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Prevalence and Risk Factors Associated with *Campylobacter* Infection in Diarrheal Patients in Busia County, Kenya

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Abstract

Introduction: *Campylobacter* are zoonotic bacteria that cause gastroenteritis worldwide with the species, *Campylobacter jejuni* and *Campylobacter coli* commonly associated with human diarrhea. Transmission is mainly through direct contact with farm animals, consumption of chicken and contaminated water. There is paucity of data on the epidemiology of *Campylobacter* in developing countries despite its global widespread and expansion of poultry farming; hence there is the need to explore and build on the available data. This study aimed at determining prevalence and homestead risk practices associated with *Campylobacter* infection in diarrheal patients in Busia County. **Methods:** A cross-sectional study was conducted from February, 2017 to April, 2019. Stool samples were collected from patients of all ages attending Busia County referral Hospital and structured questionnaires on homestead associated risk practices administered. Isolation and identification of *Campylobacter* species was performed using standard culture method on Modified Charcoal Cefoperazone Deoxycholate medium and confirmed by mPCR. Factors associated with *Campylobacter* infection were evaluated using logistic regression analysis. **Results:** A total of 132 (11.6%) *Campylobacter* comprising 89.2% *C. jejuni* and 10.8% *C. coli* were isolated from 1200 diarrhoegenic patients sampled. Isolation rate was higher in children aged < 2 years (13.7%) as compared to 2 - 5 years (10.2%) and >5 years (9.4%). Multilevel logistic models showed that homestead poultry farming was a significant risk associated with *Campylobacter* infection in <2 years [odds ratio (OR) 9.02; 95%

CI: 3.19 - 25.47, P < 0.001], 2 - 5 years (OR 6.47, 95% CI: 2.71 - 15.45, P < 0.001) and >5 years (OR 10.05; 95% CI: 2.60 - 24.29, P < 0.001). Other homestead risk practices linked to children < 2 years were drinking of pond water (OR 7.43, 95% CI: 1.70 - 16.33, P < 0.001), repeated use of same food cutting board without soap wash by food handlers during food preparation (OR 3.32, 95% CI: 1.28 - 8.62, P = 0.014) and female gender (OR 6.68, 95% CI: 2.51 - 17.75, P < 0.001). However, use of toilet (OR 0.08, 95% CI: 0.02 - 0.27, P < 0.001) and breast feeding practices (OR 0.24, 95% CI: 0.11 - 0.52, P < 0.001) were protective. Patients aged 2 - 5 years who had contact with domestic pets (OR 5.72, 95% CI: 1.21 - 10.04, P = 0.016), fed on chicken meat (OR 2.83, 95% CI: 1.32 - 6.04, P = 0.007), drunk untreated pond water (OR 6.51, 95% CI: 1.57 - 13.59, P = 0.001) and female gender (OR 8.25, 95% CI: 3.43 - 19.81, P < 0.001) were at risk of *Campylobacter* infection while those who lived in urban areas (OR 0.47, 95% CI: 0.20 - 0.82, P = 0.041) were protected. Contact with infected diarrheal person from the same household (OR 4.72, 95% CI: 2.10 - 10.52, P = 0.006) and consumption of raw milk (OR 7.14, 95% CI: 1.96 - 18.24, P = 0.001) posed risk among those aged > 5 years respectively. **Conclusion:** *Campylobacter jejuni* is the leading cause of *Campylobacter* infections in diarrheal patients. Personal hygiene awareness of mothers/caregivers and proper animal husbandry especially where livestock-human interaction is common are important practices which require the County government support. Further studies are required on sex specific age difference, other social economic factors, domestic animals and the role played by the environment in the transmission of *Campylobacter* infection. These would advance knowledge and understanding on source attribution and transmission dynamics for effective control and management of the infection.

Keywords

Risk Factors, Prevalence, *Diarrhea*, *Campylobacter*

1. Introduction

The genus *Campylobacter* is a zoonotic foodborne pathogen that causes diarrheal disease; the two major species are *Campylobacter jejuni* and *Campylobacter coli* [1]. *Campylobacter jejuni* is responsible for over 90% of human campylobacteriosis and is frequently isolated from poultry (81%) while *C. coli* causes less than 10% of human disease and is most frequently isolated from pigs (100%) [2] [3].

Campylobacteriosis is a problem and an economic burden to human population worldwide with an estimated 96 million cases annually, exceeding those of salmonellosis, shigellosis and *E. coli*: 0157H7 combined [4]. It is the third most common cause of hospitalization globally after *Rotavirus* and Salmonella infections and is associated with the loss of up to 7.5 million disability-adjusted life years [5] [6]. Human *Campylobacter* infections are categorized into gastrointestinal conditions, including inflammatory bowel diseases (IBD), diarrhea, Barrett's esophagus and colorectal cancer [7] and extragastrointestinal manifestations including bacteremia, lung infections, brain abscesses, meningitis, and reactive arthritis [8]. Incubation period is 1 - 10 days and clinical symptoms range from acute abdominal pain, watery diarrhoea, fever, nausea, vomiting, headache and muscle pain [9] which may persist for a week or longer [10]. The severity of illness varies from mild malaise to dehydration sufficient to warrant hospitalization and can be life-threatening [11].

