ST-ETEC	*STp*	F: TGAATCACTTGACTCTTCAAAA R: GGCAGGATTACAACAAAGTT P: TGAACAACACATTTTACTGCT	(1)	95.0 (89.4, 101.3)	0.990
EIEC/*Shigella*	*ipaH*	F: CCTTTTCCGCGTTCCTTGA R: CGGAATCCGGAGGTATTGC P: CGCCTTTCCGATACCGTCTCTGCA	(1)	91.9 (91.1, 92.7)	1.000
Salmonella enterica	*ttr*	F: CTCACCAGGAGATTACAACATGG R: AGCTCAGACCAAAAGTGACCATC P: CACCGACGGCGAGACCGACTTT	(1)	N/A[4]	N/A
V. cholerae	*hlyA*	F: ATCGTCAGTTTGGAGCCAGT R: TCGATGCGTTAAACACGAAG P: TCGATGCGTTAAACACGAAG	(1)	N/A[4]	N/A

[1]PCR efficiency and R^2 of the dilution series curve was determined using pooled known positives from child stool samples in four-fold dilutions with four replicates at each dilution. Abbreviations: C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *Escherichia coli (E. coli)*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; ST-ETEC, heat-stable enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli*, *V. cholerae*, *Vibrio cholerae;* CI, Confidence Interval; qPCR, quantitative Polymerase Chain Reaction

[2]All probes were double-quencher probes with a 5' 6-FAM™ fluorophore, internal ZEN™ quencher, and 3' Iowa Black® Fluorescent Quencher.

[3]Base pairs CGA were added to the 5' end of the probe sequence to increase melting temperature.

[4]An insufficient number of positive samples precluded running a PCR efficiency curve.

References

1. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, Mduma E, Platts-Mills J, Boisen N, Nataro J, et al. Optimization of quantitative PCR Methods for enteropathogen detection. PLoS One. 2016;11:e0158199. DOI: 10.1371/journal.pone.0158199
2. Taniuchi M, Walters CC, Gratz J, Maro A, Kumburu H, Serichantalergs O, Sethabutr O, Bodhidatta L, Kibiki G, Toney DM, et al. Development of a multiplex polymerase chain reaction assay for diarrheagenic *Escherichia coli* and *Shigella spp.* and its evaluation on colonies, culture broths, and stool. Diagn Microbiol Infect Dis; 2012;73:121–8. DOI: 10.1016/j.diagmicrobio.2012.03.008

Table A.2 Pathogen gene target detection and Ct values among positive samples detected by qPCR in stool of children (n=262)

Pathogen (Gene)	Number of Positive Samples	Ct Mean ± SD	Ct Median	Ct Range
C. jejuni/C. coli (*cadF*)	29	28.0 ± 4.8	28.4	17.8-34.5
EPEC (*eae*)	163	28.1 ± 4.5	29.0	17.4-34.8
EPEC (*bfpA*)	22	26.0 ± 4.7	25.8	17.2-34.8
STEC (*stx1*)	17	32.7 ± 1.8	32.4	28.7-34.9
STEC (*stx2*)	13	32.8 ± 1.7	32.1	29.6-34.9
EAEC (*aatA*)	140	27.3 ± 5.1	28.3	13.8-34.7
EAEC (*aaiC*)	78	27.1 ± 5.1	26.3	18.7-34.9
LT-ETEC (*LT*)	47	29.1 ± 3.8	29.2	21.4-34.9
ST-ETEC (*STh*)	9	25.6 ± 7.3	21.8	17.7-34.2
ST-ETEC (*STp*)	12	29.2 ± 4.1	30.2	20.2-33.6
EIEC/*Shigella* (*ipaH*)	40	29.1 ± 3.9	29.8	20.7-34.9
Salmonella (*ttr*)	2	34.6 ± 0.6	34.6	34.2-34.9
V cholerae (*hlyA*)	0	-	-	-

Results from qPCR for 45 cycles with a cut-off at Ct≤35. Ct (cycle threshold) values are inversely proportional to the pathogen load. Under conditions of 100% PCR efficiency, a change in Ct of 3.3 cycles is equivalent to a 10-fold difference in concentration.
Abbreviations: C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EAEC, enteroaggregative *Escherichia coli (E. coli)*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; LT-ETEC, heat-labile enterotoxin-producing *E. coli*; ST-ETEC, heat-stable enterotoxin-producing *E. coli*; STEC, Shiga toxin-producing *E. coli*, *V. cholerae*, *Vibrio cholerae;* SD, standard deviation; qPCR, quantitative Polymerase Chain Reaction

Table A.3 Pathogen gene target detection and Ct values detected by qPCR in fecal samples from chickens (n=156)

Pathogen (Gene)	Number of Positive Samples	Ct Mean ± SD	Ct Median	Ct Range
C. jejuni/C. coli (*cadF*)	70	31.9 ± 4.0	32.0	22.0-40.0
EPEC (*eae*)	88	31.4 ± 3.9	31.9	20.5-37.7
EPEC (*bfpA*)	10	32.6 ± 3.5	32.1	27.0-37.0
STEC (*stx1*)	23	34.5 ± 2.6	34.8	26.6-38.0
STEC (*stx2*)	18	33.8 ± 3.1	35.0	24.5-36.8
Salmonella (*ttr*)	6	31.0 ± 3.1	31.8	26.6-34.2

Results from qPCR for 45 cycles with a cut-off at Ct≤40. Ct (cycle threshold) values are inversely proportional to the pathogen load. Under conditions of 100% PCR efficiency, a change in Ct of 3.3 cycles is equivalent to a 10-fold difference in concentration.
Abbreviations: C. jejuni/coli, *Campylobacter jejuni* or *Campylobacter coli*; EPEC, enteropathogenic *Escherichia coli (E. coli)*; STEC, Shiga toxin-producing *E. coli;* SD, standard deviation; qPCR, quantitative Polymerase Chain Reaction

Table A.4 Classification of enteric pathogens by gene target presence or absence

Pathogen	Gene Target
Typical enteropathogenic *E. coli* (tEPEC)	*eae+, bfpA+, stx-1, stx2-*
Atypical enteropathogenic *E. coli* (aEPEC)	*eae+, bfpA-, stx1-, stx2-*
Shiga toxin-producing *E. coli* (STEC)	*eae+, bfpA-, stx1+ and/or stx2+*
Enteroaggregative *E. coli* (EAEC)	*aatA+ and/or aaiC+*
Heat-stable toxin enterotoxigenic *E. coli* (ST-ETEC)	*STh+ and/or STp+, LT+/-*
Heat-labile toxin ETEC (LT-ETEC)	*LT+, STh-, STp-*
Enteroinvasive *E. coli* (EIEC*)/Shigella*	*ipaH+*
Campylobacter jejuni/coli	*cadF+*
Salmonella enterica	*ttr+*
Vibrio cholerae	*hlyA+*