Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

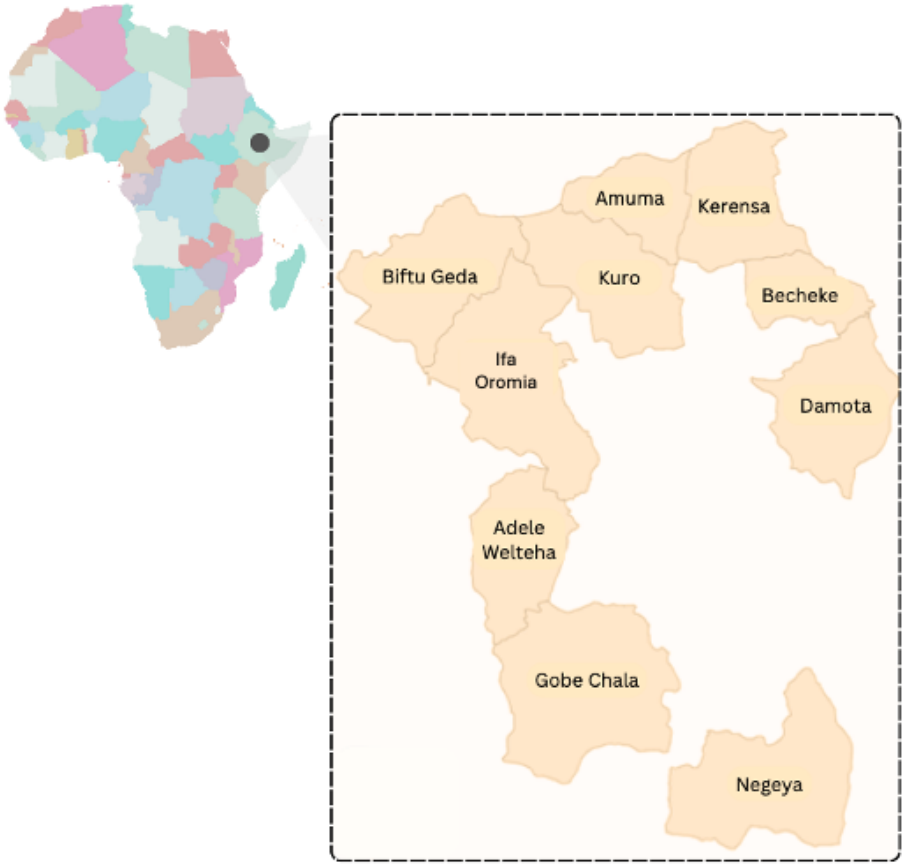


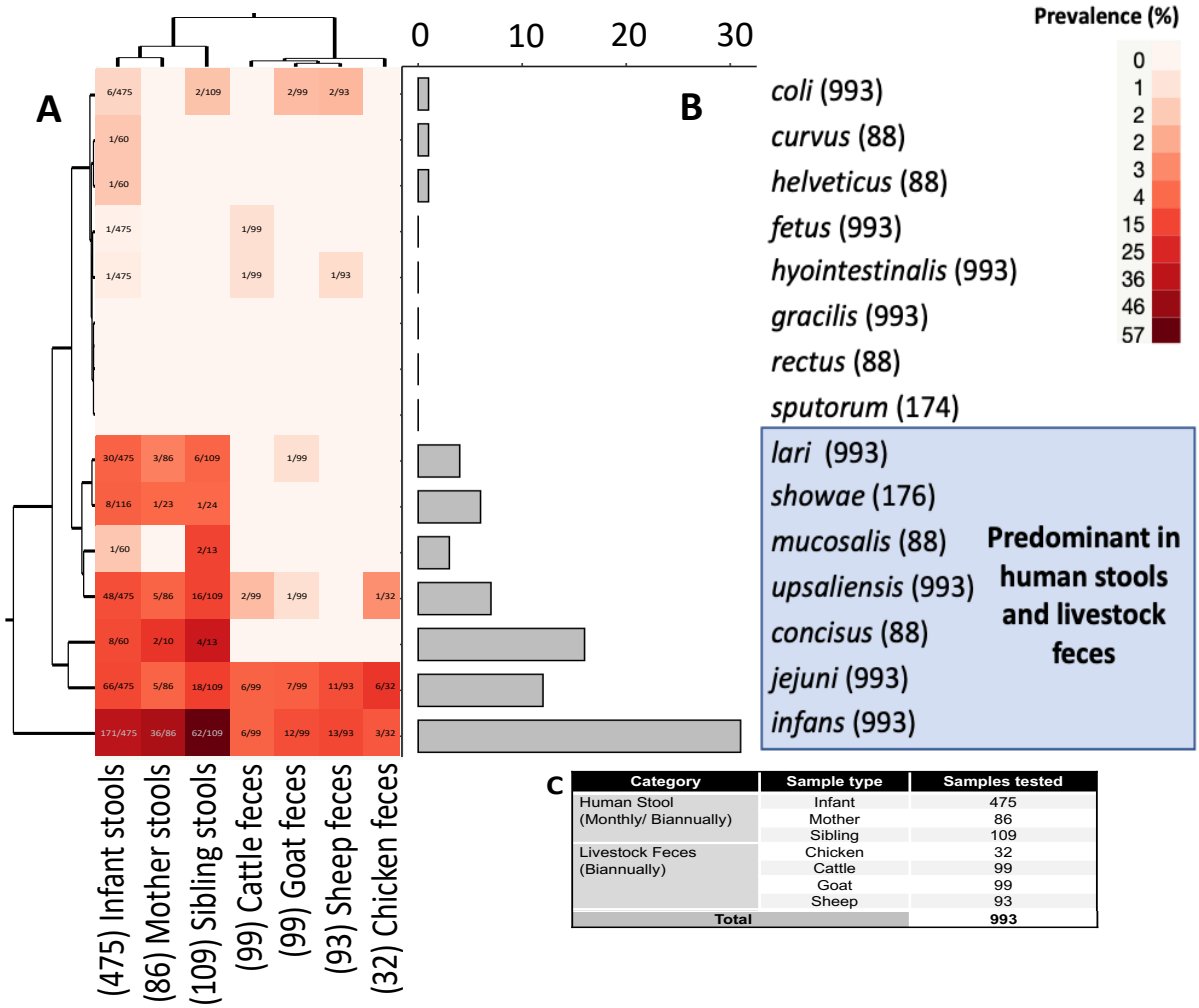
Fig. S1. Geographic locations of the households selected for the CAGED longitudinal study. A total of 106 households located within ten kebeles from the Haramaya woreda in eastern Ethiopia were selected and followed for one year. Additional details about the population size and sample types for each kebele are included in Table S1.

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

DNA Used (50ng)	Species-specific primers														
	C. fetus	*C. helveticus*	*C. hyointestinalis*	*C. lari*	*C. sputorum*	*C. upsaliensis*	*C. concisus*	*C. rectus*	*C. showae*	*C. infans*	*C. jejuni*	*C. gracillis*	*C. curvus*	*C. mucosalis*	*C. coli*
C. coli ATCC33559	42	40	36	42	41	37	42	42	42	42		42	42		15
C. fetus #33293	13	37	37	33	36	35	42	42	38	42	35	42	42	42	
C. helveticus #51209	33	14	34	34	36	27	42	41	39	40	42	42	42	42	
C. hyointestinalis #35217	37	36	13	42	37	36	42	39	39	42	42	42	42	42	
C. jejuni 81-176	42	42	42	42	42	42	42	42	42	42	13	42	42	42	
C. lari #43675	36	38	36	13	36	37	39	42	42	42	33	42	42	42	
C. sputorum #49916	38	38	37	36	14	37	38	42	42	42	42	42	42	42	
C. upsaliensis #49816	34	18	34	28	38	12	41	37	42	42	34	42	42	42	
C. concisus #51561	42	42	42	42	41	42	18	42	42	32	42	42	42	42	
C. rectus #33238	42	42	42	42	38	37	38	24	38	42		42	42	42	
C. showae #51146	42	40	38	38	38	42	40	38	20	36	35	42	42	42	
C. hominus #15827	42	42	41	42	42	42	42	42	42						
C. infans						42				14.3	42	42	42	42	

