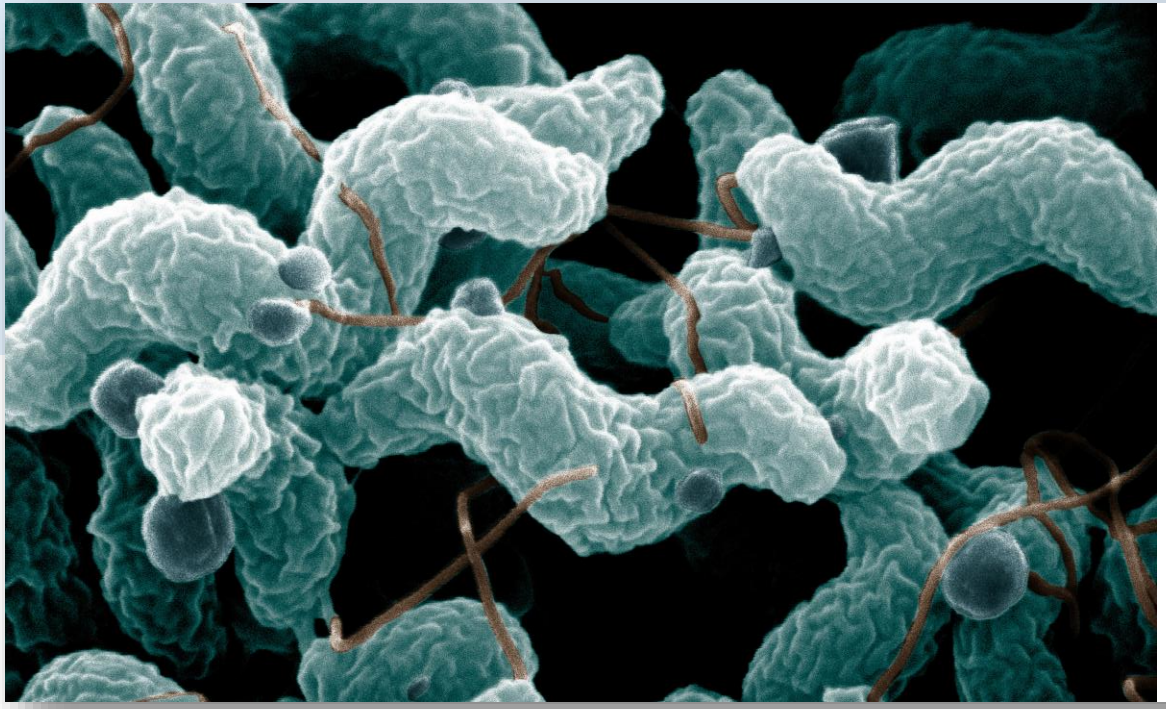


CAGED:

Campylobacter genomics and environmental enteric dysfunction

Principal Investigator

- Arie Hendrik Havelaar, University of Florida

Co-PI and Collaborators

- University of Florida: Dr. Sarah Mckune, Co-PI; Dr. Nitya Singh; Dr. Yang Yang; Dr. Volker Mai; Dehao Chen, Xiaolong Li, Amanda Ojeda
- Haramaya University: Dr. Jemal Yousuf, Co-PI; Dr. Abdulmuen Mohammed, Project Manager; Dr. Negassi Ameha; Dr. Mengistu Ketema; Dr. Nega Assefa; Dr. Yitagele Terefe; Dr. Kedir Teji Roba
- Ohio State University: Dr. Wondwossen Gebreyes, Co-PI; Dr. Getnet Yimer; Dr. Gireesh Rajashekara (Co-PI), Dr. Loic Deblais
- Washington University in St. Louis: Dr. Mark Manary, Co-PI; Dr. Isabel Ordiz
- U.S. Food and Drug Administration: Dr. Marc Allard
- Massey University of New Zealand: Dr. Nigel French

Objectives

1. Assess the prevalence of stunting, environmental enteric dysfunction (EED), and Campylobacter colonization in young children
2. Characterize the socio-demographic background of study participants
3. Design a longitudinal study that builds on this formative research

Campylobacter, EED, and stunting in Ethiopia

Chen D, McKune S, Singh N et al.