Although campylobacteriosis is largely perceived as a foodborne infection with consumption and handling of poultry and poultry-related products being the major source of all *Campylobacter* infections [12], there is increasing evidence of additional routs of transmission including direct contact with other farm animals, consumption of undercooked foods and contaminated environment [13] [14]. Wild birds also play a role as reservoirs and can spread *Campylobacter* to humans through the environment [15]. Human-to-human transmission has been observed, although at low frequencies [16].

The epidemiology of *Campylobacter* enteritis differs between high- and low-income countries [17]. In high-income countries, clinical signs are symptomatic and occur in all age groups [18] whereas in low resource settings, most cases are asymptomatic with young children being more predisposed due to underdeveloped immunity. Adults acquire a level of protective immunity following repeated exposure and become predisposed when immunity gets low at senior age. Globally, the distribution of *Campylobacter* is attributed to asymptomatic colonization of the intestinal tract in a wide range of livestock species, use of animals manure, drinking contaminated water and poor hygiene conditions [19] [20] [21]. Studies have suggested that the incidence of *Campylobacter* enteritis is on the rise globally [22]. This increase has been noted much more in developed countries due to improved awareness and advances in detection practices [23].

In low and middle income countries (LMICs) such as Kenya, sources of *Campylobacter* infections have been less well documented due to lack of awareness and advances in detection practices to undertake national surveillance programmes and build a National epidemiological data banks of the disease. However, the available data indicate that the infection is endemic and on the rise [23]. The limited reports on *Campylobacter* are estimates from laboratory-based surveillance studies of pathogens responsible for diarrhoea [24] [25]. In Tanzania, the prevalence of human *Campylobacter* enteritis was reported at 21.6% [26] and between 12% - 12.6% in Kenya [27]. Because of the importance of this disease and the rise in poultry farming in Kenya, this study was carried out with an aim of determining the prevalence and homestead risk practices associated with *Campylobacter* infection in diarrheal patients in Busia County, a region marked by high prevalence of HIV and proliferation of small-holder livestock production with over 95% of households keeping poultry [28].

2. Materials and Methods

2.1. Study Design and Settings

This was a cross-sectional study conducted between February 2017 and April, 2019 in Busia County referral Hospital that provides health care services to the population of Busia County in Western Kenya. Busia County has an area 1696 km^2 and borders Uganda to the north, north-east and west, Lake Victoria to the south west, Siaya to the south and south-east and Kakamega and Bungoma to the east. The County has a population of 893,681 people (47% male and 53% female), according to the 2019 National Census. Agriculture is the mainstay for the economy and contributes approximately 50% of the household incomes [29]. The region has the highest rural human and livestock population densities and interaction of human and livestock in East Africa, operating in a mixed smallholder livestock production system [28]. Most households keep livestock, with the most common species being indigenous chickens. In addition to high rates of household livestock ownership, the community commonly slaughter and prepare chicken meals at home and rarely buy chicken from the few available retail outlets consequently leading to high exposures and transmission of zoonotic diseases [28] with regards to urban population, only Busia and Malaba towns in the County meet the minimum population threshold of 10,000 people for an urban center as per the section 8 and 9 of the Urban Areas and Cities Act 2011.

2.2. Study Population

The study population consisted of all patients presenting with diarrhea who sought treatment at the outpatient department at Busia County Hospital from February 2017 to April 2019.

2.3. Recruitment Strategy

After obtaining consent/assent from the adult participants and guardian/child, a systematic recruitment of the diarrhoeal (defined as passing of loose stool at least 3 times a day) patients [30] was conducted by requesting for stool sample in a clean plastic poly pot or rectal swab from children who could not pass stool. Semi-structured questionnaire was administered to obtain data on exposures in the 10 days before the onset of symptoms. The questionnaire comprised several sections, each representing different exposure groups and was pre-tested before the main recruitment for consistency. The interview was conducted in Kiswahili, English or Luhya depending on the language that was best understood by the respondent. Parent/guardians of the participating children were asked to complete the questionnaire on behalf of children.