Fig. S2. *Species-specific primer specificity among Campylobacter species.* A total of 15 *Campylobacter* species-specific primers were tested against several thermophilic and non-thermophilic species, using specific *Campylobacter* primers targeting *hipO* or the *cpn60* gene (Chaban et al, 2009)—50 ng of DNA per reaction with three replicates per species tested. Blank cells reflect species that were not tested. DNA was unavailable for *C. gracillis*, *C. curvus*, and *C. mucosalis*. The cell color corresponds to the Ct value (dark purple to blue = low to high). #refers to American Type Culture Collection (ATCC) number.

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.



C

Category	Sample type	Samples tested
Human Stool (Monthly/ Biannually)	Infant	475
	Mother	86
	Sibling	109
Livestock Feces (Biannually)	Chicken	32
	Cattle	99
	Goat	99
	Sheep	93
Total		993

Fig. S3. Findings tiered approach to *Campylobacter* species testing. (**A**) *Campylobacter* species prevalence in human stools and livestock feces pilot test using two-way clustering plot**.** The color of the cells is proportional to the prevalence values (positive samples / total population tested). Numbers in parentheses indicate the overall population size tested. Empty cells: species not detected. (**B**) Total Prevalence % of *Campylobacter* spp. Detected. Prevalence values were determined by calculating (positive samples / total population tested). The total population tested per species varied based on the number of samples tested. Ct value cut-off of 35 was used to detect *Campylobacter* in the field samples across all species. (**C**) Sampling summary of *Campylobacter* species pilot test**.**

