Prevalence of *Campylobacter* species and associated risk factors for contamination of dairy products collected in dry season from major milk sheds in Ethiopia

Abera Admasie[1,5], Adane Eshetu[1], Tesfaye Sisay Tessema[1], Jessie Vipham[2], Jasna Kovac[3] and Ashagrie Zewdu[4]

[1]Institute of Biotechnology, New Graduate Building, Addis Ababa University, P.O. Box 1176

[2]Department of Animal Science and Industry, Kansas State University, 247 Weber Hall, Manhattan, KS 66506, United States

[3]Department of Food Science, The Pennsylvania State University, 437 Erickson Food Science Building, University Park, PA 16802, United States

[4]Center for Food Science and Nutrition, Addis Ababa University, New Graduate Building, College of Natural Sciences, P.O. Box 1176, Addis Ababa, Ethiopia

[5]Department of Biology, Arba Minch University, College of Natural Sciences, P.O. Box 21, Arba Minch, Ethiopia

Authors' contribution:

AA: Conceptualization, investigation, formal analysis, data collection, data analysis, writing - original draft, writing - review and editing.

AE: Investigation.

TS: Conceptualization, methodology, supervision, writing - review and editing.

JK: Conceptualization, methodology, fund acquisition, writing - review and editing.

JV: Conceptualization, methodology, fund acquisition, writing - review and editing.

AZ: Conceptualization, methodology, data curation, fund acquisition, writing - review and editing.

Abstract

A cross-sectional study was conducted to investigate the prevalence and risk factors for contamination of Ethiopian dairy products with *Campylobacter*. A total of 912 dairy food samples were collected from establishments of 682 study participants that were interviewed. Samples were tested for *Campylobacter* by following the ISO 10272-1:2017 standard and PCR confirmation. *Campylobacter* was detected in 11% of tested food samples and all detected *Campylobacter* were *C. jejuni*. The highest prevalence of *C. jejuni* was found in raw milk (16%), followed by pasteurized milk (9%) and cottage cheese (2%) (P<0.001). Using warm water and soap for cleaning cow udders and teats on farms reduced the likelihood of detecting *Campylobacter* in milk (AOR=0.3, P=0.023). Filtering milk with a cloth, using a plastic filter (AOR=0.065, P=0.005), and storing milk in an aluminum container (AOR=0.23, P=0.027) reduced the likelihood of detecting *Campylobacter* in milk at the collection facilities. In contrast, *Campylobacter* detection was significantly more likely in milk collected at collection centers with concrete floors (AOR=5.2, P=0.004). The odds of detecting *Campylobacter* in milk were 17 times greater (AOR=17, P=0.007) in milk processing facilities that did not calibrate a pasteurizer on an annual basis. Finally, having a separate refrigerator for milk storage reduced the odds of detecting *Campylobacter* in retail (AOR=0.29, P=0.021).

Keywords: *Campylobacter jejuni*, raw milk, pasteurized milk, cottage cheese, dairy products, Ethiopia, risk factors, contamination

1. Introduction

Campylobacter is among the leading bacterial foodborne pathogens, causing a high foodborne disease burden worldwide (1, 2). *C. jejuni* is responsible for the majority of campylobacteriosis cases and *C. coli* is the second most common cause of human campylobacteriosis (3, 4) .These species are recognized as a cause of gastroenteritis that can result in severe abdominal pain, fever, nausea, headache, muscle pain, and diarrhea (5, 6). Furthermore, infections with *Campylobacter* can cause Guillain-Barré syndrome with symptoms of muscle weakening or paralysis (7, 8).

According to the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the WHO, *Campylobacter* is one of the four main global causes of diarrheal infections, causing estimated 550 million foodborne disease cases annually (9). In high-income countries, the incidence of campylobacteriosis is well documented through surveillance systems. For example, in the EU, 40.35 per 100,000 people in the European Union had campylobacteriosis in 2020 (13). However, due to minimal surveillance systems for *Campylobacter* in low- and middle-income countries, the incidence of campylobacteriosis in Africa remains largely unknown. A systematic review and meta-analysis reported an average campylobacteriosis incidence of 8.3% in diarrheic and non-diarrheic patients seen in hospitals, basic healthcare clinics, or community cohorts in Sub-Saharan Africa (15).

The ingestion of contaminated food or water and direct contact with feces from infected animals have been reported as the main modes of transmission of *Campylobacter* (18). *C. jejuni* is part of a normal intestinal microbiota of many wild and domesticated animals, including livestock, such as poultry, cattle, and swine (19). Among these animal reservoirs, poultry has been identified as the principal reservoir and source of human *Campylobacter* infections, followed by ruminants, including cattle and sheep (20). Raw milk was identified as the second most common source of *Campylobacter* infections, after chicken meat (21, 22).

Unless appropriately handled, milk can be contaminated by microorganisms at multiple points between production and consumption (23). Microbial contamination of milk can originate from a variety of sources, including feed, the environment, cow's udder, milking equipment (26), and surface water (27) utilized for cleaning milking containers (28). The level of hygienic handling of milk throughout the value chain can therefore affect the safety and quality of milk and dairy products (24, 25).

Risk factors such as poor herd hygiene, the health status of the cattle, production environment, milking environment, and milk preservation practices at dairy farms have previously been associated with general bacterial contamination (29). For example, proper udder and teat cleaning before milking plays an important role in the production of safe milk (30). Similarly, an environment soiled with animal feces has been reported as one of the risk factors for microbial contamination of milk during milking (31). Given that milk is commonly consumed raw in Ethiopia, introduction of pathogens at the farm level, prior to pasteurization, presents a considerable risk for foodborne exposure to *Campylobacter* (32).

Several research studies on the prevalence of *Campylobacter* among humans (33-42), livestock (38, 43-46), and meat (38, 47, 48) have been conducted in Ethiopia. However, there is a knowledge gap in understanding the prevalence of *Campylobacter* in milk and dairy products. This study was therefore conducted to characterize the prevalence of *Campylobacter* and the potential exposure of the Ethiopian public to *Campylobacter* via consumption of milk and cottage cheese. Importantly, this study provides insight into the regional and value chain differences in *Campylobacter* prevalence in Ethiopia. To improve the understanding of risk factors for contamination of dairy products with *Campylobacter* in Ethiopia, this study also reports findings gained through structured interviews with participating dairy farmers, milk collectors, and retailers. The results reported here can inform the development and

implementation of *Campylobacter* control measures in the dairy value chain in Ethiopia and other African countries with similar dairy value chains.

2. Materials and methods

2.1. Study areas and sample size

This study was carried out in three Ethiopian regions, including Oromia, Southern Nation Nationalities Peoples (SNNP), and Amhara during the dry season between January and March 2020 (Figure 1). These regions were selected for inclusion in the study due to their substantial milk production potential. The sample size was calculated based on the following formula: $\mathbf{N = Z^2\ P\ (1\text{-}P)/\ (D^2)}$, where **z** = 1.96 at a 95% confidence interval, **D** is the tolerated margin of sampling error (5% marginal error was used), **p** is an estimated prevalence of *Campylobacter* in the population. Since the prevalence of *Campylobacter* in dairy products in Ethiopia was not known, p was assumed to be 50% for the population. This resulted in a minimum sample size of 384.

The three study regions have different production capacities: Oromia produces an estimated ~52%, SNNP ~23%, and Amhara ~20% of milk produced in Ethiopia (49). The relative number of samples collected from each region was therefore proportional to the relative milk production potential. In the Oromia region, 480 samples were collected from the towns of Assela, Fiche, Debre Zeit, and Walmara. In the SNNP region, 240 samples were collected from Wolayita, Dilla, Hawassa, and Yirgalem; and in the Amhara region, 192 samples were collected from Bahirdar, Debre Berhan, Gondar, and Debre Markos (Figure 1). Thus, a total of 912 dairy food samples were collected from 682 study participants, including dairy farmers, milk collectors, processors, and retailers. Study participants were randomly selected from the list of existing potential participants that was assembled with the help of agricultural development agents. The number of participants was lower than the number of collected

samples since multiple samples were collected from the same milk collectors and processing facilities.

2.2. Sample collection

A total of 250 ml of each raw milk sample was collected into a sterile plastic bottle at each of the 184 participating dairy farms (n = 184) and 58 participating milk collection centers (n = 184). A total of 500 ml of each pasteurized milk sample was collected from each of the 12 participating processors (n = 184) and retailer (n = 184). A total of 500 g of each cottage cheese sample was collected with a sterile plastic pouch from each participating producer (n = 88) and retailer (n = 88). All collected samples were kept at 4°C in a portable refrigerator (Dometic group) until delivery to the laboratory. After samples were delivered at the lab, the laboratory analysis was initiated within an hour. Samples were kept at 3°C in the laboratory until analyses were carried out.

2.3. Enrichment and isolation of *Campylobacter*

Milk and cottage cheese samples were enriched for *Campylobacter* by following the ISO 10272-1:2017 method B. This method was followed because Ethiopian milk and milk products have a relatively high concentration of background microflora (50). A total of 10 g of cottage cheese or 10 ml of milk were homogenized (Nasco, Whirl-Pak) with 90 ml of Preston broth (OXOID nutritional broth No. 2, CM0067) supplemented with 5% laked horse blood (Hardy Diagnostics, 10052-808) and a modified Preston *Campylobacter* selective supplement (OXOID, SR0204E), by hand massaging in homogenization bags. Homogenized samples were incubated at 41.5°C for 24 ± 4 hours in a microaerobic environment (CampyGen, Oxoid AGS). A loopful of undiluted enrichment was streaked on mCCDA agar after enrichment. After 44 ± 4 hours of incubation at 41.5°C in a microaerobic environment (CampyGen, Oxoid, AGS),

streaked mCCDA plates were examined for the presence of presumptive *Campylobacter* colonies.

