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FEED THE FUTURE INNOVATION LAB FOR LIVESTOCK SYSTEMS

KEY FINDINGS FROM FORMATIVE RESEARCH OF THE CAGED PROJECT

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Background

Undernutrition has been identified as an underlying cause in 45% of all under-five mortality. Animal-source foods, including milk, meat, and eggs, have been shown to dramatically decrease malnutrition in children under five. In order to meet Ethiopia's increasing demand and nutritional need for animal-source foods, the Livestock Master Plan, adopted by the Growth and Transformation Plan of the Government of Ethiopia in 2015, aims to significantly increase livestock production. However, the relationships between livestock ownership and malnutrition and stunting are complex, and exposure to livestock—particularly poultry—has been documented as an important risk factor for stunting. *Campylobacter* bacteria can colonize all warm-blooded animals, but they are specifically associated with poultry and are abundant in poultry excreta. A high prevalence of *Campylobacter* in primarily asymptomatic children in eight low-resource settings has been associated with stunting, increased intestinal permeability, and intestinal and systemic inflammation. Thus, while increasing livestock production may be beneficial for improving diets of children in Ethiopia, these benefits may be offset or even negated by increased exposure to *Campylobacter*.

The *Campylobacter* Genomics and Environmental Enteric Dysfunction (CAGED) project in Ethiopia is examining the links between livestock reservoirs of *Campylobacter* species and colonization of young children to ultimately inform intervention studies.

Key Takeaways

- Malnutrition contributes to nearly half of under-five child deaths. Nutrient dense animal-source foods can help reduce malnutrition.
- Pathogens from livestock feces, specifically poultry, are associated with intestinal permeability and inflammation as well as chronic malnutrition/stunting.
- Dietary diversity among children involved in the study is low. Half consume some animal-source foods, most of that is dairy, and only 5% consume eggs.
- Half of the women participants were empowered, as defined by a five domain, aggregated indicator generated by the A-WEAI.
- The formative research did not provide sufficient evidence to perform a cluster randomized controlled trial as planned. More research is needed to understand the species composition, reservoirs, and transmission pathways of all *Campylobacter* species.

This brief describes key findings of formative research aimed to inform the design of an intervention trial to reduce exposure of young children to chicken droppings. The research findings did not fully confirm the hypothesis that chicken droppings were the main source of exposure, so we also present here updated plans for a research study to increase understanding of the complex reservoirs and transmission pathways of these bacteria in a low-resource setting. The formative research was conducted in Haramaya woreda (district) in the East Hararghe zone, Oromia region of Ethiopia.

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Methodology and project area

Ethnographic research was conducted in four kebeles (wards) over a twelve-week period from April through June 2018. The aim was to better understand local community contexts, socio-cultural beliefs and practices, and social organization in relation to poultry, dietary intake, water, sanitation and hygiene (WASH), and child growth as they pertain to *Campylobacter* epidemiology. The rapid ethnographic research generated in-depth data on subject matter relevant to the subsequent formative research activities. Findings were used in the development and improved implementation of the cross-sectional study, the design of ongoing studies, and the establishment of working relationships at the community, regional, and national levels necessary for data collection in this area. A **cross-sectional study** was conducted in five kebeles and involved randomly selecting 102 children 12-16 months of age who were examined for the prevalence of infection with *Campylobacter* spp. in their stool, environmental enteric dysfunction (EED), and stunting. Fecal samples were obtained from livestock (chickens, cattle, and goats) in the households of these children. Questionnaire-based information was collected through interviews with mothers or guardians of the study participants and their male partners on demographics; livelihoods; wealth; animal ownership, management, and diseases; WASH conditions; health and nutrition; and women's empowerment, as defined by the Women's Empowerment in Agriculture Index. Data analysis included descriptive, univariate, and bivariate statistics and general mixed-effects regression models.

Results

Diarrhea and fever were frequently reported. Rates of breastfeeding at birth and at the time of data collection were high, and most children were introduced to other foods after six months of age. Only half of the children met the definition of having been exclusively breastfed, due to the common practice of giving some other food directly after birth. Dietary diversity of most children did not meet the minimum threshold for minimum dietary diversity standards for infants and young children. A majority of children consumed grains, roots, and tubers, and legumes and nuts. Just over half of children had consumed dairy products in the previous 24 hours, while 5% had consumed eggs. Sanitation was generally poor, with high levels of unimproved latrines and open defecation. Most households had access to an improved source of drinking water, with 42% needing more than 30 minutes to collect that water. Approximately half of households had no chickens or did not keep any in the house overnight. Of the households who kept chickens in the house overnight, approximately half did not confine the animals. Half of the women achieved an aggregate score indicating empowerment, and the lowest levels of empowerment were in the domains of 'Time and Access' and 'Decisions about Credit'.