Introduction

- Stunting poses far-reaching negative impacts on children's health
- ASF can reduce stunting, but poor livestock management may increase others risks
- Exposure to livestock increases the potential of Campylobacter colonization, EED, and stunting in children

Methods

1. We conducted a cross-sectional study in Haramaya woreda in rural eastern Ethiopia
2. 102 children (age: 360 - 498 days) were randomly sampled for participation
3. Survey, measurements, and specimens were taken between Sep. & Dec. 2018

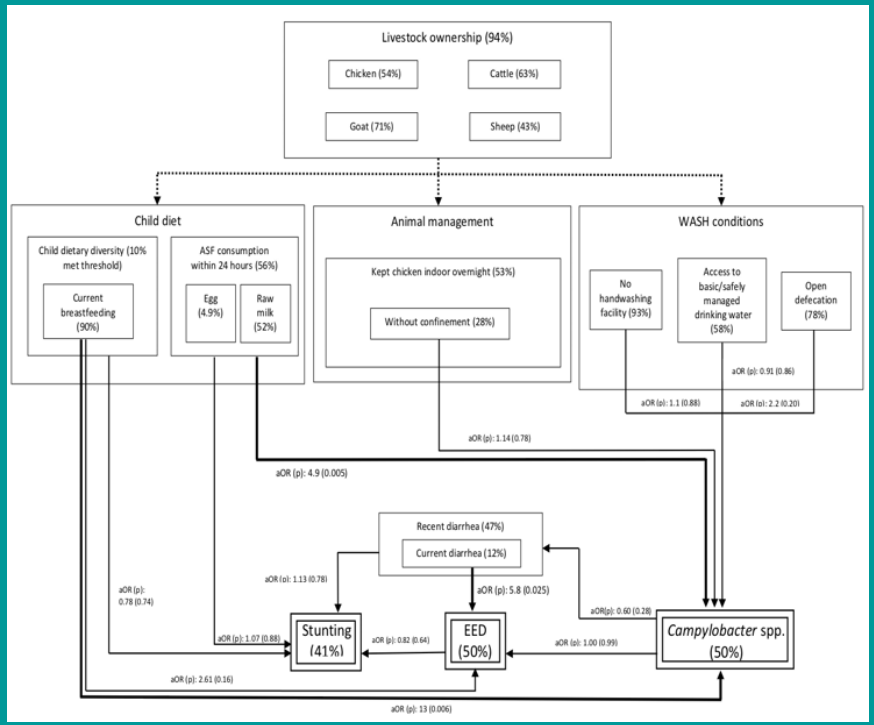
Results

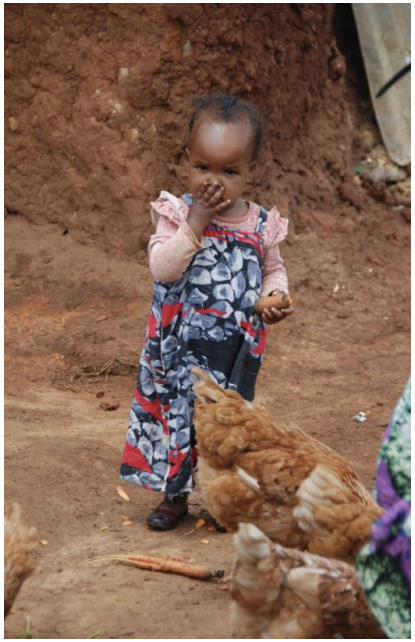
1. Prevalence of C. spp, EED, and stunting was 50%, 50%, and 41%, respectively
2. Current breastfeeding and ASF consumption increase the odds of Campylobacter detection, while improved drinking water decreases the odds of EED.

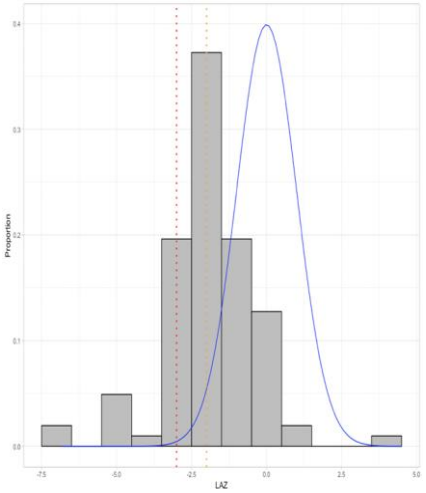
Next Steps

- Longitudinal study and exposure assessment have been planned to explore reservoirs and transmission pathways of Campylobacter.