2.4. DNA Extraction for PCR confirmation of *Campylobacter* spp.

Two presumptive *Campylobacter* colonies were collected from each mCCDA plate and streaked onto brain heart infusion (BHI) agar and incubated at 37°C for 44 hours in microaerobic conditions (CampyGen, Oxoid AGS). DNA was extracted by heat-lysing a colony of each freshly cultivated isolate in 100 µl of sterile nuclease-free water (Ambion, USA) for 10 minutes at 95°C. Cell lysis was followed by centrifugation at 13,000 g for 5 minutes to sediment cell debris (Kamei, Asakura, et al. 2014). The extracted DNA was stored at -20°C until used in a PCR reaction.

2.5. Confirmation of *Campylobacter* species using PCR

Multiplex PCR was used to confirm the genus and species of presumptive *Campylobacter* spp. isolates obtained from mCCDA agar. Table 1 lists the primer sequences as well as the size of the target PCR products (Wang, Clark, et al. 2002). PCR was performed using a thermal cycler (Bio-Rad T100TM Thermal Cycler, Singapore) in 25 µl reactions consisting of 2.5 µl of DNA template, 12 µl of GoTaq Green Master Mix (Promega), 0.125 µl of forward and reverse primers (100 µM) targeting the *C. jejuni hipO* gene, 0.25 µl of forward and reverse primers (100 µM) targeting the *C. coli glyA* gene, 0.05 µl of each forward and reverse primer (100 µM) targeting *Campylobacter*-specific 23S rRNA sequence, and 9.65 µl nuclease-free water. The PCR thermal cycling protocol included the initial denaturation phase at 95°C for 6 minutes, followed by 30 cycles of amplification, each consisting of 0.5 minutes of denaturation at 95°C, 0.5 minutes of annealing at 59°C, and 0.5 minutes of extension at 72°C. The PCR was completed with a 7-minute final extension at 72°C. Each PCR run included a positive control

(DNA extracted from *Campylobacter jejuni* ATCC 29428) and a negative control (nuclease-free water).

2.6. Gel electrophoresis

Gel electrophoresis was performed using a 1.5% w/v agarose gel (Thermo Scientific, 17852) prepared with a trisboric acid/EDTA (TAE) buffer and 5 µl of GelRed (5 mg/ml stock concentration, Biotium) were used to stain DNA. DNA was separated at 120 volts for 40 minutes. Gel Doc EZ Gel Documentation System (Bio-Rad Laboratories) was used to view and record the gel images. Bands of 650, 323, and 126 base pairs were interpreted as a confirmation of *Campylobacter* spp., *C. jejuni*, and *C. coli*, respectively. Each electrophoresis run included a 100 bp DNA ladder, as well as positive and negative controls.

2.7. Questionnaire survey

A questionnaire survey was carried out face-to-face using a Kobo Toolbox by interviewing a farmer, milk collector, processor, or retailer at each sampling location. Data on pre- and post-harvest dairy product handling practices such as barn type and cleaning practices, source of water used for cleaning of the udder, hygiene of a milker, sanitation of milk utensils, and housing for animal management information was collected. At each sampling location, respondents were also asked to provide information on the type of milk and milk product transportation system they use. In addition to administering a questionnaire, direct observation of general cleanliness, hygienic practices, and pasteurized milk and cottage cheese packing material was carried out and recorded. After the questionnaires were completed, milk or cottage cheese samples were collected for laboratory analysis.

2.8. Data management and analysis

Descriptive statistics were performed using SPSS version 26.0 software after raw data was loaded into a Microsoft Excel spreadsheet. The chi-squared test was used to compare the prevalence of *Campylobacter* among different regions, sample types (i.e., raw milk, pasteurized milk, cottage cheese), as well as the prevalence of *Campylobacter* at different points in the value chain. A P value of 0.05 was considered statistically significant. Unadjusted and adjusted odds ratios were calculated to investigate the associations between *Campylobacter* spp. contamination and contamination risk factors obtained through the survey. To calculate the unadjusted odds ratio of each variable with reference to *Campylobacter* spp. detection, standard logistic regression was utilized. The multivariate analysis included variables that were significant at a P value of 0.2 in the bivariate analysis. The final model that forecasts *Campylobacter* spp. recovery was developed using a forward selection with a P value of 0.05.

2.9. Ethical clearance

The Addis Ababa University Ethics Committee approved surveys used in this study (IRB/42/2019).

3. Results

3.1. Prevalence of *Campylobacter* spp. in different regions

Campylobacter spp. growth and morphological characteristics on selective media (i.e., glossy light gray colonies) were used to select putative *Campylobacter* colonies and confirm the genus and species using a multiplex PCR. We confirmed *Campylobacter* spp. in 96 samples collected in a dry season, resulting in a prevalence of 11% (Table 2). All *Campylobacter*-positive samples were contaminated with the species *C. jejuni* (*C. coli* was detected in tested samples).

The highest prevalence of *Campylobacter* was detected in SNNP region 15%, followed by Amhara (11%), and Oromia regional states (8%). The differences in the prevalence of *C. jejuni* among the three studied regions were statistically significant (P = 0.011).

3.2. Prevalence of *Campylobacter* species in different dairy food types at different points in the dairy value chain

Prevalence of *Campylobacter* spp. was assessed in raw milk and milk pasteurized using High Temperature Short Time (HTST), as well as in cottage cheese (Table 2). Of the 368 raw milk samples tested, 16% were contaminated with *C. jejuni.* The prevalence of *C. jejuni* in raw milk samples collected from dairy farmers and milk collectors did not differ significantly (P = 0.88). Compared to the raw milk samples, the prevalence of *C. jejuni* was significantly lower (P = 0.004) among 368 tested pasteurized milk samples (9%) collected from milk processors and retailers. However, the prevalence (9%) of *C. jejuni* did not significantly differ in pasteurized milk samples collected from processors and retailers (P = 0.85). Lastly, the lowest prevalence of *C. jejuni* (2%) was found among 176 tested cottage cheese samples (P = 0.0001) that were collected at dairy farms and retailers. Noteworthy, the cottage cheese samples collected from retailers had a significantly lower prevalence (1%) of *C. jejuni* compared to cottage cheese samples collected from dairy farmers (3%). Overall, as outlined above and summarized in Table 2, the prevalence of *C. jejuni* differed significantly by sample type (P < 0.0001) and point in the value chain (P = 0.013).

3.3. Regional differences in *Campylobacter* species prevalence in different sample types

We further examined the regional differences in the prevalence of *C. jejuni* among different sample types tested in this study (Table 3). *C. jejuni* was detected in 13% of tested raw milk samples, 5% of tested pasteurized milk samples, and 3% of tested cottage cheese samples in

the Oromia region. In the Amhara region, *C. jejuni* was present in 15% of the raw milk samples, 11% of the pasteurized milk samples, and 3% of the cottage cheese samples. In the Southern Nation Nationalities People region, 15% of the 240 samples tested were contaminated with *C. jejuni*. Of these 36 *Campylobacter*-positive samples, 23% were raw milk samples and 14.6% were pasteurized milk samples. Unlike in milk samples, no *Campylobacte*r was detected in any of the cottage samples collected in SNNP. Overall, in the Oromia (P = 0.003) and Amhara regions (P = 0.001), the prevalence of *C. jejuni* was significantly different among different sample types, whereas in SNNP, the prevalence of *C. jejuni* did not significantly differ among sample types (P = 0.204).

3.4. Differences in *Campylobacter* species prevalence at different points along the dairy value chain

The prevalence of *C. jejuni* differed significantly at different points in the dairy value chain in the Oromia, Amhara, and SNNP regions ($P \leq 0.0001$) (Table 4). In Oromia, *C. jejuni* was detected in 13% of samples collected from producers (dairy farmers), 14% of samples collected from milk collectors, 5% samples collected from milk processors, 5% samples collected from pasteurized milk retailers, and 6 % of the cottage cheese collected from the producer. However, none of the cottage cheese collected from retailers was contaminated with *Campylobacte*r species. The prevalence of *C. jejuni* significantly differed at different points in the dairy value chain in the region (P = 0.022).

In Amhara, *C. jejuni* was detected in 7%, 23%, 8%, 15 % and 6% of samples collected from milk producers (dairy farmers), milk collectors, milk processors, pasteurized milk retailers, and cottage cheese, respectively (Table 4). Unlike in Oromia, the prevalence of *C. jejuni* in the Amhara region did not significantly differ at different points in the dairy value chain (P = 0.108).

In the Southern Nation Nationalities Regional State, *C. jejuni* was detected in 31%, 15%, 19%, 10 % of raw milk samples collected from producers (dairy farmers), milk collectors, milk processors, and milk retailers, respectively. Similar to Oromia, the prevalence of *C. jejuni* varied significantly at different points in the dairy value chain in the region (P = 0.001).