The exposure variables were chosen on the basis of biological plausibility and previous knowledge on the risks associated with *Campylobacter* infection in other settings [13] [20] [21], and included socio-demographic factors such as age, residence, educational level and occupation. The occupation was divided into business which referred to those who earned livelihood by engagement in income generating activities such as working in hotels, local markets, shops and public transportation; the employed which referred to those who were salaried and worked in government, companies or NGOs; the unemployed which referred to those without any work with consistent income and finally those involved in poultry farming as their main upkeep; Behavioral and socio-economic factors included regular usage of toilet facilities within the homestead, types of foods commonly eaten and where purchased, exposure to infected diarrheal person(s) within the same household, pets and local and or international travel, types of houses lived in, source of cooking and lighting energy. The clinical section comprised of symptoms of *Campylobacter* enteritis and status on expanded immunization program (EIP) vaccine, any long term medication and immunosuppressive treatment based on a 4-week history.

2.4. Inclusion Criteria

Diarrheal patients of all ages seeking treatment at the outpatient department, Busia County Hospital who consented/assented to participate in the study and who self-reported to have not been on antibiotics for the previous 5 days.

2.5. Exclusion Criteria

Non-diarrhoeal cases and those that declined to participate.

2.6. Specimen Collection and Isolation of *Campylobacter* Species

Fresh stool/rectal swab samples were collected into a clean plastic poly-pot from diarrheal patients and transported in Amies media within 2 hours in cool box to the Centre for Infectious and Parasitic Diseases Control Research (CIPDCR), KEMRI, Busia laboratory for *Campylobacter* isolation. Standard microbiological techniques were used to culture and isolate *Campylobacter jejuni and Campylobacter coli* as described before [31]. Briefly; a gram of faecal sample or rectal swab was cultured on Exeter enrichment broth containing nutrient broth supplemented with lysed horse blood and incubated at 42˚C aerobically for 24 hours. A sub-culture was done by passing 100 µl of the enrichment broth culture on 0.45 µm cellulose nitrate filter onto modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA) supplemented with SR155 (Oxoid) and incubated at 42˚C ± 1˚C for 48 ± 4 h under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) generated by CampyGen™ (Oxoid CN0035). Identification of *Campylobacter* was by characteristic appearance on the culture medium (moist, creamy-grey and flat-spreading), gram stain, oxidase test, catalase reaction hydrolysis of hippurate and indoxyl acetate and confirmed by multiplex PCR (*m*PCR). The reference standard strain *C. jejuni* (LMG 13646) was used for quality control [32].

2.7. Molecular Identification of *Campylobacter*

Multiplex PCR (*m*PCR) was used for confirmation of the suspected *Campylo-*

bacter isolates by using genus and species specific primers as described by Bang *et al.*, and Linton *et al.*, [33] [34]. For genus identification of *Campylobacter*, 16S rRNA region was amplified using *C412F* and *C1228R* primers. Species-specific identification of *C. jejuni* and *C. coli* was done by targeting the *hip*O gene which codes for hippuricase enzyme with a 344 bp, and the *asp*K gene coding for aspartokinase with a 500 bp, respectively (**Table 1**). The PCR products were separated and identified by gel electrophoresis using 1.8% agarose containing 0.1 μg/ml ethidium bromide.

2.8. Data Management and Analysis

The data were entered and analyzed using SPSS (IBM Corp., Armonk, NY, USA) version 21. Binary logistic regression was used to model predictors of *Campylobacter* infection adjusting for factors identified to be significant at $P < 0.05$ in bivariate analysis. Backward conditional logistic regression was used with removal of predictors at $P < 0.05$. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to compare association between detection of *Campylobacter* species and predictor variables in the study.

3. Results

3.1. Socio-Demographic Characteristics

A total of 1200 consenting/assenting diarrheal outpatients seeking health care services at the Busia County Hospital were enrolled. The gender distribution was even between male and female (48.1% vs 51.9%) with a median age of 3.2 years (age range 1 month to 70.2 years). Patients aged less than 5 years constituted the largest group (79.6%; 955) and were stratified as <2 years 535 (44.6%), 2 - 5 years (442; 36.8%) and the older group > 5 years (223; 18.6%). Majority (62.3%) of the study subjects were from rural setting (**Table 2**).

3.2. Prevalence of *Campylobacter* Species

The overall prevalence of the *Campylobacter* species of interest, *C. jejuni* (CJ) and *C. coli* (CC) was 11.6% from which *C. jejuni* and *C. coli* had 89.2% and

Table 1. Characteristics of primers used in the study.

Target species	Primer code	Primer sequence (5' - 3')	Amplicon size (Bp)	Reference
Campylabacter genius.	16S-F	GGAGGCAGCAGTAGGGAATA	1062	[33]
	16S-R	TGACGGGCGGTGAGTACAAG		
C. coli spp	CC18F CC519R	GGTATGATTTCTACAAAGCGA	500	[34]
		ATAAAAGACTATCGTCGCGTG		
C. jejuni spp	hipO-F	GACTTCGTGCAGATATGGATGCTT	344	[33]
	hipO-R	GCTATAACTATCCGAAGAAGCCATCA		

Table 2. Distribution of *Campylobacter* infection in diarrheal patients attending Busia County Hospital, Kenya.