High prevalence rates of *Campylobacter* spp., EED, and stunting were found in young children (11-18 months) in eastern Ethiopia, where poor sanitation, hygiene, and livestock management were typical of small holder households.







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ETH_CAGED

Campylobacter species *prevalence*, diversity and co-occurrence in children from eastern Ethiopia

Authors: *L. Deblais, Y.T. Mekonnen, D. Lokesh, M. Ghanem, Y. Mohamed, D. Chen, N. Singh, V. Ahyong, K. Kalantar, G. Yimer, J.Y. Hassen, A. Mohammed, S. McKune, M. Manary, I. Ordiz, W. Gebreyes, A. Havelaar, G. Rajashekara*

Introduction

- *Campylobacter* is one of the most prevalent zoonotic pathogens causing gastroenteritis and growth failure in children
- High *Campylobacter* prevalence during early childhood is associated with environmental enteric dysfunction (EED) and stunting in developing countries in Middle East and Africa
- EED is a subclinical disorder of the small intestine characterized by villous atrophy, crypt elongation, inflammatory cells infiltration of the crypts and a loss of barrier function or increased permeability

The objective of this study was to assess the prevalence of *Campylobacter* spp. in children from Ethiopia and its association with EED, as part of the *Campylobacter* Genomics and EED (CAGED) project (Terefe et *al.*, 2020)

Methods

- Urine and stool samples were collected from 100 children (12-18 months old) from 5 villages in Haramaya district, Eastern Ethiopia (Fig. 1)
- EED was assessed by measuring both gut permeability and inflammation by measuring lactulose (L%) and myeloperoxidase (MPO), respectively in the urine samples
- Meta-total RNA sequencing (MeTRS) was used to analyse the microbiome composition of the children stools
  - ✓ Library preparation: NEBNext®Ultra™ II RNA Library Prep
  - ✓ Sequencing: Illumina NextSeq (~400M reads per run)
  - ✓ Data analyses: IDseq pipeline version 3.7 https://github.com/chanzuckerberg/idseq-web/wiki

Research gaps or future opportunities

- A detailed longitudinal study is needed to better understand the relationship between *Campylobacter* and EED/stunting
- Studies are needed to identify different environmental reservoirs of *Campylobacter* responsible for infections of children
- Need optimization of microbiology and molecular biology methods to assess the role of non-thermophilic *Campylobacter* in EED/stunting

Main findings: Overall, across the 100 children studied:

- Approximately 50% of the children had moderate or severe EED (Fig. 1)
- *Campylobacter* was detected in 88% of the children's stools (Fig. 1), with multiple *Campylobacter* species in a given stool sample (average of 11 species)
- Four of the 27 classified *Campylobacter* species (*C. jejuni, C. upsaliensis, C. hyointestinalis, C. coli)* were highly prevalent (>40%) and abundant (1.76 log-rpm/stool) in the stool samples (Fig. 2)
- *Co-occurence data highlight that children might be infected from more than one Campylobacter* reservoir (Fig. 3)

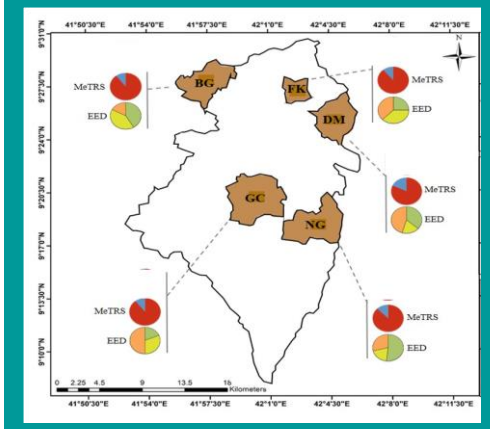


Figure 1. *Campylobacter* and EED prevalence in the 100 children from Haramaya district (Eastern Ethiopia). Pie charts made of red (positive for *Campylobacter*) and blue (negative for *Campylobacter*) represent the *Campylobacter* prevalence based on children stool samples collected from the designated kebeles (villages in brown) using MeTRS (meta-total RNA sequencing). Pie charts made of green (normal), yellow (moderate EED), and orange (severe EED) represent the severity of the EED for the designated kebeles. GC, Gobe Chala; NG, Negaya; FK, Finkle; DM, Damota; BG: Biftu Geda