3.5. Risk factors for milk contamination with *Campylobacter* at the milk production level

We found that 54% of dairy farms had cattle barn floor made of concrete, while the remaining 46% had barn floor made of soil. A total of 76% of the surveyed dairy farmers had cattle barns that were in poor sanitary conditions (e.g., floor soiled with manure, contaminated feed, and accumulated dirty water). Before milking, 95% of the surveyed farmers cleaned the cow teats. Among those who cleaned cow teats, 63% used warm water and 32% used cold water. Furthermore, 64% of the surveyed farmers used a dry cloth to dry the cleaned cow udder and teats before milking. At the time of a survey, 56% dairy farms reported having at least one cow that was suffering from mastitis. Regarding the equipment used for milking and milk storage, 58% of farmers used tap water to clean milk storage equipment. For milk handling, 89% of the surveyed farmers used plastic containers, 7% used aluminum cans, and 4% used Mazzi can (i.e., a wide-mouth plastic container designed to be easy to clean). Among surveyed farmers, 74% did not refrigerate milk before selling it. According to the survey results shown in Table 5, cleaning cow udders and teats with warm water and soap reduced the risk of milk contamination with *Campylobacter* (AOR = 0.3 (0.1 - 0.8), P = 0.023).

3.6. Risk factors associated with contamination of raw milk with *Campylobacter* at a milk collection point

The majority (97%) of survey participants did not maintain milk cool while transporting it to the collection center. Upon delivery to a collection center, milk was refrigerated using a

refrigerator at 62% of the surveyed collection centers. The milk was filtered by 83% of the surveyed collection centers at the milk receipt. Plastic filters, bits of cloth, and wire mesh were used for milk filtering by 67%, 10%, and 5% by collectors, respectively. The majority, 91%, of the surveyed collection centers had a concrete floor, while the rest had a floor made of soil. In collection centers, 97% and 3% of collectors used tap water and ground water for equipment washing, respectively. Plastic milk containers were used by 78% of the surveyed participants at the milk collection point. Furthermore, 26% of milk collection centers were using aluminum cans to collect milk. Mazzi can was not used in any of the surveyed collection centers. In this study, 31% of surveyed collectors cleaned their equipment with cold water and soap at the collection point. In addition to this, 47% were using warm water and soap for equipment washing. However, none of the collectors washed equipment with only water. During observation by our study team, the milk storage equipment was stored upside down on a shelf by 69% of milk collectors, 45% of the collectors stored it upright open, 66% stored it upright, and 64% stored it covered and upside down in contact with the ground.

The risk of milk contamination with *Campylobacter* at the milk collection center was lower when milk was filtered through a cloth (AOR =0.053 (0.7-0.38), P=0.003), through a plastic filter (AOR = 0.065 (0.009 - 0.04), P = 0.005), or stored in an aluminum container (AOR = 0.23 (0.064 - 0.84), P = 0.027). *Campylobacter* contamination of milk samples was also five times more likely to occur in milk collected in collection facilities with concrete floors compared to those with soil floors (AOR = 5.2 (1.7 - 16), P = 0.004) (Table 6).

3.7. Risk factors for milk contamination with *Campylobacter* species at the milk processing level

To investigate risk factors for *Campylobacter* contamination in milk, we used a structured questionnaire to survey an employee at 12 different milk processing plants about their milk

processing practices. The survey was conducted at the time of sample collection. We found that 92% of surveyed milk processors reported that they had previously gone through food safety training. Further, we carried out an observational survey of the milk processing environment and found soiled and untidy areas (e.g., with pieces of cartons, broken plastic pouches, plastic boxes contaminated with droplets of milk, and droplets of milk on the ground) in 8% of the surveyed milk processing facilities. Among surveyed facilities, 33% used groundwater for washing of equipment, while 67% used tap water. To ensure the effectiveness of pasteurization, 92% of milk processing facilities used a cleaning in place (CIP) system, and 92% dismantled the pasteurizer in the milk processing plant to clean it. However, we did not ask how frequently these cleaning processes were carried out. We further found that 67% of surveyed milk processors calibrated their milk pasteurizer once a year to ensure that the target temperature is reached and held for the required time during pasteurization. Among surveyed processors, 50% and 33% did microbiological and phosphate tests to assess pasteurization efficacy, respectively. Most of the surveyed milk processors (92%) reported that they prohibit milk handlers from working with milk when sick. Lastly, 50% of milk processors maintained a cold chain during distribution from the processing facility to the retailing shop, as shown in Table 7. The likelihood of detecting *Campylobacter* in was 17 times higher (AOR = 17 (2.2 - 131), P = 0.007) in milk processing facilities that did not calibrate the pasteurizer annually (Table 7).

3.8. Assessment of risk factors associated with contamination of pasteurized milk with *Campylobacter* at the retail level

In terms of training, 95% of pasteurized milk retailers did not receive any milk safety training. Sixty-four percent of the pasteurized milk retailers reported transporting milk using four-wheel-drive vehicles at ambient temperature, whereas the rest of the **retailers**

reported transporting milk by maintaining a cold chain. Furthermore, 59% of retailers did not keep milk products at a refrigerator temperature during delivery to the retailing point (a shop or a supermarket). Refrigerators and deep freezers were used for milk storage until the milk was sold by 99% and 1% of surveyed retailers, respectively. We found that 70% of retailers did not have a separate refrigerator for milk and had stored milk together with other foods. As shown in Table 8, the likelihood of *Campylobacter* contamination was lower in pasteurized milk kept in a separate refrigerator than in milk stored with other food items (AOR = 0.29 (0.1 - 0.8), P = 0.021) (Table 8).

4. Discussion

4.1. The prevalence of *Campylobacter* in Ethiopia compared to its prevalence in other African countries

This study is the first to report the prevalence of *Campylobacter* species in dairy foods collected in a dry season in Ethiopia, where *C. jejuni* was detected in 11% of tested dairy product samples. Ethiopia has tropical climate with a dry season that typically runs from October to April (51-53) and a wet season that typically runs from June to mid-September (54). Samples analyzed in this study have been collected exclusively in dry season months. Given that changes in temperature and precipitation have previously been shown to affect the prevalence of *Campylobacter* (55), the prevalence reported here may not be representative of a wet season. Due to limited data from countries that have comparable income, level of agricultural development, livestock size, and food safety culture, we compared the prevalence of *Campylobacter* found in our study with its prevalence in other countries. The prevalence of *Campylobacter* found in this study is similar to findings reported by Zeinhom et al. (2021), who detected *Campylobacter* species in 9.5 % (n = 19/200) of the tested dairy product samples

356 collected in Egypt (56). The higher prevalence, 20.4 % (n = 51/250), of *Campylobacter* species
357 was reported in Egypt by El-Kholy et al. (2016) (57).

358 During the study period, of the 368 raw milk samples tested, 16% were contaminated
359 with *Campylobacter* species, which is related to Zeinhom et al. (2021), who reported that 18%
360 (n = 9/50) in milk samples in Egypt (Zeinhom et al., 2021). In Tanzania, Kashoma et al. (2016)
361 reported a related finding, 13 % (n = 38/284) in raw milk samples (58). Furthermore, in the
362 Eastern Cape Province of South Africa, Igwaran and Okoh (2020) observed a higher prevalence
363 compared to our finding. They reported 37 % (n = 59/159) prevalence of *Campylobacter*
364 contamination in milk samples (59). Mabote et al. (2011) reported a substantially higher
365 prevalence of *C. jejuni* in raw milk in Koster (96 %) and Dellareyville regions (100 %) of South
366 Africa (60).

367 Pasteurization of raw milk is designed to inactivate foodborne pathogens. Gram
368 negative bacteria such as *Campylobacter* species are particularly susceptible to pasteurization
369 (61). The fact that viable *C. jejuni* was detected in 9 % of pasteurized milk samples collected
370 from milk processors and retailers in Ethiopia suggests that the pasteurization is not always
371 carried out at the target temperature and/or for the recommended duration, or that cross-
372 contamination occurs during post-pasteurization processing. Similar data was found in Nigeria,
373 where the prevalence of *Campylobacter* in pasteurized milk was even higher (16%), as reported
374 by Ogbomon et al. (62). Several studies reported no *Campylobacter* in pasteurized milk,
375 although there have been reports that claim that *Campylobacter* was found in patients who
376 consumed pasteurized milk that has not been sufficiently thermally treated (63, 64).

377 In our study, 2% of the 176 tested cottage cheese samples collected, across all three
378 regions, were contaminated with *C. jejuni*, which is similar to the 2 % (n = 8/288) prevalence
379 of *Campylobacter* reported by Omara et al., who analyzed Quraish cheese in Egypt (65). Even
380 higher prevalence of *Campylobacter* (8%; n = 14/180) was recently reported from Egypt by

Barakat et al. (66). El-Kholy et al. reported that 52% of Kareish cheese, 18% of Domiati cheese, and 6% of ice cream were contaminated with *Campylobacter* species in Egypt (57).

We found a lower prevalence of *Campylobacter* in cottage cheese compared to milk, which is likely due to the sensitivity of *Campylobacter* to low pH. This is likely due to organic acids produced by lactic acid bacteria (e.g., lactic, acetic, formic acids) during cottage cheese fermentation, which lower pH in cottage cheese (67). The reduction of pH due to organic acid production to 4.6 or below is likely to inactivate *Campylobacter*, which explains the low prevalence of *Campylobacter* in cottage cheese samples (68).

Overall, the prevalence of *Campylobacter* detected in Ethiopian dairy products was similar or lower compared to that reported in other African countries, although other African countries may not have comparable dairy production and processing systems, or hygiene and food safety culture. Given that milk is often consumed raw in Ethiopia, the 11% prevalence of *Campylobacter* in milk represents a public health concern.