Variables	Positive	Negative	Total	OR (95% CI)	P-value
	N (%)	N (%)	N (%)		
Age groups					
<2 years	73 (13.7)	462 (86.3)	535 (44.6)	1.56 (0.94,2.6)	0.088
2 - 5 years	45 (10.2)	397 (89.8)	442 (36.8)	1.04 (0.6,1.8)	0.884
>5 years	21 (9.4)	202 (90.6)	223 (18.6)	Ref	
Gender					
Female	69 (11.1)	554 (88.9)	623 (51.9)	0.90 (0.63, 1.28)	0.568
Male	70 (12.1)	507 (87.9)	577 (48.1)	Ref	
Residence					
Village	87 (11.6)	661 (88.4)	748 (62.3)	1.01 (0.70,1.46)	0.947
Urban	52 (11.5)	400 (88.5)	452 (37.7)	Ref	

OR: Odds ratio, N: total number of participants, CI = confidence interval, Ref: Reference.

10.8% respectively. The recovery rate of *Campylobacter* species was relatively higher in children < 2 years (13.7%) as compared to other age groups and showed an overall general progressive downward trend with age (Figure 1). However the difference were not significant (P = 0.088) (Table 2).

3.3. Possible Risk Factors Associated with *Campylobacter* Infection

Of all the potential risk variables associated with household practices investigated, higher risk proportions were observed in children < 5 years. Occupational exposure to poultry farming showed significant association with *Campylobacter* infection in all the selected group categories of patients with children < 2 years being 9 times more at risk (OR 9.02; 95% CI: 3.19 - 25.47, P < 0.001); 2 - 5 years (OR 6.47, 95% CI: 2.71 - 15.45, P < 0.001) and the older group > 5 years (OR 10.05; 95% CI: 2.60 - 24.29, P < 0.001).

Other established age specific household exposure variables significantly associated with *Campylobacter* infection in children < 2 years were use of same food cutting board without proper cleaning during food preparation by the mothers/caregivers (OR 3.32, P = 0.014) however, we noted that regular use of toilets (OR 0.08, P < 0.001) and breast feeding (OR 0.24, <0.001) were protective.

The significant risk variables specific to children between 2 - 5 years included contact with domestic pets (OR 5.72, P = 0.016) and feeding on chicken meat (OR 2.83, P = 0.007). Generally, it was noted that chicken meat consumption, was the most fed on in this community (78.2%) followed by fish (74.6%), Raw milk (70.3%), eggs (69.3%), beef (42.5%) and pork (39.5%) mainly from local markets (88.6%) and small hold individual farms (10.2%). However, urban

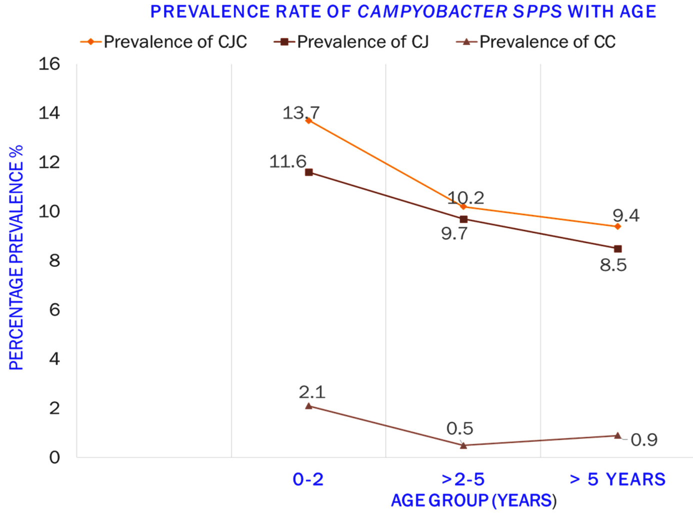

Figure 1. Prevalence of *Campylobacter jejuni* and *C. coli* by multiplex PCR from diarrheal children and adults in Busia County Hospital, February, 2017 to April, 2019. Key: CJC; *C. jejuni* and *C. coli)*, CJ; *C. jejuni* and CC; *C. coli*.

dwelling was protective (OR 0.47, P = 0.041) in this group (2 - 5 years) compared to those from the village.

The most common source of drinking water in this area was borehole (49.5%) followed by treated tap water from municipality (28.4%), river (20.1%), pond (1.9%) with a small percentage from the lake (0.1%). Our analysis showed drinking of untreated pond water, an additional significantly risk to children < 5 years (OR 7.43, P < 0.001) together with female gender (OR 8.25, P < 0.001). Likewise, we noted specific risk variables in >5 years as drinking of raw milk (OR 7.14, P = 0.001) and contacts with infected (diarrheal) person(s) from the same household (OR 4.72, P = 0.006) (Table 3).