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

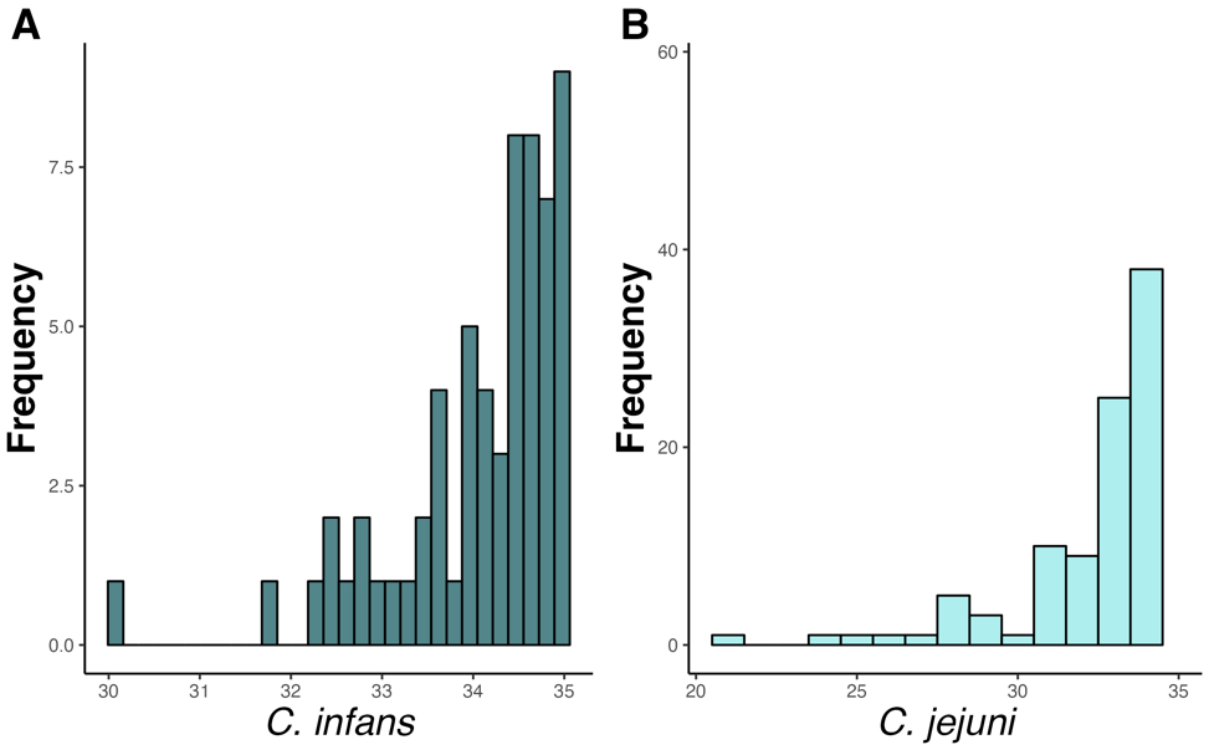


Fig. S4. CT Distribution of Livestock samples positive for (A) *C .infans* and (B) *C. jejuni*.

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

Table S1. Sample size by source for *Campylobacter* spp. Testing using real-time quantitative PCR per kebele.

KEBELES (N=10)	HOUSEHOLDS SELECTED	SAMPLES TESTED	ENVIRONMENT					Human		
			Soil	Cattle	Goat	Chicken	Sheep	Infant	Mother	Sibling
NEGEYA	12	236	32	22	22	15	20	102	11	12
DAMOTA	12	232	26	23	21	13	17	99	17	16
GOBE CHALLA	9	177	29	14	17	8	16	70	10	13
BIFTU GEDA	11	217	22	18	17	10	18	102	13	17
IFA OROMIA	10	192	28	19	18	10	19	79	9	10
AMUMA	11	205	32	17	16	10	18	94	7	11
KURO	11	186	32	18	16	10	14	78	10	8
ADELE WALTA	10	181	6	17	16	9	16	87	16	14
QERENSA DEREBA	12	249	20	21	22	14	23	119	16	14
BACHAQE	8	170	21	14	14	5	13	77	12	14
TOTAL	**106**	**2045**	**248**	**183**	**179**	**104**	**174**	**907**	**121**	**129**

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

Table S2. Determinants of *Campylobacter* species load.

