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Prevalence and species diversity of *Campylobacter* in infants and livestock reservoirs in rural households in Eastern Ethiopia

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Introduction

Asymptomatic infections with *Campylobacter* species have been associated with an increased risk of linear growth faltering in infants under 2 years of age (Rogawski *et al.*, 2018)

In 2018, a cross-sectional study conducted in rural Eastern Ethiopia as part of the CAGED project (*Campylobacter* Genomics and Environmental Enteric Dysfunction; Terefe *et al.*, 2020 and Chen *et al.*, 2021) showed that;

- *Campylobacter* was detected in 88% (88/100) of the stools collected from children (<2years of age) using meta-total RNA sequencing
- An average of 11 *Campylobacter* species were detected per positive child stool
- Non thermotolerant species (*i.e., hyointestinalis*, RM6137 and RM12175) in addition to thermotolerant C. *upsaliensis C. jejuni* and *C. coli* were predominant in child stools

However, little is known about thermophilic and non-thermophilic *Campylobacter* reservoirs in Ethiopia and transmission pathways of *Campylobacter* to infants within household

Objectives

- **Determine the composition of thermotolerant and non-thermotolerant *Campylobacter* species** in infants during 12 first months of age
- **Identify potential *Campylobacter* reservoirs** within selected households (*i.e.*, family, livestock & environment) potentially associated with early infections of infants with *Campylobacter*

Methodology

Research location: rural Eastern Ethiopia, Haramaya district

Timeframe: A longitudinal study involving 106 households was conducted from December 2020 until June 2022

Study Recruitment: Participants were selected randomly from a birth registry covering ten kebeles (villages) of Haramaya district. Informed consent was obtained from both parents

Sample collection:

- Infant stools (n=1,158) were collected monthly from birth until 12 months of age (Time Points 1-13; infant age between 0 - 399 day old)
- Environmental (soil & water), livestock feces (cattle, chicken, goat & sheep) and other human stool (mother & sibling) samples were collected biannually (TP A & B between 1-6 and 7-12 months of age, respectively; n=1,744) from the same households

Detection of *Campylobacter* in the field samples:

- DNA was extracted using QIAGEN PowerFecal or PowerSoil Pro kits, as recommended by the manufacturer
- DNA quality and yield was assessed using Mettler UV5Nano Nanodrop
- *Campylobacter* genus- (Taqman approach; target: 16S rRNA; Platts-Mills *et al.*, 2014) and species-specific (Sybr green approach; target: *hipO* or *cpn60;* Ivanova *et al.*, 2014, Chaban *et al.*, 2009, 2010 & Hill *et al.*, 2006) QPCR were conducted

Statistics:

- R v4.2.1 (https://cran.r-project.org/) and JMP Pro16 (SAS, Cary, NC) were used for the analyses of the QPCR data
- Ct value cut off of 35 was used for the detection of *Campylobacter* in the field samples (= average Ct value of nuclease free water – 2.5x standard deviation [SD])
- Pure *Campylobacter* DNA was used to generate standard curves to convert Ct values into *Campylobacter* genome copies

Results – Genus-specific QPCR

Campylobacter was detected in all households selected in this study (n=106). A total of 71% (1,939/2,718) of field samples were positive for *Campylobacter* at the genus level

Campylobacter was detected in 61% (657/1,074) of infant stools at the genus level (Fig. 1)

- *Campylobacter* prevalence in the infant stools significantly increased over time; 29% between 0 and 50 days of age versus 87% between 250 and and 399 days of age (r^2= 0.94; P<0.001)
- *Campylobacter* load (genome copies per 50 ng of DNA) in the positive infant stools significantly increased over time; 2.89 ±0.85(SD)-log between 0 and 50 days of age versus 4.00 ±1.00(SD)-log between 250 and 399 days of age (P<0.001)