Among the clinical symptoms of *Campylobacter* infection analyzed, fever (77.7%) and vomiting (53.8%) were the main reported clinical signs. Bivariate analysis revealed that crumps (OR 2.23, P < 0.001), chills (OR 2.77, P < 0.001) and fatigue (OR 2.37, P < 0.001) were significantly associated with the infection in <5 years. However, there was no significant (P > 0.05) association found at the multivariable level.

The childhood expanded immunization program take up was high (84.4%) in this area while very few people (0.5%) were under long term medication. The literacy level for the study participants was high with only 9% reporting no formal education. No significant association (P > 0.05) was found between the level of education of the participants and *Campylobacter* infections.

The analysis of social economic factors and the participant responses were; type of house lived in (permanent 32.3%, semi-permanent 60.9% and thatched 6.8%), lighting system used (paraffin 36.3%, electricity 41.0% and solar 22.7%) and the cooking fuel (firewood 44.2%, charcoal 52.8%, paraffin 2.5%, electricity 0.4% and gas 0.2%) in the households. However, none of the above social economic factors were significantly associated with *Campylobacter* infection.

Table 3. *Campylobacter* infection and associated risk factors among the diarrheal patients attending Busia County Hospital, Kenya - February, 2017 to April, 2019.

Variables	<2 years		2 - 5 years		>5 years	
	aOR 95% (L - U)	P-value	aOR 95% (L - U)	P-value	aOR 95% (L - U)	P-value
Gender						
Female	6.68 (2.51 - 17.75)	<0.001	8.25 (3.43 - 19.81)	<0.001		
Male	Ref		Ref			
Occupation of the respondents						
Business	2.31 (0.82 - 6.52)	0.114	1.3 (0.55 - 3.04)	0.546	1.08 (0.22 - 5.14)	0.497
Employed	0.75 (0.22 - 2.54)	0.645	0.95 (0.36 - 2.52)	0.925	1.22 (0.19 - 4.08)	0.212
Poultry farming	9.02 (3.19 - 25.47)	<0.001	6.47 (2.71 - 15.45)	<0.001	10.05 (2.60 - 24.29)	<0.001
Unemployed	Ref		Ref		Ref	
Regular use of toilet in homestead						
Yes	0.08 (0.02 - 0.27)	<0.001				
No	Ref					
Source of drinking water						
Borehole	1.46 (0.55 - 3.84)	0.447	1.22 (0.61 - 2.44)	0.185		
Lake water	UD	UD	UD	UD		
Pond water	7.43 (1.70 - 16.33)	<0.001	6.51 (1.57 - 13.59)	0.001		
River water	2.90 (0.92 - 7.70)	0.058	3.47 (0.62 - 7.19)	0.063		
Tap water	Ref		Ref			
Breast milk feeding						
Yes	0.24 (0.11 - 0.52)	<0.001				
No	Ref					
Repeated use of same food cutting board without soap wash in household kitchen						
Yes	3.32 (1.28 - 8.62)	0.014				
No	Ref					
Residence						
Urban			0.47 (0.20 - 0.82)	0.041		
Village			Ref			

Continued

Contact with domestic pets in the house				
Yes	5.72 (1.21 - 10.04)	0.016		
Not sure	UD	UD		
No	Ref			
Eating chicken meat				
Yes	2.83 (1.32 - 6.04)	0.007		
No	Ref			
Drinking raw milk				
Yes			7.14 (1.96 - 18.24)	0.001
No			Ref	
Contact with infected diarrheal person(s) in the same homestead 10 days before onset of diarrhea				
Yes			4.72 (2.10 - 10.52)	0.006
Not sure			UD	0.999
No			Ref	

aOR: Adjusted Odds Ratio, L: Lower limit, U: Upper, P value: Probability value, Ref: Reference, UD: Undefined.

4. Discussion

In this study, the high number of hospital visits from children < 5 years of age (81.4%) suggests diarrhea, which *Campylobacter* is mostly associated with, was largely a pediatric disease in this region.

Our overall prevalence of *Campylobacter* infection among diarrheal patients was 11.6%, comprising of 89.2% *C. jejuni* and 10.8% *C. coli*. This prevalence was higher compared to reports from studies in Nairobi 3.1% [31] and Kisii 5.8% [35] in Kenya, Tanzania 9.3% [36] and Egypt 9% [20], consistent to those reported from Ethiopia 11.6% [37], and lower than those found in Nigeria 16.5% [38] and Algeria 17.7% [39]. These variations may arise due to differences in the set predictive values of test systems, social and cultural practices, geographical location, population immunity as well as differences in public health standards, intervention strategies, surveillance systems, food safety practices, and the prevalence of *Campylobacter* in natural reservoirs in different regions [23] [40].