Campylobacter prevalence in children was 50% by conventional Polymerase Chain Reaction (PCR). Speciation by PCR identified *C. jejuni* in 13 children, *C. coli* in 2 children, and other *Campylobacter* species in at least 36 children. Metagenomic sequencing confirmed the presence of the *C. jejuni* and *coli* species in as much as 88% of the children, and it also identified several non-thermotolerant *Campylobacter* species, mainly related to *C. hyointestinalis*. EED prevalence was 50%, based on combined results of the dual sugar absorption test and concentration of fecal myeloperoxidase, 41% of children were stunted, and 5% were wasted. Current breastfeeding and animal source food consumption (mainly raw cow's milk) were associated with higher rates of *Campylobacter* colonization in children, while improved drinking water supply decreased the odds of EED. No risk factors were significantly associated with stunting.

Implications

The formative research did not fully confirm the primary hypothesis underlying the CAGED study: that exposure to *Campylobacter* bacteria from chickens is a major driver of EED and stunting. While the study results have confirmed a high prevalence of *Campylobacter* species in children, attribution to animal reservoirs has yet to be achieved. The metagenomic sequencing results confirm that the thermotolerant species *C. jejuni* and *C. coli*, which are commonly associated with chickens (but also other livestock and animals, including wild birds), are frequently found at high abundance levels in children. Little is known about the reservoirs of the non-thermotolerant species, but they are usually associated with mammalian rather than avian species. More information is needed on the genetic diversity of all *Campylobacter* species infecting children in the study region and their attribution to different animal species, as well as the biological and social processes that lead to infections.

Based on these findings, the CAGED study team will implement a second phase of research through a longitudinal study with the following objectives:

1. assess the prevalence, species composition, and genomic diversity of thermotolerant and non-thermotolerant *Campylobacter* species in young children, adults, livestock and other reservoirs in the Haramaya woreda;
2. determine the attribution of *Campylobacter* infections in young children to humans, livestock, and other reservoirs (i.e. drinking water, soil) based on the genetic population structure of *Campylobacter* species circulating in these reservoirs;
3. assess the associations among the presence of *Campylobacter* species, gut microbiota, and the health status of children.

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Abbreviations

A-WEAI Abbreviated Women's Empowerment in Agriculture Index
CAGED *Campylobacter* Genomics and Environmental Enteric Dysfunction
EED Environmental Enteric Dysfunction
LAZ Length-for-Age Z-score

Disclaimer

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Annex

Table 1. *Campylobacter* prevalence in children and animals, by Polymerase Chain Reaction, PCR

Source	Number of samples	Number positive	Prevalence (95% CI)
Children	101	51	50% (41% - 60%)
Chickens	100	36	36% (27% - 46 %)
Cattle	101	29	29% (21% - 38%)
Goats	94	61	65% (55% - 74%)

Table 2. *Campylobacter* species detected in child feces, by shotgun metagenomic sequencing

Species	Prevalence	Mean $\log_{10}$(RPM*)	SD $\log_{10}$(RPM)
Campylobacter genus	0.88&	3.42	0.98
Campylobacter jejuni	0.68	2.19	1.12
Campylobacter hyointestinalis	0.65	2.75	1.13
Campylobacter coli	0.62	1.76	1.16
Campylobacter sp. RM6137	0.53	2.08	1.00
Campylobacter upsaliensis	0.50	1.79	1.03
Uncultured Campylobacter spec.	0.46	1.85	1.05
Campylobacter sp. RM12175	0.41	2.79	1.24

* Abundance (Reads Per Million)
& The table is ordered by prevalence. Only species with prevalence > 0.40 and mean $\log_{10}$(RPM) > 1.5 are included.

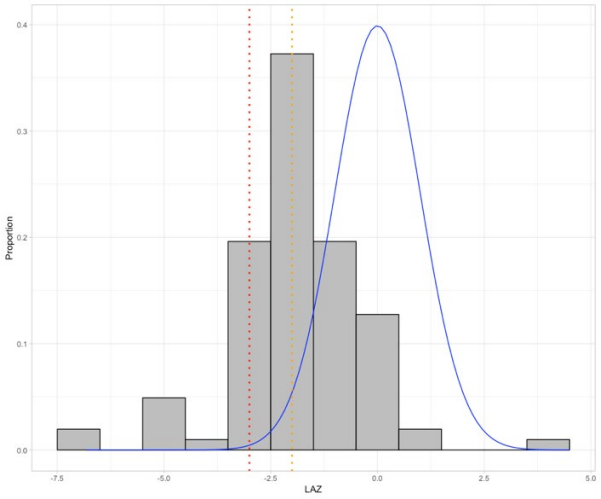

Figure 1: *Distribution of LAZ compared to WHO normal growth standards*
The histogram shows the distribution of LAZ in the sample; the blue curve shows a standard normal distribution ($\mu = 0$, $\sigma = 1$). Orange and red dotted lines indicate thresholds for stunting (orange) and severe stunting (red), respectively.