4.2. The prevalence of *Campylobacter* in Ethiopia compared to its prevalence in Asian countries

The 11% prevalence of *Campylobacter* in our study is comparable to the findings reported by Almashhadany, (2021) and Rahimi et al., (2013), who found *Campylobacter* species in 13% (n = 44/350) and 9% (n = 13/552) of tested dairy product samples collected in Iraq and Iran, respectively (69, 70). In Pakistan, Mahmood et al. (61) reported a higher prevalence of *Campylobacter*, 83% (n = 100/120) among tested milk and milk products. In India (Gujarat state), a substantially lower prevalence of *Campylobacter* compared to our finding was reported by Modi et al. who reported that 3% (n = 7/240) of tested milk and milk product samples were contaminated with *Campylobacter* species (22).

Our study detected *Campylobacter* in 16% of the 368 tested raw milk samples from Ethiopia. This finding is similar to a recent study conducted in Iraq by Almashhadany (2021), who reported that 16% (n = 19/120) of the raw milk was contaminated with *C. jejuni*. In Pakistan, Mahmood et al. (61) reported a lower prevalence compared to us, with 12 % (n = 14/120) of the milk samples contaminated with *Campylobacter*. Another study conducted by Hussain et al. found a similar prevalence to ours in Pakistan, where 10% (n = 13/127) of raw milk samples were contaminated with *Campylobacter* species (71). Khanzadi et al., (2010) of Iran and Yang et al. of China reported that *Campylobacter* prevalence was 8% (n = 16/200) and 3% (n = 3/120) in milk samples, respectively, which was lower than what we found in Ethiopia (72, 73).

In Ethiopia, we detected *C. jejuni* in 9% of pasteurized milk samples collected from milk processors and retailers. In contrast to our finding, in Pakistan, UHT and pasteurized packaged milk samples were found to be free of *Campylobacter* (61). However, pasteurized unpackaged and chocolate milk samples were contaminated with *Campylobacter* at rates of 3 and 6%, respectively, in Pakistan (61).

In this study, *C. jejuni* was detected in 3% of the 176 cottage cheese samples across all study regions, which is comparable to the finding reported by Rahimi et al. who found that 5% of traditional cheese in Iraq was contaminated with *Campylobacter* species (70). A higher prevalence than that found in our study was reported by Hussain et al. who reported that 11% of the cheese sample in Pakistan were contaminated with *Campylobacter* species, resulting in a substantially higher prevalence as compared to our study (71).

Lastly, in Asia, Mahmood et al. found that 6, 6, 6, 6, 4, 4, 3, and 3% of plain yogurt, ice cream, chocolate milk, mayonnaise, commercially packaged cheese, skimmed milk powder, flavored yogurt, and pasteurized unpackaged milk were contaminated with *Campylobacter* species (61).

Overall, the prevalence of *Campylobacter* in Ethiopian raw milk is comparable to that reported in Asian countries. However, the prevalence of *Campylobacter* is substantially higher in Ethiopian pasteurized milk compared to pasteurized milk in Asian countries. This may be explained by economic and cultural differences among countries.

4.3. The prevalence of *Campylobacter* in Ethiopia compared to European and North American countries

The prevalence of *Campylobacter* species in dairy foods in Ethiopia was similar to the prevalence found by Andrzejewska et al. among 454 samples of raw milk and unpasteurized milk products (12%) purchased from individual suppliers in Poland (74). We detected *C. jejuni* in 16% of the 368 raw milk samples, which is also similar to a study reported by Bianchini et al., (2014), who reported 12% (n = 34/282) prevalence of *Campylobacter* among tested bulk milk samples collected in Italy (75). Likewise, Artursson et al. reported that 9 % of raw milk samples collected in Sweden were contaminated with *Campylobacter* species, which is again similar to what we found in Ethiopia (76). In contrast to this study, a lower prevalence of *Campylobacter* (5%) was reported by Elmalı and Can who tested milk samples collected in Hatay, Turkey (77). A lower prevalence of *Campylobacter* was also reported in Russia (5%) and Poland (5%) by Efimochkina, and Wysok et al. (78, 79). In the USA, Jayarao et al. reported an even lower prevalence of *C. jejuni* among raw milk samples collected in Pennsylvania (2%) (80).

The presence of *Campylobacter* in raw milk is not surprising and it emphasizes the risks of raw milk consumption. It also points out the need for milk pasteurization. Pasteurization is namely one of the most effective means of controlling pathogens, such as *Campylobacter,* in milk (81). Nevertheless, *C. jejuni* was found in 9% of pasteurized milk samples collected from milk processors and retailers in Ethiopia, which suggests incomplete pasteurization or potential

post-pasteurization contamination. In England, Fernandes et al. tested and examined internal dairy equipment components and revealed mechanical faults that could have led to incomplete pasteurization of a portion of the milk (82). Fahey et al. also reported the failed milk pasteurization as a cause of an outbreak of campylobacteriosis (83). The causes for contamination of pasteurized milk with *Campylobacter* in Ethiopia are unclear and warrant further investigation to mitigate contamination at the milk processing level.

4.4 Risk factors associated with contamination of raw milk and pasteurized milk by *Campylobacter* at milk production, collection, processing, and retail levels

We discovered that farmers who wash cow udders with warm water are less likely to have milk contaminated with *Campylobacter* compared to those who wash them with cold water. Similarly, study conducted in Ethiopia reported a reduced risk of contamination with bacteria in farms that washed milk containers using hot water with a detergent (84).

According to this study, at collection center, filtering milk with pieces of cloth and plastic filter, and storing milk in an aluminum container all reduce the likelihood of finding *Campylobacter* in milk at the collection facilities. Similarly, the aluminum cans had the maximum microbial load decrease, and the type of container was significant ($P = 0.001$) in the reduction of microbial pollutants (85). The concrete floor in milk storage area was linked with a significant increase in the odds of detecting *Campylobacter* in raw milk. Noteworthy, most collection centers had concrete flooring. According to our research, milk collected in a room with a concrete floor is 5 times more likely to be contaminated with *Campylobacter* than milk collected in a room with soil floor. During our visit, we observed that the concrete floor in most collection centers was covered with mud, which may have contributed to this finding as a confounding factor.

At the processing level, it was found that failing calibrate the pasteurization system on an annual basis was linked with an increased risk for detecting *Campylobacter* in pasteurized milk. Despite temperature records showing effective pasteurization, additional testing may reveal mechanical flaws likely to result in incomplete pasteurization of some of the milk (82). Nada et al., (2012) showed that after a dairy plant implemented a HACCP system, the presence of bacterial contaminants in pasteurized milk decreased. They had shown that the additional investments in the pasteurization unit and automated cleaning and disinfection system resulted in a significant reduction of bacterial contaminates in pasteurized eight months after the HACCP implementation (91).

Lastly, we found that keeping pasteurized milk in a separate refrigerator at retail can reduce the risk of pasteurized milk contamination with *Campylobacte*r. This suggests that cross-contamination may be an important factor affecting the prevalence of *Campylobacter* in milk at retail in Ethiopia. Indeed, Marler (2009) showed that pasteurized milk from various sources could be cross-contaminated from other foods stored with milk (93).

5. Conclusion

The 11% prevalence of *Campylobacter* in Ethiopian dairy products presents a considerable food safety risk, particularly given that most of the milk is consumed raw in Ethiopia. Detection of *Campylobacter* in pasteurized milk suggests the need for improved manufacturing practices to ensure adequate pasteurization and prevention of post-pasteurization contamination. Our analysis of risk factors associated with increased odds of *Campylobacter* contamination suggests that simple changes in production, collection, processing, and retailing of dairy products may lead to reduction in contamination. These practices include cleaning cow udders and teats with warm water at the farm level, using aluminum milk can container, cloth and

plastic filters at the collection level, annually calibrating a pasteurizer at the processor level, and storing milk and dairy products separately from other foods in retail stores. The findings reported in this study can be used to develop food safety training and prioritize investments in the dairy value chain that can result in improved dairy safety.

6. Recommendation

Given the prevalence of *Campylobacter* contamination in milk, awareness of the risks associated with consumption of raw milk should be raised at a regional and national level. Producers, collectors, processors, and retailers of milk and milk products would benefit from regular training on the safe handling of milk and milk products, to contribute to the improvement of milk safety. Milk producers (dairy farmers) should be made aware of the sources of milk contamination with *Campylobacter* and provided with training on hygienic milk production. Milk processors are advised to validate and verify the pasteurizer performance to ensure proper pasteurization. Post-pasteurization, it is advised to store milk at or below 4°C and to maintain the cold chain during transportation to retail locations. Milk vendors are advised to keep pasteurized milk in a separate refrigerator, away from other foods. Lastly, the public should be made aware of the existing health risk associated with *Campylobacter* species and encouraged to avoid the consumption of raw milk.

Data availability statement

All data used in the analyses presented here are available in the Supplementary Material (Table S1).

Acknowledgments

This work was supported, in whole or in part, by the Bill & Melinda Gates Foundation and the UK Foreign, Commonwealth, and Development Office, grant number INV-008459. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission. JK was partially funded by the USDA National Institute of Food and Agriculture, Hatch Appropriations under Project #PEN04646 and Accession #1015787. We would like to thank the Oromia, Amhara, and SNNP research participants for their willingness to participate in the survey. We would also like to thank the agricultural development agents at each study site for their assistance with the subject identification and enrollment, as well as questionnaire administration. Thank you also to the Department of Microbiology personnel of the Ethiopian Conformity and Assessment Enterprise for their great technical and moral assistance during the laboratory analyses.