Results and Recommendations:

- A high percentage (88%) of children in Eastern Ethiopia are positive for *Campylobacter*
- A high diversity of *Campylobacter* species were detected in children's stools, suggesting diverse environmental sources of infection
- The microbiome and *Campylobacter* species compositions in the children's stools were associated with the diarrhea and EED.
- Future studies should consider assessing the role of non-thermophilic *Campylobacter* in EED and stunting

- **Children's stools harbored a high diversity and abundance of *Campylobacter* species**

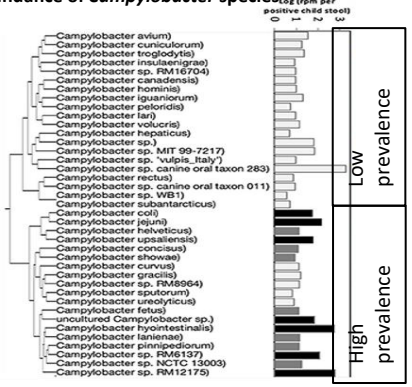
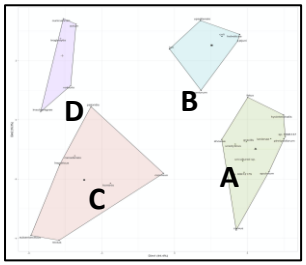


Figure 2. Prevalence and abundance of *Campylobacter* spp. in children stools White: prevalence <40% and abundance >0.95-log rpm/stool sample. Gray: prevalence >40% and abundance <1.76-log rpm/stool sample. Black: prevalence >40% and abundance >1.76-log rpm/stool sample. rpm: read per million

- **Four distinct co-occurrence clusters of *Campylobacter* species was observed in the children's stools**



Cluster A:
C. fetus
C. hyointestinalis
C. sputorum
C. showae
C. gracilis
C. curvus
C. pimmipediorum
C. ureolyticus

Cluster B:
C. jejuni
C. coli
C. lari
C. upsaliensis
C. helveticus
C. iguaniorum

Cluster C:
C. concisus
C. hominus
C. rectus
C. subantarcticus
C. hepaticus
C. peloridis

Cluster D:
C. avium
C. cuniculbrum
C. volucris
C. troglodytis
C. insulaenigrae

Figure 3. Co-occurrence of *Campylobacter* species in the stool samples. The plot was created using K-means clustering analysis using the multivariate analysis data (r^2) based on the prevalence of *Campylobacter* spp.

ETH_CAGED

Development of optimal standard operation protocols to detect, isolate, and quantify *Campylobacter* spp. in environmental samples

Authors: *L. Deblais, D. Lokesh, Y.T. Mekonnen, A.E. Ojeda, A. Havelaar, and G. Rajashekara*

Introduction:

- Data obtained through the Formative Research of the CAGED project in Ethiopia highlighted that children's stools harbored a broad diversity of thermophilic and non- thermophilic *Campylobacter* species
- *Campylobacter* is a challenging bacteria to isolate and quantify using conventional microbiology approaches due to specific and expensive equipment needed to reproduce optimal growing conditions in LMICs

The objective of this study is to standardize the detection, isolation and quantification of thermophilic and non-thermophilic *Campylobacter* using conventional microbiology and molecular approaches

Methods:

- Different selective media (CHROMagar, Mueller Hinton (MH) and Columbia agar supplemented with 5% sheep blood, and trypticase agar (TA) and tryptic soy agar (TSA) supplemented with 6% sodium formate and fumarate) and incubation conditions (microaerophilic and anaerobic gas-packs) were tested to identify the optimal conditions to grow 12 thermophilic and non- thermophilic *Campylobacter* species (Table 1)
- *Campylobacter* 16S RNA genus-specific primer using SYBR (Malde *et al.,* 2014) or Taqman PCR (Platt-Mills *et al.,* 2014), and *Campylobacter* species-specific primers (Chaban *et al.,* 2009)using SYBR PCR were tested for detection of 12 thermophilic and non- thermophilic *Campylobacter* species (Table 2 and 3)