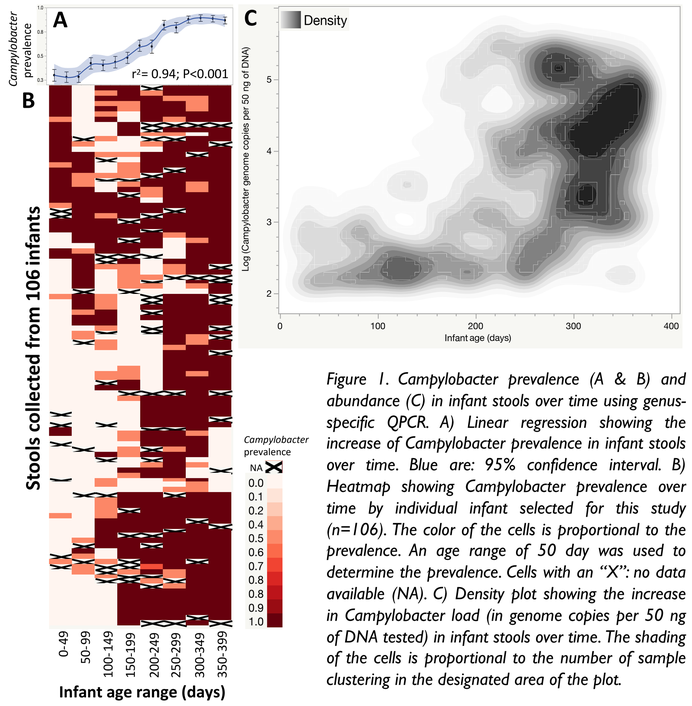
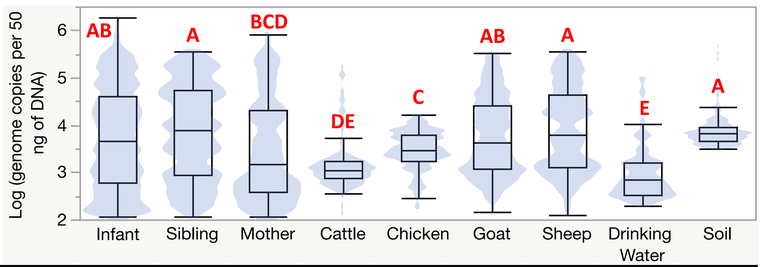


Figure 1. Campylobacter prevalence (A & B) and abundance (C) in infant stools over time using genus-specific QPCR. A) Linear regression showing the increase of Campylobacter prevalence in infant stools over time. Blue are: 95% confidence interval. B) Heatmap showing Campylobacter prevalence over time by individual infant selected for this study (n=106). The color of the cells is proportional to the prevalence. An age range of 50 day was used to determine the prevalence. Cells with an "X": no data available (NA). C) Density plot showing the increase in Campylobacter load (in genome copies per 50 ng of DNA tested) in infant stools over time. The shading of the cells is proportional to the number of sample clustering in the designated area of the plot.

In addition, *Campylobacter* was detected in 78% (1,282/1644) of the livestock feces and environmental samples collected from the 106 selected households at the genus level (Fig. 2);

- *Campylobacter* was the most frequently detected (>89%) in the livestock feces (cattle, chicken, goat & sheep) and sibling stools
- *Campylobacter* was the most abundant (>3.73-log *Campylobacter* genome copies) in soil, sibling & infant stools, and goat & sheep feces



Samples tested	1,074	147	136	200	161	199	197	148	456
***Campylobacter* +**	657	131	107	193	149	196	193	49	264
Prevalence	61%↓	89%↑	79%	97%↑	93%↑	98%↑	98%↑	33%↓	58%↓
Lower CI 95%	58%	84%	72%	94%	88%	97%	96%	25%	53%
Upper CI 95%	64%	94%	86%	99%	97%	100%	100%	41%	62%

Figure 2. Campylobacter prevalence and load in environmental samples. the boxplot shows the Campylobacter load (Campylobacter genome copies per 50 ng of DNA tested) per sample type. Blue shade indicates the distribution of the Campylobacter load data within the selected sample type. Letter (A-E) indicate statistical differences (P<0.05). The table displays Campylobacter prevalence (with lower and upper 95% confidence interval) for the associated samples. Red and blue arrows indicates whether the prevalence is significantly higher and lower, respectively compared to the Campylobacter prevalence across all the samples (71.4%).

