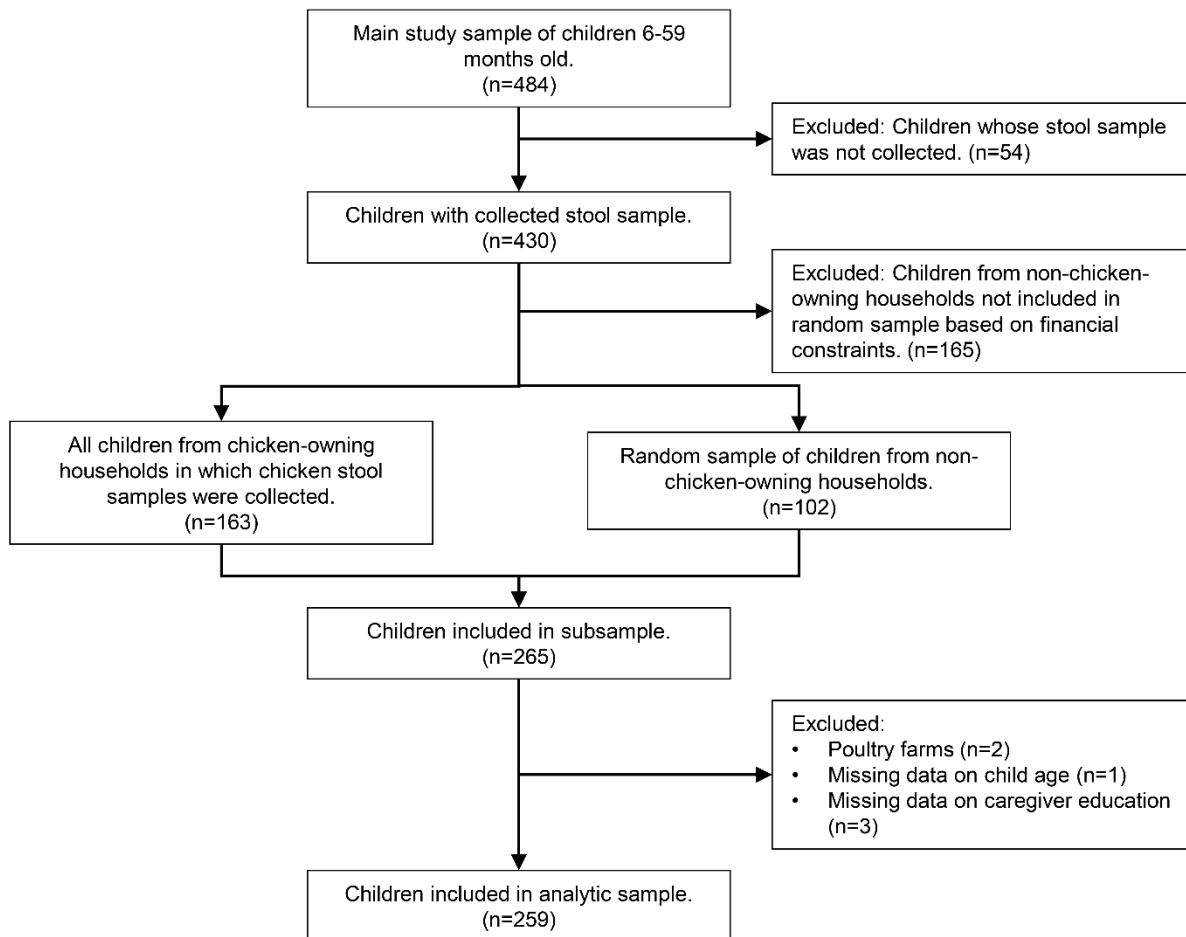**Ruminant-related risk factors are associated with Shiga toxin-producing *Escherichia coli* infection in children in southern Ghana**
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Supplemental Figure 1. Flow chart describing sample selection.

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Supplemental Figure 2. Chicken from a household's flock entering a stool sample collection pen.

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Supplemental Table 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
Salmonella enterica	*ttr*	F: CTCACCAGGAGATTACAACATGG R: AGCTCAGACCAAAAGTGACCATC P: CACCGACGGCGAGACCGACTTT	(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*; EPEC, enteropathogenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*; 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

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Supplemental Table 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 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)

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Supplemental Table 3. Full adjusted logistic regression models predicting odds of enteropathogen detection in children 6-59 months old by livestock-associated risk factors[1]

	Cattle ownership	Goat or sheep ownership	Poultry ownership, with other livestock	Poultry ownership only	Pathogen detected in chicken stool	Animal feces observed around household	Fresh cow's milk consumed ≥1 time/week	Chicken meat consumed ≥1 time/week
	(n=259)	(n=259)	(n=259)	(n=259)	(n=156)	(n=256)	(n=259)	(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** (0.61, 1.35)
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)

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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, 6.16)	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.

[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 Osoduku district.