Research Gaps and Future Opportunities

- Little information is available concerning the detection and isolation of *Campylobacter* species in the LMICs
- Optimization of microbiology and molecular methods are needed to assess the impact of different *Campylobacter* species in EED and stunting in LMICs
  - Isolation of thermophilic and non-thermophilic *Campylobacter* from various samples
  - Improved quantification using genus-specific combined with *species*-specific real time PCRs

Main findings

Microbiology approach:

- CHROMagar supported the growth of thermophilic *Campylobacter* (*C. jejuni* and *C. coli*), as well as few non-thermophilic *Campylobacter* (*C. fetus, C. hyointestinalis, C. lari,* and *C. upsaliensis;* Table 1)
- Columbia agar and Mueller Hinton agar supplemented with 5 % sheep blood supported the growth of most non-thermophilic *Campylobacter* (*C. fetus, C. hyointestinalis, C. helveticus, C. sputorum, C. lari,* and *C. upsaliensis*) as well as both thermophilic *Campylobacter* (Table 1). However, both media also showed growth of background bacteria. Future studies will optimize the concentration of antimicrobials (vancomycin, polymyxin B, trimethoprim, and amphotericin B) needed to reduce/remove background bacteria from field samples (feces, soil, water, milk, fomites, and hand wash)
- Both microaerobic and anaerobic gas-packs supported the growth of 8 *Campylobacter* species mentioned above using similar selective media

Molecular Biology approach:

- Taqman 16S *Campylobacter* genus-specific primers detected all the thermophilic and non-thermophilic *Campylobacter* species tested (n=12), while SYBR 16S *Campylobacter* genus-specific primers detected only 4 (Table 2)
- *Campylobacter* species-specific primers (SYBR) had the highest sensitivity towards their specific DNA (Table 3); however, *C. helveticus* primers also detected *C. upsaliensis* DNA; *C. upsaliensis* primers also detected *C. helveticus* DNA; and *C. lari* primers also detected *C. upsaliensis* DNA

Recommendations

- Thermophilic *Campylobacter* can be isolated on CHROMagar at 42°C in microaerophilic condition for at least 48 hrs
- Non-thermophilic *Campylobacter* can be optimally isolated on Columbia agar supplemented with 5% sheep blood at 37°C in anaerobic condition for at least 72 hrs
- Taqman 16S real time PCR using *Campylobacter* genus-specific primers combined with SYBR real time PCR using *Campylobacter* species-specific primers can provide better quantification and speciation of *Campylobacter* present in various samples.

Table 1. Growth of thermophilic and non-thermophilic *Campylobacter* species using different selective media and incubation conditions

Growing conditions	Selective media	*Campylobacter* spp.											
		C. jejuni 81-176	*C. coli* ATCC33559	*C. fetus* #33293	*C. hyointestinalis* #35217	*C. helveticus* #51209	*C. lari* #43675	*C. sputorum* #49916	*C. upsaliensis* #49816	*C. showae* #51146	*C. concisus* #33237	*C. hominis* #BAA-381	*C. rectus #33238*
Microaerophilic	CHROMagar	+++++	+++++	+++++	++	0	+++++	0	0/+				
	MH with 5% blood	+++++	+++++	+++++	+++++	+++	+++++	+++++	+++++				
	Columbia agar with 5% blood	+++++	+++++	+++++	+++++	+++	+++++	+++++	+++++				
Microaerophilic + H2	MH with 5% blood	+++++	+++++	+++++	+++++	+++	+++++	+++++	+++++				
	Columbia agar with 5% blood	+++++	+++++	+++++	+++++	+++	+++++	+++++	+++++				
	TA+6%SF+6%F									+++	+++		
	TSA+6%SF+6%F											0/+	
Anaerobic	TA+6%SF+6%F									+++++	+++++		
	TSA+6%SF+6%F											+	