References

1. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, et al. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS medicine. 2015;12(12):e1001923.

2. Mughini Gras L, Smid JH, Wagenaar JA, de Boer AG, Havelaar AH, Friesema IH, et al. Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. PloS one. 2012;7(8):e42599.

3. Lambert E, Hogan N. The importance of job satisfaction and organizational commitment in shaping turnover intent: A test of a causal model. Criminal Justice Review. 2009;34(1):96-118.

4. Hsieh Y-H, Sulaiman IM. Campylobacteriosis: An emerging infectious foodborne disease. Foodborne diseases: Elsevier; 2018. p. 119-55.

5. Fitzgerald C. Campylobacter. Clinics in laboratory medicine. 2015;35(2):289-98.

6. El-Zamkan MA, Hameed KG. Prevalence of Campylobacter jejuni and Campylobacter coli in raw milk and some dairy products. Vet World. 2016;9(10):1147-51.

7. Jackson B, Zegarra JA, Lopez-Gatell H, Sejvar J, Arzate F, Waterman S, et al. Binational outbreak of Guillain–Barré syndrome associated with Campylobacter jejuni infection, Mexico and USA, 2011. Epidemiology & Infection. 2014;142(5):1089-99.

8. El-Zamkan MA, Hameed KGA. Prevalence of Campylobacter jejuni and Campylobacter coli in raw milk and some dairy products. Veterinary World. 2016;9(10):1147.

9. WHO. World Health Organization (WHO). Campylobacter, World Health Organisation web page, 2020. Available at: https://www.who.int/news-room/fact-sheets/detail/campylobacter. [Accessed: 9 September 2022. 2020.

10. Authority EFS, Prevention ECfD, Control. The European Union one health 2020 zoonoses report. EFSA Journal. 2021;19(12):e06971.

11. Huang JY, Henao OL, Griffin PM, Vugia DJ, Cronquist AB, Hurd S, et al. Infection with pathogens transmitted commonly through food and the effect of increasing use of culture-independent diagnostic tests on surveillance—Foodborne Diseases Active Surveillance Network, 10 US sites, 2012–2015. Morbidity and Mortality Weekly Report. 2016;65(14):368-71.

12. Lake I, Colon-Gonzalez FJ, Takkinen J, Rossi M, Sudre B, Dias JG, et al. exploring campylobacter seasonality across Europe using the European surveillance system (TESSy), 2008 to 2016. Eurosurveillance. 2019;24(13):1800028.

13. Authority EFS, Prevention ECfD, Control. The European Union one health 2019 zoonoses report. EFSA Journal. 2021;19(2):e06406.

14. Group OW. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet network, 2010. Communicable diseases intelligence quarterly report. 2012;36(3):E213-E41.

15. Fletcher SM, Stark D, Ellis J. Prevalence of gastrointestinal pathogens in Sub-Saharan Africa: systematic review and meta-analysis. Journal of public health in Africa. 2011;2(2).

16. Supply WUJW, Programme SM. Progress on sanitation and drinking water: 2015 update and MDG assessment: World Health Organization; 2015.

17. Hlashwayo DF, Sigaúque B, Noormahomed EV, Afonso SM, Mandomando IM, Bila CG. A systematic review and meta-analysis reveal that Campylobacter spp. and antibiotic resistance are widespread in humans in sub-Saharan Africa. PloS one. 2021;16(1):e0245951.

18. Zenebe T, Zegeye N, Eguale T. Prevalence of Campylobacter species in human, animal and food of animal origin and their antimicrobial susceptibility in Ethiopia: a systematic review and meta-analysis. Annals of clinical microbiology and antimicrobials. 2020;19(1):1-11.

19. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global Epidemiology of Campylobacter Infection. Clinical Microbiology Reviews. 2015;28(3):687-720.

20. Cody AJ, Maiden MC, Strachan NJ, McCarthy ND. A systematic review of source attribution of human campylobacteriosis using multilocus sequence typing. Eurosurveillance. 2019;24(43):1800696.

21. Davis KR, Dunn AC, Burnett C, McCullough L, Dimond M, Wagner J, et al. Campylobacter jejuni infections associated with raw milk consumption—Utah, 2014. Morbidity and Mortality Weekly Report. 2016;65(12):301-5.

22. Modi S, Brahmbhatt M, Chatur Y, Nayak J. Prevalence of Campylobacter species in milk and milk products, their virulence gene profile and anti-bio gram. Veterinary world. 2015;8(1):1.

23. Bereda A, Yilma Z, Nurfeta A. Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. Wudpecker Journal of Agricultural Research. 2012;1(11):459-65.

24. Keba A, Rolon ML, Tamene A, Dessie K, Vipham J, Kovac J, et al. Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. International dairy journal. 2020;109:104762.

25. CHATTERJEE S, Bhattacharjee I, Chatterjee S, Chandra G. Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. African Journal of Biotechnology. 2006;5(15).

26. Boor KJ, Wiedmann M, Murphy S, Alcaine S. A 100-year review: microbiology and safety of milk handling. Journal of Dairy Science. 2017;100(12):9933-51.

27. Mulder AC, Franz E, de Rijk S, Versluis MA, Coipan C, Buij R, et al. Tracing the animal sources of surface water contamination with Campylobacter jejuni and Campylobacter coli. Water Research. 2020;187:116421.

28. Mpatswenumugabo JPM, Bebora LC, Gitao GC, Mobegi VA, Iraguha B, Shumbusho B. Assessment of bacterial contamination and milk handling practices along the raw milk market chain in the north-western region of Rwanda. African Journal of Microbiology Research. 2019;13(29):640-8.

611 29. Velazquez-Ordoñez V, Valladares-Carranza B, Tenorio-Borroto E, Talavera-Rojas M, Varela-
612 Guerrero J, Acosta-Dibarrat J, et al. Microbial contamination in milk quality and health risk of the
613 consumers of raw milk and dairy products. 2019.

614 30. Skrzypek R, Wójtowski J, Fahr R-D. Hygienic quality of cow bulk tank milk depending on the
615 method of udder preparation for milking. Archives Animal Breeding. 2003;46(5):405-11.

616 31. Gillespie B, Headrick S, Boonyayatra S, Oliver S. Prevalence and persistence of coagulase-
617 negative Staphylococcus species in three dairy research herds. Veterinary microbiology. 2009;134(1-
618 2):65-72.

619 32. Davys G, Marshall J, Fayaz A, Weir R, Benschop J. Campylobacteriosis associated with the
620 consumption of unpasteurised milk: findings from a sentinel surveillance site. Epidemiology &
621 Infection. 2020;148.

622 33. Asrat D, Hathaway A, Ekwall E. Studies on enteric campylobacteriosis in Tikur Anbessa and
623 Ethio-Swedish children's hospital, Addis Ababa, Ethiopia. Ethiopian medical journal. 1999;37(2):71.

624 34. Beyene G, Haile-Amlak A. Antimicrobial sensitivity pattern of Campylobacter species among
625 children in Jimma University Specialized Hospital, southwest Ethiopia. Ethiopian Journal of Health
626 Development. 2004;18(3):185-9.

627 35. Terefe Y, Deblais L, Ghanem M, Helmy YA, Mummed B, Chen D, et al. Co-occurrence of
628 Campylobacter Species in Children From Eastern Ethiopia, and Their Association With Environmental
629 Enteric Dysfunction, Diarrhea, and Host Microbiome. Frontiers in Public Health. 2020;8:99.

630 36. Budge S, Barnett M, Hutchings P, Parker A, Tyrrel S, Hassard F, et al. Risk factors and
631 transmission pathways associated with infant Campylobacter spp. prevalence and malnutrition: A
632 formative study in rural Ethiopia. PloS one. 2020;15(5):e0232541.

633 37. Mitike G, Kassu A, Genetu A, Nigussie D. Campylobacter enteritis among children in Dembia
634 district, northwest Ethiopia. East African Medical Journal. 2000;77(12).

635 38. Ewnetu D, Mihret A. Prevalence and antimicrobial resistance of Campylobacter isolates from
636 humans and chickens in Bahir Dar, Ethiopia. Foodborne pathogens and disease. 2010;7(6):667-70.

637 39. Tafa B, Sewunet T, Tassew H, Asrat D. Isolation and antimicrobial susceptibility patterns of
638 Campylobacter species among diarrheic children at Jimma, Ethiopia. International journal of
639 bacteriology. 2014;2014.

640 40. Getamesay M, Getenet B, Ahmed Z. Prevalence of Shigella, Salmonella and Cmpylobacter
641 species and their susceptibility patters among under five children with diarrhea in Hawassa Town, South
642 Ethiopia. Ethiopian journal of health sciences. 2014;24(2):101-8.

643 41. Mulatu G, Beyene G, Zeynudin A. Prevalence of Shigella, Salmonella and Cmpylobacter
644 species and their susceptibility patters among under five children with diarrhea in Hawassa town, South
645 Ethiopia. Ethiopian journal of health sciences. 2014;24(2):101.

646 42. Gedlu E, Aseffa A. Campylobacter enteritis among children in north-west Ethiopia: a 1-year
647 prospective study. Annals of tropical paediatrics. 1996;16(3):207-12.

43. Kassa T, Gebre-Selassie S, Asrat D. Antimicrobial susceptibility patterns of thermotolerant Campylobacter strains isolated from food animals in Ethiopia. Veterinary Microbiology. 2007;119(1):82-7.