From an African perspective, where *Campylobacter* is endemic and chicken meat on top feeding list, our reported prevalence (11.6%) in human is considered significant especially in this era of HIV/AIDS. Although this study was limited in establishing the role of HIV in *Campylobacter* infection, it is important to consider *Campylobacter* infection in a different perspective because of its close association with HIV/AIDS. Studies have predicted an upsurge of several diseases associated with immune-suppression and reported incidences of *Campylobacter* infection in patients with HIV/AIDS as 39 times higher than the infection rate in the general population [41]. There might be a possible future increase of *Campylobacter* infection since HIV infection has been reported high (7.7%) in this area with an incidence of 52,800 cases across all ages annually [42].

Although the diagnosis and treatment of diarrhea was not captured in our questionnaire, the health management strategies adopted by the Ministry of Health (MoH) Kenya since 2010 on appropriate measures for treatment and management of diarrheal diseases both in hospitals and home based care at community level recommends ORS and Zinc for mild diarrhea. Antibiotics are rarely given unless in severe cases of dysentery, cholera, giardiasis and amoebiasis. Detection of these diarrheal etiologies require proper laboratory systems which are absent in most hospitals in Buisa and most patients are treated empirically [43].

Even though there was a general downward trend of prevalence of *Campylobacter* infection from children < 2 years (13.7%) through 2 - 5 years (10.2%) and >5 years (9.4%), this general progressive decline of the infection with age might be due to age-related acquisition of protective immunity as a result of close proximity and frequent interactions with livestock hence frequent exposure to *Campylobacter* infection [17] [18] [43]. However, other studies have shown a decline of this immunity at an advanced age > 60 years [44].

This study analyzed the potential risk exposures associated with household practices from several exposure variables of social demographic, clinical, economic and behavioral factors considered on the basis of biological plausibility and previous knowledge on the risks associated with *Campylobacter* infection [13] [20] [21]. Our findings suggest that homestead practices of poultry farming, eating chicken meat, drinking raw milk, breast feeding, use of untreated pond water, contact with infected diarrheal individuals and domestic pets, use of same food cutting board without washing with soap, use of toilets, residential area and gender were significantly associated with human *Campylobacter* infection in Busia County. Although this study did not comprehensively evaluate all the plausible social economic factors, the few that were analyzed did not yield any significant association with *Campylobacter* infection.

Majority of the participants presented with fever (77.7%) and vomiting (53.8%) as the main clinical signs. Similarly none of the analyzed clinical signs and symptoms were found to be associated with *Campylobacter* infection. This is likely because majority of *Campylobacter* infections in low resource settings are mainly asymptomatic [22].

Poultry farming was significantly associated with *Campylobacter* infection across all age divide of the participants. This might be because of the fact that most of the household livelihoods in Busia County where the study was conducted, depend majorly on livestock farming with chicken being the most commonly kept livestock that offers quick sale and food in an environment where water sanitation and hygiene (WASH) pose a lot of challenges in the livestock sector [29]. *Campylobacter* enters food chain through poultry regarded as the main source through which transmissions occur and once established in chicken caeca, the number proliferates rapidly to high levels (109 CFU/g) and can remain carriers until slaughter [45] [46]. During this period, high number of *Campylobacter* are shaded through feces and contaminates the surrounding environment where the birds live and in turn, allow the pathogen transmission to humans through fecal oral rout [47]. Likewise, there has been increasing evidence that contact with farm animals offer significant risk exposures to human *Campylobacter* infection [13] [48] with poultry being the major source of food-related transmission and reservoir of *Campylobacter* species to humans [49]. In addition, source-attribution studies have linked zoonotic transmission of human *Campylobacter* to poultry exposures and consumption of chicken, dairy products and contaminated water [13] [14] [17].

We noted at the same time that household use and drinking of untreated pond water was a significant risk for *Campylobacter* infection among children < 5 years. This environmental surface water (pond) is served by several contaminated streams from free-range small hold livestock farm systems and can remain stagnant for a long period of time. Consequently such cumulative contamination by *Campylobacter* species may present potential reservoir and transmitting vehicles for *Campylobacter* [50] [51] [52] [53]. Studies have shown many possible sources of *Campylobacter* infection in humans including contaminated water [54]. In Kenya, approximately 17,100 children under 5 years die each year from diarrhea, with 90% of these deaths being attributed to poor water, sanitation and hygiene [55]. According to Busia County Development Plan, 2018-2022, the main source of drinking water is from borehole (46%), other sources including springs, rivers, piped and pond water. Apart from urban treated water supplied by Busia Water and Sewerage Company with a supply capacity of only 12.5%, the larger percentage of water used is untreated. Mothers or caregivers possibly struggle for clean and safe water in this area and a number of them likely end up using untreated pond water to care for children whose immunity may have not developed as much.