SF: sodium formate, F: fumarate; Bacteria were grown at 37 °C . "0" indicates no growth and "0/+" indicates slow growth (colonies growing after 48hrs or longer). The scale from 0 to 5 (+++++) indicates the level of growth with 48 hrs of incubation

Table 2. Detection of thermophilic and non-thermophilic *Campylobacter* species using Taqman or SYBR real time PCR using 16S *Campylobacter* genus specific primers (Malde *et al.,* 2014 and Platt-Mills *et al.,* 2014)

DNA used (50 ng)	16S	
	Taqman	SYBR green
C. coli ATCC33559	13.91	19.9
C. fetus #33293	13.82	42
C. helveticus #51209	15.51	22.2
C. hyointestinalis #35217	14.28	42
C. jejuni 81-176	12.31	17.9
C. lari #43675	14.53	37.3
C. sputorum #49916	15.48	42
C. upsaliensis #49816	12.43	20.17
C. showae #51146	18.2	42
C. concisus# 51561	16.1	42
C. rectus #33238	20.2	42
C. hominis#15827	27.4	42
Water control	42	42

The cell color corresponds to the Ct value (red to blue = low to high)

Table 3. Detection of thermophilic and non-thermophilic *Campylobacter* species using SYBR real time PCR using species-specific *Campylobacter* primers (Chaban *et al.,* 2009)

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. coli ATCC33559	42	40.1	36.3	42	40.6	36.9	42	42	42
C. fetus #33293	12.9	37.3	37.4	33.3	35.5	34.7	42	41.6	37.8
C. helveticus #51209	33.1	13.9	33.7	33.6	36	27.1	41.7	40.5	38.8
C. hyointestinalis #35217	37	36.4	12.6	42	36.7	36	42	42	39.4
C. jejuni 81-176	42	42	42	42	42	42	38.6	42	42
C. lari #43675	36.2	38.3	35.7	12.9	36.4	36.6	38.4	42	42
C. sputorum #49916	37.9	38.4	33.9	37.4	14.2	37	42	40	42
C. upsaliensis #49816	34	18.4	34.1	27.9	37.9	11.7	40.87	36.7	42
C. concisus #51561							17.5	38	41
C. rectus #33238							39.8	24.2	37.8
C. showae #51146							39.4	38.3	19.69
C. hominis #15827							42	40.7	42
Water control	36.3	38.1	42	37.5	38	38	42	42	40

The cell color corresponds to the Ct value (red to blue = low to high)

Women's empowerment in livestock ownership, child's nutrition and growth

Singh N, McKune S, Chen D, Rabil A and Havelaar A

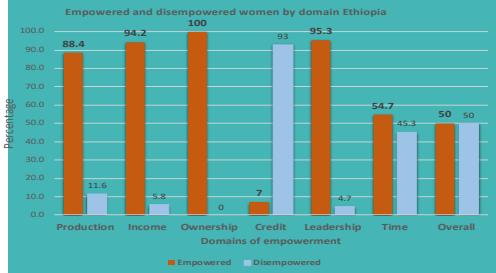
Introduction

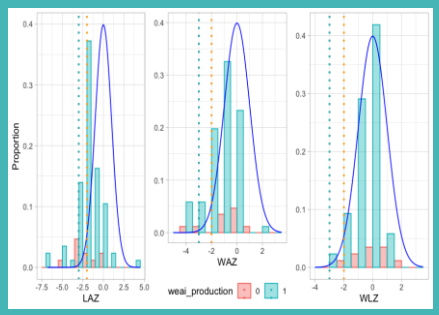
- *Women's empowerment* in various domains of livelihood can play an important role in child nutrition especially animal source food(ASF) consumption, and hence child growth.
- Six domains of A- WEAI(Abbreviated Women Empowerment in agriculture Index) and are great quantitative tools to assess the women empowerment

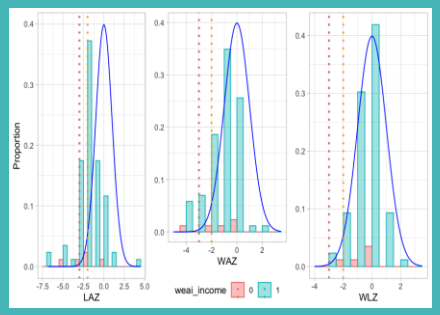
Methods

1. Status of dis-/empowerment was calculated in all 6 indicator domains of WEAI for all 102 female participants enrolled in CAGED study from Ethiopia.
2. 86 women with no missing score in any of 6 indicator domains, were categorized as empowered/dis-empowered based on aggregated score.
3. Proportions of Overall empowered women were explored over variables quantifying Child growth, Consumption of Food groups, Food security, Chat production in livelihood, Female livestock ownership.