44. Abamecha A, Assebe G, Tafa B, Wondafrash B. Prevalence of thermophilic Campylobacter and their antimicrobial resistance profile in food animals in Lare District, Nuer Zone, Gambella, Ethiopia. Journal of Drug Research and Development. 2015;1(2):1-6.

45. Nigatu S, Mequanent A, Tesfaye R, Garedew L. Prevalence and Drug Sensitivity Pattern of Campylobacter jejuni Isolated from Cattle and Poultry in and Around Gondar Town, Ethiopia. Glob Vet. 2015;14(1):43-7.

46. Hailemariam S. Prevalence, associated risk factors and antimicrobial susceptibility pattern of thermophilic Campylobacter spp. of ovine carcass at Addis Ababa Abattoir Enterprise, Ethiopia: Addis Ababa University; 2014.

47. Dadi L, Asrat D. Prevalence and antimicrobial susceptibility profiles of thermotolerant Campylobacter strains in retail raw meat products in Ethiopia. Ethiopian journal of health development. 2008;22(2):195-200.

48. Faris G. Identification of Campylobacter species and their antibiotic resistance patterns from raw bovine meat in Addis Ababa, Ethiopia. International Journal of Multimedia Information Retrieval. 2015;4(1):1-5.

49. CSA. Agricultural Sample Survey, 2010/11 (2003 EC), Volume II: Report on Livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 505. Central Statistical Agency (CSA) Federal Democratic Republic of Ethiopia …; 2011.

50. Standardization IOf. Microbiology of the Food Chain-Horizontal Method for Detection and Enumeration of Campylobacter Spp: ISO; 2017.

51. Aerts R, Van Overtveld K, November E, Wassie A, Abiyu A, Demissew S, et al. Conservation of the Ethiopian church forests: threats, opportunities and implications for their management. Science of the Total Environment. 2016;551:404-14.

52. Ambachew S, Assefa M, Tegegne Y, Zeleke AJ. The Prevalence of Intestinal Parasites and Their Associated Factors among Diabetes Mellitus Patients at the University of Gondar Referral Hospital, Northwest Ethiopia. Journal of Parasitology Research. 2020;2020.

53. Fazzini M, Bisci C, Billi P. The climate of Ethiopia. Landscapes and landforms of Ethiopia: Springer; 2015. p. 65-87.

54. Walker B. Seasonal Weather Assessment for Ethiopia during March–July 2016. London: Government of the United Kingdom. 2016.

55. Kalupahana RS, Mughini-Gras L, Kottawatta S, Somarathne S, Gamage C, Wagenaar J. Weather correlates of Campylobacter prevalence in broilers at slaughter under tropical conditions in Sri Lanka. Epidemiology & Infection. 2018;146(8):972-9.

56. Zeinhom MM, Abdel-Latef GK, Corke H. Prevalence, Characterization, and Control of Campylobacter jejuni Isolated from Raw Milk, Cheese, and Human Stool Samples in Beni-Suef Governorate, Egypt. Foodborne Pathogens and Disease. 2021;18(5):322-30.

687 57. El-Kholy A, Meshref A, El-Gedawy A, Esam R. Prevalence of Campylobacter species in milk
688 and some dairy products. Journal of Veterinary Medical Research. 2016;23(2):133-42.

689 58. Kashoma IP, Kassem II, John J, Kessy BM, Gebreyes W, Kazwala RR, et al. Prevalence and
690 antimicrobial resistance of Campylobacter isolated from dressed beef carcasses and raw milk in
691 Tanzania. Microbial drug resistance. 2016;22(1):40-52.

692 59. Igwaran A, Okoh AI. Occurrence, Virulence and Antimicrobial Resistance-Associated Markers
693 in Campylobacter Species Isolated from Retail Fresh Milk and Water Samples in Two District
694 Municipalities in the Eastern Cape Province, South Africa. Antibiotics. 2020;9(7):426.

695 60. Mabote KI, Mbewe M, Ateba CN. Prevalence of Campylobacter contamination in fresh chicken
696 meat and milk obtained from markets in the North-West Province, South Africa. Journal of Human
697 Ecology. 2011;36(1):23-8.

698 61. Mahmood MS, Hussain I, Arshad MI, Ali S, Aktar M, Khan A, et al. Seasonal prevalence of
699 Campylobacter species in milk and milk products in Pakistan. Pak J Zool Suppl Ser. 2009;9:227-31.

700 62. Ogbomon EO, Akpomie OO, Enenya RP, Obanor O, Morka E. Prevalence and Antibiotic
701 Susceptibility Patterns of Campylobacter Species in Locally Pasteurized Milk Product (Nunu) Sold in
702 Zaria Metropolis, Kaduna State, Nigeria. International Journal of Microbiology and Biotechnology.
703 2018;3(3):89.

704 63. Djuretic T, Wall P, Nichols G. General outbreaks of infectious intestinal disease associated
705 with milk and dairy products in England and Wales: 1992 to 1996. Communicable disease report CDR
706 Review. 1997;7(3):R41-5.

707 64. Galbraith N, Forbes P, Clifford C. Communicable disease associated with milk and dairy
708 products in England and Wales 1951-80. Br Med J (Clin Res Ed). 1982;284(6331):1761-5.

709 65. Omara ST, El Fadaly H, Barakat A. Public health hazard of zoonotic Campylobacter jejuni
710 reference to Egyptian regional and seasonal variations. Research Journal of Microbiology.
711 2015;10(8):343.

712 66. Barakat AM, Abd El-Razik KA, Elfadaly HA, Rabie NS, Sadek SA, Almuzaini AM.
713 Prevalence, molecular detection, and virulence gene profiles of Campylobacter species in humans and
714 foods of animal origin. Veterinary World. 2020;13(7):1430.

715 67. Ibrahim SA, Ayivi RD, Zimmerman T, Siddiqui SA, Altemimi AB, Fidan H, et al. Lactic acid
716 bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods.
717 2021;10(12):3131.

718 68. Doyle M, Roman D. Growth and survival of Campylobacter fetus subsp. jejuni as a function of
719 temperature and pH. Journal of Food Protection. 1981;44(8):596-601.

720 69. Almashhadany DA. Isolation, biotyping and antimicrobial susceptibility of Campylobacter
721 isolates from raw milk in Erbil city, Iraq. Italian Journal of Food Safety. 2021;10(1).

722 70. Rahimi E, Sepehri S, Momtaz H. Prevalence of Campylobacter species in milk and dairy
723 products in Iran. Revue de Médecine Vétérinaire. 2013;164(5):283-8.

724 71. Hussain I, Mahmood MS, Akhtar M, Khan A. Prevalence of Campylobacter species in meat,
725 milk and other food commodities in Pakistan. Food microbiology. 2007;24(3):219-22.

72. Khanzadi S, Jamshidi A, Soltaninejad V, Khajenasiri S. Isolation and identification of Campylobacter jejuni from bulk tank milk in Mashhad-Iran. World Applied Sciences Journal. 2010;9(6):638-43.

73. Yang C, Jiang Y, Huang K, Zhu C, Yin Y. Application of real-time PCR for quantitative detection of Campylobacter jejuni in poultry, milk and environmental water. FEMS Immunology & Medical Microbiology. 2003;38(3):265-71.

74. Andrzejewska M, Szczepańska B, Śpica D, Klawe JJ. Prevalence, virulence, and antimicrobial resistance of Campylobacter spp. in raw milk, beef, and pork meat in Northern Poland. Foods. 2019;8(9):420.

75. Bianchini V, Borella L, Benedetti V, Parisi A, Miccolupo A, Santoro E, et al. Prevalence in bulk tank milk and epidemiology of Campylobacter jejuni in dairy herds in Northern Italy. Applied and environmental microbiology. 2014;80(6):1832-7.

76. Artursson K, Schelin J, Lambertz ST, Hansson I, Engvall EO. Foodborne pathogens in unpasteurized milk in Sweden. International Journal of Food Microbiology. 2018;284:120-7.

77. Elmalı M, Can HY. Antimicrobial susceptibility and virulence-associated genes in Campylobacter isolates from milk and wastewater in Hatay, Turkey. Ciência Rural. 2019;49.

78. Efimochkina N. Evaluation of the role of Campylobacter spp. in the occurrence of foodborne diseases and modern methods to detect the pathogen. Voprosy Pitaniia. 2015;84(6):5-18.

79. Wysok B, Wiszniewska-Łaszczych A, Uradziński J, Szteyn J. Prevalence and antimicrobial resistance of Campylobacter in raw milk in the selected areas of Poland. Polish journal of veterinary sciences. 2011(3).

80. Jayarao BM, Donaldson SC, Straley BA, Sawant AA, Hegde NV, Brown J. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. Journal of dairy science. 2006;89(7):2451-8.

81. Robinson D. Infective dose of Campylobacter jejuni in milk. British medical journal (Clinical research ed). 1981;282(6276):1584.

82. Fernandes AM, Balasegaram S, Willis C, Wimalarathna HM, Maiden MC, McCarthy ND. Partial failure of milk pasteurization as a risk for the transmission of Campylobacter from cattle to humans. Clinical Infectious Diseases. 2015;61(6):903-9.

83. Fahey T, Morgan D, Gunneburg C, Adak G, Majid F, Kaczmarski E. An outbreak of Campylobacter jejuni enteritis associated with failed milk pasteurisation. Journal of Infection. 1995;31(2):137-43.