Domain		Determinant[$]	*C.infans* load *(P-value)*		*C.jejuni* load *(P-value)*		*C. upsaliensis* load *(P-value)*	
			Unadjusted	Adjusted[^]	Unadjusted	Adjusted[^]	Unadjusted	Adjusted[^]
Immediate Causes	Inadequate Dietary intake	**Time-varying determinants**						
		Achieved MDD[#]	0.094 (0.688)	-0.136 (0.555)	0.280 (0.409)	0.098 (0.769)	0.696 (0.014)*	0.363 (0.195)
	Disease	**Baseline determinants**						
		WAZ[#] at enrollment	-0.077 (0.249)	-0.061 (0.358)	-0.059 (0.472)	-0.068 (0.409)	-0.140 (0.127)	-0.145 (0.118)
		Underweight at enrollment	0.330 (0.170)	0.280 (0.236)	0.032 (0.913)	0.063 (0.831)	0.212 (0.521)	0.237 (0.479)
Underlying Causes	Household food security	**Time-varying determinants**						
		HFIAS[#]	0.049 (0.001)*	0.024 (0.087)	0.068 (0.000)*	0.040 (0.024)*	0.024 (0.178)	0.010 (0.584)
		Food Secure (i.e., HFIAS = 1)	-0.634 (0.000)*	-0.171 (0.313)	-0.968 (0.000)*	-0.259 (0.246)	-0.296 (0.149)	0.120 (0.557)
	Inadequate care and feeding practices	**Baseline determinants**						
		Colostrum feeding	-0.281 (0.422)	-0.526 (0.253)	4.108 (0.9969)	4.043 (0.997)	5.978 (0.997)	5.885 (0.997)
		Pre-lacteal feeding	-0.092 (0.713)	-0.13 (0.615)	-4.620 (0.994)	-4.536 (0.994)	-6.724 (0.994)	-6.602 (0.994)
		Age/duration of feeding practices						
		Current breastfeeding	0.093 (0.907)	0.225 (0.763)	3.795 (0.997)	0.256 (0.999)	5.523 (0.997)	0.3733 (0.999)
	Unhealthy household environment and inadequate health services[$]	**Baseline determinants**						
		Access to drinking water[%]	-0.269 (0.391)	-0.330 (0.291)	-0.764 (0.050)*	-0.743 (0.061)	0.624 (0.142)	0.604 (0.164)
		Access to any sanitation[%]	-0.223 (0.201)	-0.207 (0.236)	-0.365 (0.095)	-0.392 (0.079)	0.185 (0.441)	0.159 (0.517)
		Access to any hygiene facilities[%]	-0.099 (0.590)	-0.150 (0.415)	-0.082 (0.723)	-0.062 (0.794)	0.214 (0.394)	0.200 (0.437)
		Handwashing after cleaning a baby's bottom/changing a baby's nappy	-0.256 (0.215)	-0.226 (0.270)	-0.152 (0.557)	-0.173 (0.511)	0.165 (0.563)	0.133 (0.646)
		Handwashing before preparing/handling food	0.121 (0.675)	0.139 (0.624)	-0.239 (0.507)	-0.250 (0.491)	0.118 (0.765)	0.094 (0.813)
		Handwashing before feeding an infant/eating	-0.218 (0.280)	-0.220 (0.282)	-0.147 (0.561)	-0.173 (0.511)	-0.058 (0.834)	-0.123 (0.670)
		Handwashing after handling raw food	-0.066 (0.669)	-0.033 (0.827)	0.137 (0.478)	0.124 (0.526)	-0.289 (0.172)	-0.312 (0.145)
		Handwashing after handling garbage	-0.088 (0.620)	-0.076 (0.663)	-0.085 (0.700)	-0.091 (0.683)	-0.238 (0.326)	-0.247 (0.311)
		Handwashing after field work	-0.356 (0.072)	-0.304 (0.122)	-0.046 (0.852)	-0.068 (0.788)	-0.390 (0.153)	-0.403 (0.146)
		Handwashing after handling livestock	-0.053 (0.729)	-0.076 (0.620)	0.169 (0.380)	0.177 (0.367)	0.036 (0.866)	0.023 (0.914)
		Handwashing with Soap and water	-0.055 (0.759)	-0.103 (0.570)	-0.387 (0.081)	-0.383 (0.093)	-0.100 (0.687)	-0.125 (0.622)
		Mother using soap to wash hands	-0.068 (0.677)	-0.073 (0.648)	-0.469 (0.020)	-0.471 (0.020)*	-0.047 (0.835)	-0.056 (0.805)
		Cleaning infants, post-defecation	0.024 (0.894)	0.040 (0.818)	-0.021 (0.925)	-0.030 (0.894)	0.323 (0.181)	0.307 (0.208)
		Handwashing after cleaning infants	-0.071 (0.786)	0.065 (0.810)	-0.134 (0.671)	-0.207 (0.533)	-0.016 (0.963)	-0.055 (0.881)
		Disposal of infant's stool, post-defecation[%]	0.141 (0.353)	0.211 (0.164)	0.081 (0.669)	0.063 (0.747)	0.157 (0.452)	0.162 (0.450)
		Time-varying determinants						
		Putting soil/animal feces in mouth	1.103 (0.000)*	0.461(0.028)*	0.730 (0.000)*	-0.369 (0.010)*	0.694 (0.000)*	-0.027 (0.914)
		Preventing putting soil/animal feces in mouth	0.924 (0.000)*	-0.066 (0.745)	0.985 (0.000)*	0.235 (0.115)	0.796 (0.000)*	0.208 (0.405)
		Drinking from a bottle with nipple	0.844 (0.000)*	-0.067 (0.729)	0.844 (0.000)*	-0.076 (0.588)	0.611 (0.000)*	-0.073 (0.749)
Control measures		**Time-varying determinants**						
		Any antibiotic use	0.252 (0.258)	-0.330 (0.123)	1.270 (0.000)*	0.348 (0.224)	0.519 (0.039)*	0.142 (0.578)

Title: Determinants of *Campylobacter* species diversity in infants and association with family members, livestock, and household environments in rural Eastern Ethiopia.