Female children < 5 years were more at risk of *Campylobacter* infection. Similarly, studies on diarrheal correlates associated with enteric bacterial infections in Busia and Muranga Counties in Kenya showed that female between 6 - 36 months were twice more likely to be infected with enteric bacterial pathogens than their male counterparts [56] [57]. Likewise in rural Sudan, female children < 5 years were more at risk [58]. Although female subjects were found to be significantly more at risk of *Campylobacter* infections, other studies have contrasting reports with unclear reasons [59] [60]. Sex specific age differences in immunity may exist but have not been elaborately documented. More investigations are recommended for better understanding on factors such as immunological, demographic and behavioral associated with sex specific differences across the globe.

Although we did not directly assess the risk associated with each and every step in handling and preparation of food in household kitchens, assessment of use of same food cutting board without proper wash was picked as a significant risk for children < 2 years. Care givers involved in food preparation for these children were three times (AOR 3.32, P = 0.014) more likely to predispose their children to *Campylobacter* infection and this is consistent with other findings [61]. In Kenya, complementary feeding is introduced after 6 months though some mothers introduce weaning earlier. It is possible that there might have been lack of personal hygiene and inadequate cleansing of food surfaces by the mothers or caregivers and subsequent cross-contamination of *Campylobacter* during food preparation from raw food. Similarly, it has been shown that unhygienic food preparation, food storage and subsequent feeding of infants can aggravate transmission of diarrheal pathogens and pose great risk to this age cohort [62]. Otherwise self-contamination and cross transmission of *Campylobacter* infection may be minimize through personal and kitchen hygiene practices at household level.

Breast feeding and frequent use of toilet had protective effect among children < 2 years. This might be due to transfers of maternal antibodies in breast milk with health befits to the child. Open defecation predisposes water and food to fecal contamination more so when it rains and may increase the risk of *Campylobacter* transmission. Frequent use of toilets partly takes care of this public health problem and ensures standards necessary to break the vicious cycle of diarrheal pathogens spread from persons infected to the environment hence minimize the transmission of *Campylobacter* infection and offer protection to these children [39]. In addition, this study found a fairly high literacy level among the participants with 91% having at least acquired primary school education. This may have contributed towards adherence to the recommended WASH practices such as frequent usage of toilets.

Many people keep cats and dogs at home as pets and for security reasons and our study showed this interaction was significantly associated with *Campylobacter* infection in children of 2 - 5 years (OR 5.72, P = 0.016). This might have been due to their playful nature at this age and a likelihood of being physically closer to these pets and more likely than others to contaminate and put their fingers or other items into their mouths which might have aggravated the transmission of the infection. Our finding is similar to previous studies performed in Kenya [63] which showed that the incident of *Campylobacter* infection was significantly higher in children who had contact with domestic animals and more so in those who keep dogs and cats as pets [64].

On dietary habits reported at household level, consumption of chicken was more frequent (78.2%) of all the foods considered and was significantly associated with *Campylobacter* infection in children of 2 - 5 years. This age is explorative, very playful and is common when self-feeding practices occur with subsequent development of immunity due to exposures. When chicken is prepared under unhygienic way or consumed when under-cooked, it can cause *Campylobacter* infection and is considered the most common cause of diarrheal outbreaks in Europe [65]. Although *Campylobacter* infection rate was the same among rural and urban dwellers, children between 2 - 5 years of age from urban were more protected from the infection 0.47 (0.20 - 0.82) P = 0.041 as compared to those from rural. The expected improved environment and water, sanitation and hygiene (WASH) infrastructure in urban areas may have played a role and came with health benefits to these children [66].

Drinking raw milk was the third most used food item at home (70.3%) and was a risk in >5 years. Raw unpasteurized milk has largely been implicated in several *Campylobacter* infection and outbreaks [67] [68] [69]. Elsewhere, genomic analyses showed that the presence of *Campylobacter* species in milk was attributed to fecal contamination [70].

We found exposure to household member with diarrhea a risk to those > 5 years of age (AOR 4.72, P = 0.006). The mode of transmission here is thought to be through fecal-oral root from either person-to-person or exposure to a common source of infection within the homestead not connected to the exposures under investigation. We could not establish information about the exposures to risk factors associated with other household members in our study. However, similar studies have implicated the contributions of person-to-person transmission of *Campylobacter* infection and outbreaks [65] [71].

The absence of association between *Campylobacter* infection and clinical symptoms has been reported in low income countries [72] [73]. This may be due to development of defensive immunity characterized with developing countries, high sensitivity of the PCR method in detecting low number of live and dead bacteria, all of which are neither indicative of clinical disease. Furthermore, the detected *Campylobacter* can also reflect convalescent phase as excretion of *Campylobacter* may last up to 10 weeks after infection [17]. Other factors considered without significant association included medical history on expanded immunization program (EIP) vaccine coverage, education, contact with wildlife, local and or International travel, types of house lived in and any long term medication and immunosuppressive treatment based on a 4-week history.