84. Tigabu E, Asrat D, Kassa T, Sinmegn T, Molla B, Gebreyes W. Assessment of Risk Factors in Milk Contamination with S taphylococcus aureus in Urban and Peri-Urban Small-Holder Dairy Farming in Central E thiopia. Zoonoses and Public Health. 2015;62(8):637-43.

85. Wafula WN, Matofari WJ, Nduko MJ, Lamuka P. Effectiveness of the sanitation regimes used by dairy actors to control microbial contamination of plastic jerry cans' surfaces. International Journal of Food Contamination. 2016;3(1):1-8.

86. Giacometti F, Serraino A, Finazzi G, Daminelli P, Losio MN, Tamba M, et al. Field handling conditions of raw milk sold in vending machines: experimental evaluation of the behaviour of Listeria monocytogenes, Escherichia coli O157: H7, Salmonella Typhimurium and Campylobacter jejuni. Italian Journal of Animal Science. 2012;11(1):e24.

87. Hudson A, King N, Lake R, Cressey P. Risk profile: Campylobacter jejuni/coli in raw Milk. ESR (Eur Surg Res). 2014:1-77.

88. Vahedi M, Nasrolahei M, Sharif M, Mirabi A. Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. Journal of preventive medicine and hygiene. 2013;54(2):120.

89. Nebiyu R, Beyene F, Giorgis Y, Kassa B, Kass F. Impacts of technology adoption on the quality of milk from smallholder farms in Wolayta, Southern Ethiopia. Livestock Research for Rural Development. 2012;24(12).

90. Kutsara L, Metsa M, Rättob M, Salob S, Veskusa T, Wirtanenb G. Evaluation of Cleanliness of Dairy Plants and Innovations for Improving Hygiene. RISK MANAGEMENT BY HYGIENIC DESIGN AND EFFICIENT SANITATION PROGRAMS. 2009:136.

91. Nada S, Ilija D, Igor T, Jelena M, Ruzica G. Implication of food safety measures on microbiological quality of raw and pasteurized milk. Food control. 2012;25(2):728-31.

92. Al-Farsi M, Al-Gharibi I, Al-Abri A, Al-Humaimi A, Al-Nabhani F, Al-Hashmi H, et al. Evaluating the shelf-life of pasteurized milk in Oman. Heliyon. 2021;7(3):e06555.

93. Marler W. Comparing the food safety record of pasteurized and raw milk products–Part 3. 2009.

94. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, et al. Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. Journal of clinical microbiology. 2002;40(12):4744-7.

Figure and Tables

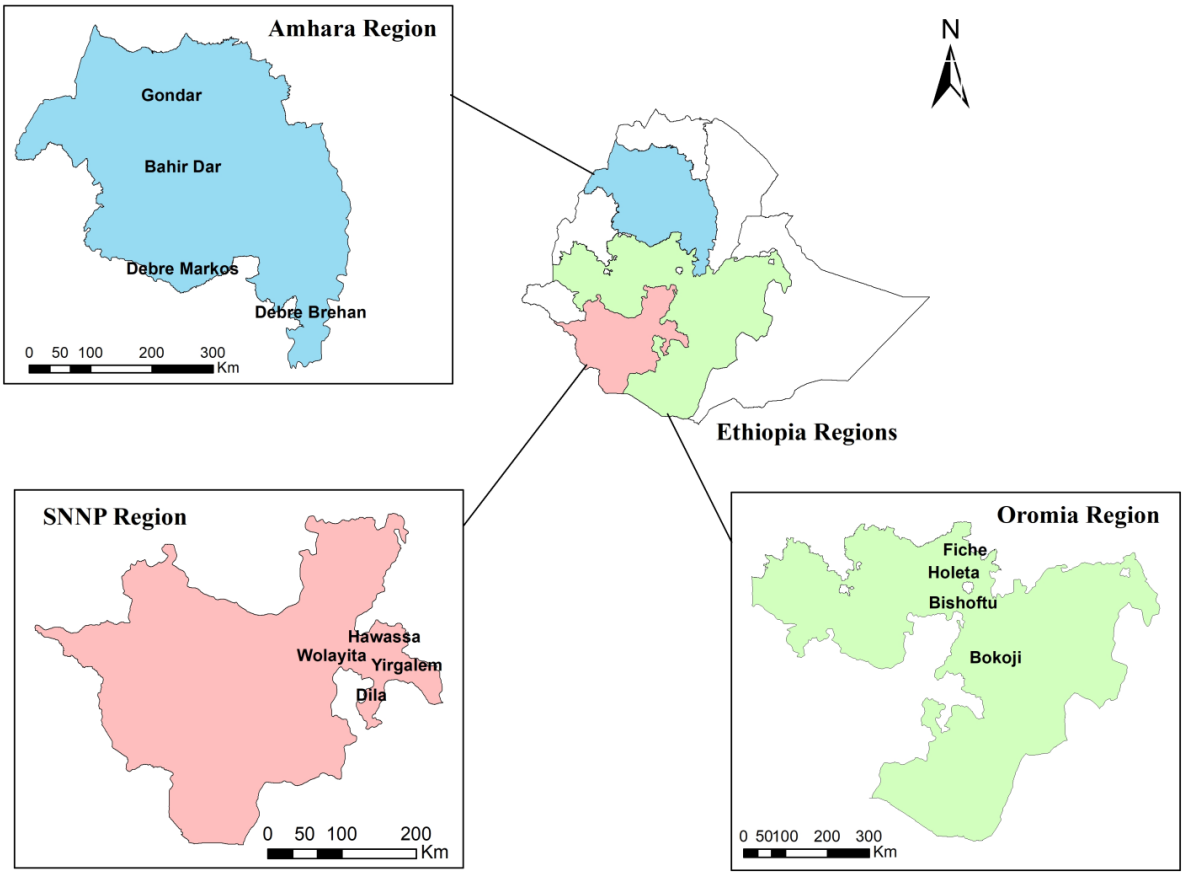

Figure 1: Map of the study areas. Milk and cottage cheese samples were collected from four sites in each of the three Ethiopian regions, including Amhara, SNNP, and Oromia. Study sites within each region are listed on maps of individual regions.

793 **Table 1**: List of primers for confirmation of *Campylobacter* genus and species.

Primer[a]	Size (bp)	Sequence (5′-3′)[a]	GenBank accession no.	Target gene	Gene location (bp)
CJR	323	ACTTCTTTATTGCTTGCTGC	Z36940	*C. jejuni hipO*	1662-1681
		GCCACAACAAGTAAAGAAGC			1984-1965
CCF	126	GTAAAACCAAAGCTTATCGTG	AF136494	*C. coli glyA*	337-357
CCR		TCCAGCAATGTGTGCAATG			462-444
23SF	650	TATACCGGTAAGGAGTGCTGGAG	Z29326	*C. jejuni* 23S rRNA	3807-3829
23SR		ATCAATTAACCTTCGAGCACCG			4456-4435

794 [a] Primer reference: (94).

795 **Table 2**: *Campylobacter* species prevalence across Ethiopian dairy value chain.

Variables	Region, sample type, and value chain	Samples (n)	*Campylobacter* spp. (%)	P value[a]
Region	Amhara	192	22 (11.5)	0.011
	Oromia	480	38 (7.9)	
	SNNP	240	36 (15.0)	
	Total	**912**	**96 (11)**	
Value chain	Raw milk collectors	184	29 (15.8)	0.013
	Cottage cheese producers	88	3 (3.4)	
	Cottage cheese retailers	88	1 (1.1)	
	Milk processors	184	17 (9.2)	
	Milk retailers	184	16 (8.7)	
	Raw producers	184	30 (16.3)	
	Total	**912**	**96 (11)**	
Sample type	Cottage cheese	176	4 (2.3)	<0.0001
	Pasteurized milk	368	33 (9.0)	
	Raw milk	368	59 (16.0)	
	Total	**912**	**96 (11)**	

796 [a] P value indicates statistical significance.

797

798

Table 3: Regional differences in *Campylobacter* species prevalence among different sample types.

Region	Sample Type	Samples (n)	*Campylobacter* spp. (%)	P value[a]
Oromia	Cottage cheese	96	3 (3.1)	0.003
	Pasteurized milk	192	10 (5.2)	
	Raw milk	192	25 (13)	
	Total	**480**	38 (7.9)	
Amhara	Cottage cheese	32	1 (3.1)	0.001
	Pasteurized milk	80	9 (11.3)	
	Raw milk	80	12 (15.0)	
	Total	**192**	22 (11.5)	
SNNP	Cottage cheese	48	0	0.204
	Pasteurized milk	96	14 (14.6)	
	Raw milk	96	22 (22.9)	
	Total	**240**	36 (15.0)	
Total		**912**	**96 (11)**	

[a] P value indicates statistical significance.

802 **Table 4:** The prevalence of *Campylobacter* species at different points in a dairy value chain in
803 different regions.