Risks	Exposure to enteric pathogens[@]	**Baseline determinants**						
		Cattle count	0.043 (0.513)	0.052 (0.428)	0.069 (0.396)	0.067 (0.425)	0.039 (0.663)	0.023 (0.807)
		Chicken count	0.009 (0.689)	0.003 (0.879)	0.036 (0.207)	0.039 (0.181)	-0.007 (0.813)	-0.007 (0.822)
		Goat count	0.007 (0.873)	0.007 (0.875)	-0.013 (0.802)	-0.014 (0.791)	-0.022 (0.708)	-0.026 (0.653)
		Sheep count	-0.075 (0.072)	-0.075 (0.067)*	0.011 (0.831)	0.011 (0.843)	-0.041 (0.483)	-0.046 (0.433)
		Chicken daytime location risk score[%]	0.466 (0.002)*	0.443 (0.004)*	0.070 (0.715)	0.086 (0.664)	0.027 (0.900)	0.010 (0.963)
		Chicken nighttime location risk score[%]	0.412 (0.006)*	0.395 (0.010)*	-0.087 (0.647)	-0.083 (0.675)	0.111 (0.595)	0.094 (0.667)
		Cattle daytime location risk score[%]	0.187 (0.291)	0.214 (0.219)	0.114 (0.607)	0.107 (0.633)	-0.119 (0.608)	-0.116 (0.620)
		Cattle nighttime location risk score[%]	0.238 (0.118)	0.270 (0.070)	0.172 (0.369)	0.163 (0.401)	0.111 (0.599)	0.087 (0.682)
		Sheep daytime location risk score[%]	-0.312 (0.106)	-0.320 (0.095)	0.166 (0.493)	0.176 (0.476)	-0.565 (0.033)	-0.546 (0.042)*
		Sheep nighttime location risk score[%]	-0.383 (0.011)*	-0.399(0.008)*	-0.082 (0.667)	-0.087 (0.655)	-0.409(0.049)*	-0.445 (0.036)*
		Goat daytime location risk score[%]	-0.124 (0.440)	-0.086 (0.588)	-0.031 (0.878)	-0.047 (0.815)	-0.311 (0.156)	-0.332 (0.134)
		Goat nighttime location risk score[%]	0.101 (0.508)	0.070 (0.646)	0.000 (0.999)	0.009 (0.964)	0.013 (0.952)	0.003 (0.991)
		Collection of livestock waste	0.219 (0.360)	0.192 (0.425)	-0.028 (0.925)	-0.025 (0.934)	-0.482 (0.142)	-0.544 (0.106)
		Livestock dropping present in household/homestead	0.229 (0.137)	0.217 (0.152)	0.256 (0.184)	0.262 (0.177)	0.234 (0.271)	0.242 (0.256)
		Slaughtering owned livestock	-0.262 (0.084)	-0.328 (0.030)	-0.016 (0.935)	0.004 (0.986)	-0.118 (0.574)	-0.117 (0.584)
		Handwashing related to slaughtering	0.058 (0.784)	0.126 (0.530)	-0.312 (0.315)	-0.304 (0.342)	0.314 (0.322)	0.303 (0.352)
		Time-varying determinants						
		Raw milk consumption	1.677 (0.000)*	0.962 (0.002)*	1.904 (0.000)*	0.487 (0.238)	0.608 (0.107)	0.041 (0.913)
		Crawling where animal droppings might be present	1.145 (0.000)*	0.597 (0.007)*	2.091 (0.000)	0.828 (0.004)	0.843 (0.000)*	0.227 (0.395)
		Physical contact with livestock	1.090 (0.000)*	0.412 (0.034)*	1.962 (0.000)*	0.694 (0.006)*	0.723 (0.000)*	0.064 (0.789)
Benefits	Food production	**Time-varying determinant**						
		ASF[#] consumption	0.303 (0.129)	0.246 (0.196)	-0.169 (0.567)	-0.354 (0.210)	-0.034 (0.887)	-0.078 (0.730)
	Livestock income	**Baseline determinants**						
		Total annual income quartile from selling livestock	-0.080 (0.600)	-0.092 (0.554)	0.148 (0.435)	0.152 (0.441)	-0.192 (0.357)	-0.249 (0.250)
		TLU[#]	0.012 (0.885)	0.027 (0.749)	0.090 (0.384)	0.086 (0.424)	0.018 (0.876)	-0.007 (0.954)
Basic causes	Social/cultural	**Baseline determinants**						
		Residents in the home (<6 children)	-0.336 (0.205)	-0.242(0.379)	-0.063 (0.759)	-0.109 (0.612)	0.249 (0.205)	0.248 (0.212)
	Economics/livelihood	**Baseline determinants**						
		On-farm as primary livelihood	0.344 (0.233)	0.226 (0.436)	0.094 (0.794)	0.147 (0.694)	0.578 (0.144)	0.609 (0.135)
		Asset quartile	-0.097 (0.522)	0.085 (0.229)	-0.073 (0.716)	-0.068 (0.735)	-0.167 (0.367)	-0.165 (0.374)
	Human capital	**Baseline determinants**						
		Mother's age (years)	0.029 (0.912)	0.129 (0.632)	-0.60 (0.769)	-0.098 (0.646)	0.184 (0.341)	0.174 (0.373)
		Mother attended school	-0.075 (0.799)	-0.216 (0.486)	-0.229(0.342)	-0.184 (0.449)	0.003 (0.987)	0.001 (0.995)
	Basic demographics	**Baseline determinants**						
		Infant being female	0.612 (0.022)*	0.608 (0.023)*	-0.139 (0.489)	-0.137 (0.498)	-0.065 (0.724)	-0.059 (0.749)