Empowerment in production, income and leadership domains support child nutrition and growth

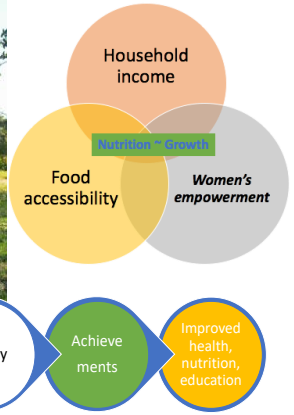






Association of Overall empowerment with nutrition, growth and food security

	Not Empowered							Empowered						
Domain Count	Overall 43	Production 16	Income 8	Owner-ship 0	Access to Credit 95	Leadership 5	Time 47	Overall 43	Production 85	Income 94	Ownership 100	Access to Credit 7	Leadership 82	Time 55
Child's ASF consumption = yes(%)	25 (58.1)	6 (60.0)	2 (40.0)	-	46 (57.5)	2 (50.0)	22 (56.4)	23 (53.5)	42 (55.3)	46 (56.8)	48 (55.8)	2 (33.3)	46 (56.1)	26 (55.3)
Dairy & Eggs= yes(%)	2 (4.7)	0 (0.0)	0 (0.0)	-	3 (3.8)	0 (0.0)	2 (5.1)	1 (2.3)	3 (3.9)	3 (3.7)	3 (3.5)	0 (0.0)	3 (3.7)	1 (2.1)
Starch = yes(%)	42 (97.7)	10 (100.0)	5 (100.0)	-	79 (98.8)	4 (100.0)	38 (97.4)	43 (100.0)	75 (98.7)	80 (98.8)	85 (98.8)	6 (100.0)	81 (98.8)	47 (100.0)
Fruits & other Vegetables= yes(%)	39 (90.7)	9 (90.0)	5 (100.0)	-	75 (93.8)	3 (75.0)	36 (92.3)	42 (97.7)	72 (94.7)	76 (93.8)	81 (94.2)	6 (100.0)	78 (95.1)	45 (95.7)
Khat livelihood aggregated (%)				-										
• No Khat production	5 (11.6)	1 (6.2)	0 (0.0)	-	9 (9.5)	0 (0.0)	5 (10.6)	3 (7.0)	8 (9.4)	9 (9.6)	9 (9.0)	0 (0.0)	8 (9.8)	4 (7.3)
• Khat only as livelihood	0 (0.0)	0 (0.0)	0 (0.0)	-	4 (4.2)	0 (0.0)	1 (2.1)	3 (7.0)	4 (4.7)	4 (4.3)	4 (4.0)	0 (0.0)	3 (3.7)	3 (5.5)
• Khat as primary livelihood	26 (60.5)	10 (62.5)	6 (75.0)	-	55 (57.9)	4 (80.0)	30 (63.8)	23 (53.5)	50 (58.8)	55 (58.5)	59 (59.0)	6 (85.7)	46 (56.1)	31 (56.4)
• Khat as primary livelihood , primary produce and irrigated	11 (25.6)	5 (31.2)	1 (12.5)	-	26 (27.4)	1 (20.0)	10 (21.3)	14 (32.6)	22 (25.9)	26 (27.7)	27 (27.0)	1 (14.3)	24 (29.3)	17 (30.9)
• Khat as primary livelihood, not primary produce, yet irrigated produce	1 (2.3)	0 (0.0)	1 (12.5)	-	1 (1.1)	0 (0.0)	1 (2.1)	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.2)	0 (0.0)
Mother's Literacy= yes(%)	11 (26.2)	3 (18.8)	0 (0.0)	-	26 (28.0)	1 (20.0)	12 (26.1)	12 (28.6)	23 (27.7)	26 (28.3)	26 (26.5)	0 (0.0)	22 (27.5)	14 (25.9)
Female livestock Ownership aggregated (%)				-										
• 0	2 (4.7)	3 (18.8)	1 (12.5)	-	5 (5.3)	0 (0.0)	2 (4.3)	2 (4.7)	3 (3.5)	5 (5.3)	6 (6.0)	1 (14.3)	4 (4.9)	4 (7.3)