Region	Point in the value chain	Samples (n)	*Campylobacter* spp. (%)	P value[a]
Oromia	Collectors	96	13 (13.5)	0.022
	Cottage cheese producers	48	3 (6.3)	
	Cottage cheese retailers	48	0	
	Pasteurized milk processors	96	5 (5.2)	
	Pasteurized milk retailers	96	5 (5.2)	
	Producers	96	12 (12.5)	
	Total	**480**	**38 (7.9)**	
Amhara	Collectors	40	9 (22.5)	0.108
	Cottage cheese producers	16	0.0	
	Cottage cheese retailers	16	1 (6.3)	
	Pasteurized milk processors	40	3 (7.5)	
	Pasteurized milk retailers	40	6 (15)	
	Producers	40	3 (7.5)	
	Total	**192**	**22 (11.5)**	
SNNP	Collectors	48	7 (14.6)	0.001
	Cottage cheese producers	24	0	
	Cottage cheese retailers	24	0	
	Pasteurized milk processors	48	9 (18.8)	
	Pasteurized milk retailers	48	5 (10.4)	
	Producers	48	15 (31.3)	
	Total	**240**	**36 (15)**	
	Total	**912**	**96 (11)**	

804 [a] P value indicates statistical significance.

805 **Table 5:** Risk factors associated with contamination of raw milk with *Campylobacter* spp. at
806 a dairy farm level in Amhara, Oromia, and SNNP region, between January and March 2020.

Variables	Category	N (%)	*Campylobacter* spp. (n)	COR[a]		AOR[a]	
				95% CI[a]	P value[a]	95% CI[a]	P value[a]
Construction material of the cattle barn floor	Concrete	100 (54)	16	0.9 (0.4 - 1.9)	0.73	-	-
	Soil	84 (46)	15				
Hygienic condition of the cattle barn	Good	45 (24)	8	0.95 (0.3 – 2.2)	0.84	-	-
	Poor	139 (76)	23				
A major source of water for washing milking equipment	Groundwater	35 (19)	8	1.4 (0.5-3.7)	0.4	-	-
	Pump water	25 (14)	2	0.4 (0.09-2)	0.28		
	Rainwater	2 (1)	0	0.00	0.99		
	River water	15 (8)	3	1.2 (0.3-4.8)	0.8		
	Tap water	107 (58)	18				
Cow udder and teats are washed	No	10 (5)	1	1.9 (0.22 - 15)	0.55	-	-
	Yes	174 (96)	30				
Type of water used for teats and udder washing	Cold water	62 (34)	5	0.3 (0.1 – 0.8)	0.020	0.3(0.1-0.8)	0.023
	Warm water	115 (63)	26				
Udder and teats are dried using a dry cloth	No	67 (36)	8	0.5 (0.2 - 1.3)	0.18	-	-
	Yes	117 (64)	23				
Owners' cows have been diagnosed with mastitis	No	81 (44)	10	1.8 (0.8 – 4.1)	0.15	-	-
	Yes	103 (56)	21				
Milk is being filtered	No	13 (7)	1	0.4 (0.04 – 3.1)	0.37	-	-
	Yes	171 (93)	30				
Material used for milk filtration	Piece of cloth	54 (29)	7	1.2 (0.2 - 6.3)	0.83	-	-
	Plastic filter	112 (61)	21	2.1 (0.4-9.9)	0.33		
	Wire mesh	18 (10)	2			-	-
Type of equipment used for milk handling	Aluminum cans	13 (7)	2			-	-
	Mazzi can	7 (4)	1	0.9 (0.07 - 12.3)	0.94		
	Plastic containers	164 (89)	28	1.13 (0.23 - 5.39)	0.87		

Refrigerator is available for milk cooling until sale	No	136 (74)	18	0.4 (0.18 – 0.9)	0.03	0.55
	Yes	48 (26)	13			

[a] COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval at 95%; P value, indicates statistical significance; - , not calculated due to COR P value being greater than 0.2.

Table 6: Analysis of risk factors associated with contamination of raw milk with *Campylobacter* species at the milk collection point in a dairy value chain in Amhara, Oromia, and SNNP regions, between January and March 2020**.**

Variables		Category	N (%)	*Campylobacter* spp. (n)	COR[a]		AOR[a]	
					95% CI[a]	P value[a]	95% CI[a]	P value[a]
Temperature is kept low during transportation		No	56 (97)	21	0.16 (0.06 – 0.47)	0.001	-	0.16
		Yes	2 (3)	8				
Milk is filtered upon receipt		No	10 (17)		0.38 (0.14 – 0.98)	0.047	-	-
		Yes	48 (83)	23				
Material used for milk filtration		Piece of cloth	6 (10)	10	0.018 - 0.64	0.014	0.053 (0.7-0.38)	0.003
		Plastic filter	39 (67)	8	0.10 - 0.36	0.002	0.065 (0.009-.04)	0.005
		Wire meshes	3 (5)	5	1	0.004	-	-
A cooling system is available for milk		No	36 (62)	11	1.3 (0.6 – 3.1)	0.461	-	-
		Yes	22 (38)	18				
Material of the collection center floor		Concrete floor	53 (91)	24	3.47 (1.2 - 9.7)	0.017	5.2 (1.7-16)	0.004
		Soil floor	5 (9)	5				
A major source of water used for equipment washing		Ground water	2 (3)	0 (0.0)	0	0.99	-	-
		Tap water	56 (97)	29				
Milk handling equipment	Plastic containers	No	13 (22)	20	20.5(0.21 – 1.15)	0.101	-	0.64
		Yes	45 (78)	9				
	Aluminum cans	No	43 (74)	6	0.39 (0.15 - 1.01)	0.054	0.23 (0.064-0.84)	0.027
		Yes	15 (26)	23				
	Mazzi cans	No	58 (100)	29	0	1	-	-
Cleaning protocol	Water only	No	58 (100)	29			-	-
	Cold water and soap	No	40 (69)	11	0.95 (0.42 - 2.1)	0.9	-	-
		Yes	18 (31)	18				
	Warm water and soap	No	31 (53)	14	0.8 (0.35 – 1.7)	0.56	-	-
		Yes	27 (47)	15				
Milk handling equipment storage	Upright and open	No	32 (55)	22	0.7 (0.26 – 1.7)	0.39	-	-
		Yes	26 (45)	7				
	Upright and covered	No	20 (34)	25	1.6 (0.5 - 5)	0.397	-	-
		Yes	38 (66)	4				
		No	21 (36)	19		0.008	-	

Upside down in contact with the ground	Yes	37 (64)	10	3.35 (1.37 - 8.2)		0.3
Upside down on a shelf	No	18 (31)	21	1.3 (0.57 – 3.29)	0.49	-
	Yes	40 (69)	8			

[a] COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval at 95%; P value, indicates statistical significance; - , not calculated due to COR P value being greater than 0.2.

Table 5: Risk factors associated with contamination of raw milk with *Campylobacter* species during milk processing in Amhara, Oromia, and SNNP region, between January and March 2020.

Risk factor	Category	N (%)	*Campylobacter* spp. (n)	COR[a]		AOR[a]	
				95 % CI[a]	P value[a]	95 % CI[a]	P value[a]
Employees attended basic food safety training	No Yes	1 (8) 11 (92)	9 8	3.69 (1.3 - 10)	0.012		0.5
Storage area is free of trash	No Yes	1 (8) 11 (92)	9 8	3.6 (1.3 -10)	0.012		0.55
Source of water for equipment washing	Ground water Tap water	4 (33) 8 (67)	11 6	1.72 (0.6-4.9)	0.303		
Milk handlers that are sick do not work with milk	No Yes	1 (8) 11 (92)	5 12	3.2 (1.03-10)	0.044		0.5
Cleaning in place (CIP) is applied	No Yes	1 (8) 11 (92)	9 8	3.6 (1.3-10)	0.012		0.55
Pasteurizer is dismantled and cleaned	No Yes	1 (8) 11 (92)	9 8	3.6 (1.3 - 10)	0.012		
Pasteurizer is calibrated annually	No Yes	4 (33) 8 (67)	16 1	17 (2.2-131)	0.007	17(2.2 - 131)	0.007
Efficacy of pasteurization is verified	No Yes	3 (25) 9 (75)	11 6	2.6 (0.9 – 7.3)	0.33	-	
Method of used for pasteurization verification Phosphatase test	No Yes	8 (67) 4 (33)	12 5	1.8 (0.6-5.5)	0.262		
Microbiological test	No Yes	6 (50) 6 (50)	12 5	3 (1.04 - 9.1)	0.042		0.73
Cold chain transportation system is in place	No Yes	6 (50) 6 (50)	9 8	3.1 (1.14-8.6)	0.027		0.88

[a] COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval at 95%; P value, indicates statistical significance; - , not calculated due to COR P value being greater than 0.2.

Table 6: Assessment of risk factors associated with contamination of pasteurized milk with *Campylobacter* species at the milk retail level in Amhara, Oromia, and SNNP region, between January and March 2020.

Risk factor	Categories	N%	*Campylobacter* spp. (n)	COR[a]		AOR[a]	
				95 % CI[a]	P value[a]	95% CI[a]	P value[a]
Employees attended food safety training	No Yes	174 (95) 10 (5)	14 2	0.35 (0.07 – 1.8)	0.21		0.4
Means of milk transportation	Cold trucks Four-wheel drives	67 (36) 117 (64)	9 7	0.41 (0.14 - 0.15)	0.092		-
Cold chain is maintained during transportation	No Yes	108 (59) 76 (41)	6 10	0.39 (0.13 – 1.1)	0.08		0.37
Equipment used to maintain cold chain	Deep freezers Refrigerator	1 (0.5) 183 (99)	0 16	0	0		-
A separate refrigerator is used for milk and dairy foods	No Yes	129 (70) 55 (30)	7 9	0.29 (0.1 – 0.83)	0.020	0.29 (0.1-0.8)	0.021

[a] COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval at 95%; P value, indicates statistical significance; - , not calculated due to COR P value being greater than 0.2.