5. Conclusion

The present study found that diarrhea is mainly a pediatric disease and children < 5 years are more predisposed to homestead related risk activities associated with *Campylobacter* infection. *Campylobacter jejuni* was found to be the leading cause of *Campylobacter* infections in the study area. Ruling out *Campylobacter* infection in patients with diarrhea would help in proper management more so in children who are most affected, and where HIV prevalence is high as in most developing countries. Personal hygiene practices and proper animal husbandry especially where livestock-human interaction is common is important. Further studies are required on sex specific age difference, social economic factors and the role played by the environment and other domestic animals in the transmission of *Campylobacter* infection. This would advance knowledge and understanding on source attribution and transmission dynamics for effective control and management of the infection.

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Data Availability

The datasets used and analyzed during this study are available from the corresponding author on request.

Ethical Consideration

The study was approved by the Scientific Ethical Review Unit (SERU), Kenya Medical Research Institute (KEMRI protocol No. 3320). Permission was obtained from County Health offices and Hospital administrators. Written informed consent was obtained from the study patients or guardians of minors/study patients. The results were shared with the duty physician for appropriate patient treatment and management.

Conflicts of Interest

No author has any competing interest to declare.

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Appendix 1: Study Questionnaire

KENYA MEDICAL RESEARCH INSTITUTE/ILRI

Questionnaire on prevalence and risk factors associated with *Campylobacter* infection in diarrheal patients in Busia County, Kenya.

Date of recruitment ……..……………… Patient id/Barcode ……..………

Personal Data:

Date of birth ………………………..…… Place of Residence __________

Gender ☐ Male ☐ Female Contact Tel/Address ……………

1. Occupation: What is your current occupation/workplace

Business

Unemployed

Poultry farming

Employed

Others ______________

2. Educational level: Which is your highest level of education?

None

Primary

Secondary

Tertiary

3. Clinical signs and symptoms

Onset of diarrhea …………………days. Temperature________ (˚C).

During illness, was there any of the following symptoms? Please tick all relevant answers

Key: *DK/NS: don't know/Not sure*	**Yes**	**No**	**DK/NS**
Vomiting……………………..…..…………………	☐	☐	☐
Diarrhoea……………………..………………….	☐	☐	☐
Stomach Cramps…………….................................	☐	☐	☐
Fever……………………….……………………….	☐	☐	☐
Chills……………………….….……………..	☐	☐	☐
Headache………………..…...…………………	☐	☐	☐
Fatigue……………………... …………………	☐	☐	☐
Consistency of stool Mucoid …………………..	☐	☐	☐
Bloody …………………..…..	☐	☐	☐
Watery …………………..	☐	☐	☐
Foul smell ………………...	☐	☐	☐
- Were you/was your child) given any antibiotics?	☐	☐	☐

- When did you/your child diarrhea last? ______ hrs. How many times/day…

- Vaccine compliant ……………………………	☐	☐	☐

4. Environmental Risk Factors

Which of the following describes where you live?

- City/Urban
- Rural/Village
- **Type of water used:**

Which type of water do you usually use:

Borehole

Tap

River

Pond

Lake

5. Dietary habit: Do you/your child usually eat the following (>3/week)

	Yes	No	DK/NS
Chicken			
Pork			
Beef			
Raw milk			
Eggs or food containing eggs			
Fish			
Raw fruits/fruit salad/vegetables			
Breast milk			

- Where do you usually purchase food for home consumption?

Grocery stores

Individual farmyard

Local market

Others, specify …….

- Do you have cutting board for food**?**

Yes

No

If yes, how do you clean it before use?

Rinse with water

Wash with detergent/soap then clean with water

Others, specify…………………….

6. Exposure information in the last 10 days before onset of diarrhea

Do you keep/rare or have contact with the following at home?

	Yes	No	DK/NS
• Domestic pets (e.g. dog, cat)			
• Person (s) suffering from diarrhea in your household?			

Are you on any long term medication or have you undergone surgery?

If yes, specify………………………………………………

- Have you/your child finished the childhood vaccines recommended by Kenya Expanded Immunization Program

7. Travel

Did you/your child travel outside the County

If yes (specify) Date ……….. Where …………………

	Yes	No
• Fecal disposal		
Do you have toilet facility if so		
Do you use the toilet regularly?		

8. Economic factors

- Type of house:

Permanent

Semi-permanent

Thatched with mud soil

- Type of lighting system in the house:

Electricity

Solar

Paraffin

- What do you use for cooking at home?

Firewood

Charcoal

Paraffin electricity

Gas

This is the end of the formal interview. Thank you very much for your time and cooperation.

Do you have any questions? ____

Thanks and Good bye.