• 1	6 (14.0)	2 (12.5)	2 (25.0)	-	16 (16.8)	2 (40.0)	9 (19.1)	10 (23.3)	17 (20.0)	18 (19.1)	19 (19.0)	4 (57.1)	15 (18.3)	11 (20.0)
• 2	22 (51.2)	8 (50.0)	5 (62.5)	-	37 (38.9)	2 (40.0)	23 (48.9)	9 (20.9)	31 (36.5)	34 (36.2)	38 (38.0)	2 (28.6)	29 (35.4)	16 (29.1)
• 3	13 (30.2)	3 (18.8)	0 (0.0)	-	37 (38.9)	1 (20.0)	13 (27.7)	22 (51.2)	34 (40.0)	37 (39.4)	37 (37.0)	0 (0.0)	34 (41.5)	24 (43.6)
Food Security Index				-										
• 0	32 (74.4)	14 (87.5)	4 (50.0)	-	67 (70.5)	5 (100.0)	33 (70.2)	28 (65.1)	55 (64.7)	66 (70.2)	69 (69.0)	3 (42.9)	56 (68.3)	37 (67.3)
• 1	4 (9.3)	0 (0.0)	1 (12.5)	-	11 (11.6)	0 (0.0)	4 (8.5)	7 (16.3)	12 (14.1)	11 (11.7)	12 (12.0)	1 (14.3)	11 (13.4)	8 (14.5)
• 2	3 (7.0)	0 (0.0)	1 (12.5)	-	6 (6.3)	0 (0.0)	3 (6.4)	3 (7.0)	7 (8.2)	6 (6.4)	7 (7.0)	1 (14.3)	6 (7.3)	4 (7.3)
• 3	2 (4.7)	2 (12.5)	2 (25.0)	-	5 (5.3)	0 (0.0)	2 (4.3)	1 (2.3)	3 (3.5)	3 (3.2)	4 (4.0)	0 (0.0)	3 (3.7)	3 (5.5)
• 4	1 (2.3)	0 (0.0)	0 (0.0)	-	2 (2.1)	0 (0.0)	1 (2.1)	1 (2.3)	2 (2.4)	2 (2.1)	2 (2.0)	0 (0.0)	2 (2.4)	1 (1.8)
• 5	1 (2.3)	0 (0.0)	0 (0.0)	-	2 (2.1)	0 (0.0)	2 (4.3)	1 (2.3)	3 (3.5)	3 (3.2)	3 (3.0)	1 (14.3)	2 (2.4)	1 (1.8)
• 6	0 (0.0)	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	1 (2.1)	1 (2.3)	1 (1.2)	1 (1.1)	1 (1.0)	1 (14.3)	1 (1.2)	0 (0.0)
• 7	0 (0.0)	0 (0.0)	0 (0.0)	-	1 (1.1)	0 (0.0)	1 (2.1)	0 (0.0)	1 (1.2)	1 (1.1)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
• 8	0 (0.0)	0 (0.0)	0 (0.0)	-	1 (1.1)	0 (0.0)	0 (0.0)	1 (2.3)	1 (1.2)	1 (1.1)	1 (1.0)	0 (0.0)	1 (1.2)	1 (1.8)



Results

- Women's empowerment in production, income and domains play critical role in decision-making about child's balanced nutrition (esp. ASF consumption) and consequentially their growth.
- Women in the dataset were observed to be Less empowered in 'access to credit' which brought down the overall empowerment scores.

Recommendations

- Continue to leverage domains of women's empowerment as a pathway to improve nutritional and growth outcomes in children.

Future opportunities

- More research is needed to understand the dynamics of male-female role in household by running AWEAI across both genders

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