Results – Species-specific QPCR

Overall, 44.6% (443/993) of the field samples tested were positive for at least one *Campylobacter* species (n=15; **Fig. 3**);

- 27.3% (121/443) of the *Campylobacter* positive samples harbored more than one species per samples (up to 4 species)
- Candidatus *C. infans and C. jejuni* were the most prevalent in both human stools (5.9-57%) and livestock feces (6.1-19%)
- *C. lari, C. showae, C. mucosalis, C. upsaliensis* and *C. concisus* were predominant in human stools (0-31%)

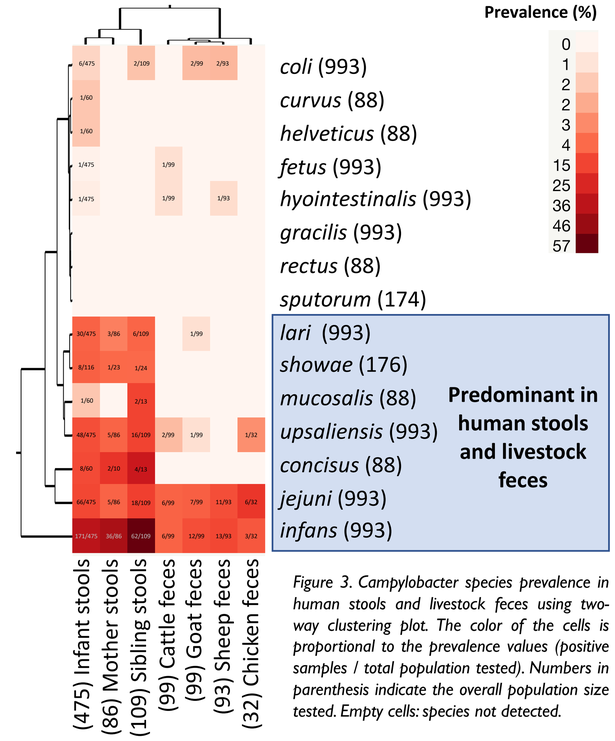


Figure 3. Campylobacter species prevalence in human stools and livestock feces using two-way clustering plot. The color of the cells is proportional to the prevalence values (positive samples / total population tested). Numbers in parenthesis indicate the overall population size tested. Empty cells: species not detected.

Conclusions & perspectives

- *Campylobacter* was highly prevalent in 106 households from eastern Ethiopia, especially in human stools and livestock feces (61-98%)
- *Campylobacter* prevalence and abundance were low in infant stools after birth, but significantly increased after 100-150 days of age
- Up to 7 *Campylobacter* species (especially *C. jejuni* and *C. infans*) were frequently detected in the human stools and livestock feces (species-specific QPCR are still being conducted; over 1,725 samples to be tested)

The *Campylobacter* reservoirs and other factors leading to the colonization of infants by *Campylobacter* are yet to be determined;

- Microbiome analyses (n=1,145) will be conducted on human stools, livestock feces and environmental samples collected from 50 households to investigate how the microbiome modulates the composition of *Campylobacter* and other enteric pathogens
- Shotgun metagenomics analyses (n=280) will be conducted on infant stools collected over time to perform functional profiling of the fecal microbiome
- Over 500 *Campylobacter* isolates recovered from human stools and livestock feces will be sequenced to conduct attribution and functional studies (virulome, resistomes and gene content), and thus, understand transmissions of *Campylobacter* early in the infancy

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