		Infant Birth order (<4)	-0.250 (0.340)	-0.149 (0.583)	-0.192 (0.357)	-0.256 (0.251)	0.293 (0.143)	0.289 (0.153)
		Time-varying determinants						
		Infant's age						
		Grp(Q[#]) 4 vs. Grp(Q) 1	1.245 (0.000)*	-	2.215 (0.000)*	-	1.041 (0.000)*	-
		Grp(Q) 3 vs. Grp(Q) 1	0.938 (0.000)*	-	1.911 (0.000)*	-	0.375 (0.026)*	-
		Grp(Q) 2 vs. Grp(Q) 1	0.363 (0.014)*	-	0.484 (0.020)*	-	0.118 (0.483)	-

[$]For baseline determinants, infant feeding indicators shortly after birth were associated with *Campylobacter* load in month one of age; other determinants were associated with the average *Campylobacter* load of the whole follow-up period12. For time-varying (longitudinal) determinants, each age quartile's average Campylobacter load was regressed on each determinant's concurrent proportion/average or an average for the HFIAS score or the *Campylobacter* load.
[%]Reference group: for binary determinants, the status of absence was treated as the reference; WaSH ladders were binarized in regressions and the categories of unimproved, open defecation, and no facility were respectively treated as reference groups for the *drinking water*, *sanitation*, and *hygiene* ladders, and other categories of each WaSH ladders were merged into a non-reference category for regressions; For *disposal of infant's stool, post-defecation*, we combined the categories of *Left in the open*, *Buried*, *Thrown into garbage* as the non-reference group, and the other three categories as the reference group in the multiple-determinant model. Definition of each category of livestock location risk score – 0: household did not have the livestock species or kept them outside the house; 1: household kept the livestock species inside the house and confined them; 2: household kept the livestock species inside the house but did not confine them.
[^]Adjusted for sex and socioeconomic status (indicated by a binary variable of household asset) at baseline, and age was further adjusted through random intercepts in linear-mixed models for time-varying determinants. [*]$p<0.05$