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Prevalence and Risk Factors of *Campylobacter* Colonization in Broiler Farms at Selected Districts of Dhaka Division, Bangladesh

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Abstract | *Campylobacter*, originating from poultry, is considered as one of the primary etiological agent of human foodborne illness. However, little is known about the *Campylobacter* spp. colonization in broilers of Bangladesh. This study aimed to investigate the prevalence of *Campylobacter* spp. colonization and its associated risk factors in the broiler farms of Munshigonj, Narayanganj, and Narsingdi Districts in Bangladesh. Cloacal swab samples were collected from 100 broiler farms. We speculated that individual samples had a higher possibility of isolating *Campylobacter*; however, five randomly selected broilers from each farm were used to create a pooled sample for this study. Standard bacteriological and molecular techniques were followed to isolate and identify *Campylobacter* spp. Data related to the poultry farm management practices were collected by using a designed questionnaire to predict the potential risk factors at the farm level. The prevalence of *Campylobacter* spp. was 24.00% irrespective of the farm locations. In the districts of Munshiganj, Narayanganj, and Narsindi, the prevalence of *Campylobacter* spp. colonization was found to be 10.00%, 27.78%, and 32.35%, respectively. In risk factor analysis, the factors significantly associated with *Campylobacter* colonization were "water supply","more than one person entering the house", "use of separate footwear to enter in to the shed", and "broiler house empty for >14 days between flocks". "Footbath facility" and "presence of rodents in the poultry house" were revealed as the factors associated with increased risk for *Campylobacter* colonization. The study gathered evidence of the presence of *Campylobacter* spp. colonization in the broiler farms and identified influencing factors which could aid to set effective interventions for controlling of *Campylobacter* infection in broiler farms to minimize *Campylobacter* infection in humans from broilers. A further extended study might provide valuable information to formulate a national control strategy.

Keywords | Broiler farms, *Campylobacter* spp., Cloacal swab, Prevalence, Risk factors, Biosecurity

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INTRODUCTION

One of the most significant human pathogens which causing diarrhea is *Campylobacter* spp. (Hughes and Cornblath, 2005; Uzoigwe, 2005). It can also cause meningitis, septicemia, reactive arthritis, and complications from Guillain-Barré syndrome. Nowadays, thermophilic *Campylobacter* species are the most frequent cause of bacterial gastroenteritis worldwide (Man, 2011). *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7 combined have caused fewer human cases than *Campylobacter*iosis (EFSA, 2012). *Campylobacter* spp. are characterized by gram-negative spiral-shaped bacteria with corkscrew motility and giving positive catalase, oxidase and indoxyl acetate reactions. It has 25 species, 8 subspecies and 2 provisional species (Man, 2011). From a food safety perspective, thermophilic *Campylobacter*s, primarily *C. jejuni* and *C. coli* are the most significant species; they can induce gastroenteritis in both humans and domestic animals (Al Hakeem *et al*., 2022; Andritsos *et al*., 2023). These two species are responsible for nearly 90% of the documented cases of *Campylobacter*iosis in humans; *C. jejuni* is responsible for over 80% of gastrointestinal infections, while *C. coli* is responsible for the remaining 10% of infections (Wagenaar and van der Graaf-van Bloois, 2018). The colonization of *C. jejuni* and *C. coli* in poultry farms increases the risk of human *Campylobacter*iosis (Al Hakeem *et al*., 2022).

Globally, the incidence of *Campylobacter*iosis has increased during the past ten years. There has been a rise in cases of *Campylobacter*iosis in North America, Europe, and Australia. Data from Asia, Africa, and the Middle East indicate that *Campylobacter* is endemic in these areas (Kaakoush *et al*., 2015). In both developed and developing nations, foodborne illnesses are becoming a bigger public health concern (Elmi, 2004). Most severe human cases of *Campylobacter* are caused by food. According to Adak *et al*. (2005) handling or consuming undercooked or raw poultry meat increases the risk of infection in humans. It has been established that poultry is the main reservoir and source of human *Campylobacter*iosis transmission. Because of the numerous steps has taken by the government to support the nation's livestock industry, Bangladesh is now self-sufficient in the production of meat, with broiler meat accounting for the majority of this output (DLS, 2020). However, human foodborne illness is a possibility if poultry meats are tainted with *Campylobacter* species. Different countries have different observed prevalence rates of *Campylobacter* in poultry. *Campylobacter* in poultry is more common in Australia (100%), Argentina (92.9%), Czech (100%), New Zealand (89.1%), and Oceanica (90.4%) than in Belgium (17%), Estonia (8.1%), Former Soviet Union and Eastern Europe (19.1%), Switzerland (25.1%), and Vietnam (30%) (Suzuki and Yamamoto, 2009). However, there have been few studies on the prevalence of *Campylobacter* colonization in Bangladeshi poultry farms, with findings ranging from 40.5% to 45% (Hasan *et al*., 2020; Logno *et al*., 2023). Earlier studies considered bacteriological, and molecular methods for the detection of *Campylobacter* at farm level (Hasan *et al*., 2020; Logno *et al*., 2023). Similarly, the present study followed bacteriological, and molecular methods for it.

Some risk factors that have been linked to the colonization of *Campylobacter* in Bangladeshi poultry farms include: cleaning the shed's surroundings; age of the shed; downtime; flock size; age of the birds; farming experience; litter materials; use of water sanitizer; feed storage; drinking water supply; wearing separate shoes or clothes; type of floor; etc. Nevertheless, the study area was restricted to Chattogram, Mymensingh, and Gazipur districts (Hasan *et al*., 2020; Logno *et al*., 2023). Dhaka is a populous city and capital of Bangladesh. Narsingdi, Narayanganj, and Munshigonj are the three important districts that supply poultry in Dhaka. The poultry rearing systems of these areas have not been well studied. Therefore, investigating the possible causes of *Campylobacter* colonization in the Dhaka division is necessary. Because of the public health significance of *Campylobacter* spp. as a foodborne pathogen, with poultry acting as the primary reservoir of *Campylobacter* isolates from both poultry and humans, the current study aimed to determine the risk factors associated with the *Campylobacter* spp. colonization in the broiler farms within three designated poultry-producing districts of Dhaka division, Bangladesh.

MATERIALS AND METHODS

Study Area, Design, and Sample Size

A cross-sectional survey was conducted between October 2020 to January 2021 in three districts of Dhaka division (Narsingdi, Narayanganj, and Munshigonj) of Bangladesh (Figure 1). A total of 100 commercial broiler farms (around 20% farm population from each district) were selected using simple random sampling and from each farm five birds were randomly sampled (pooled) for this study.

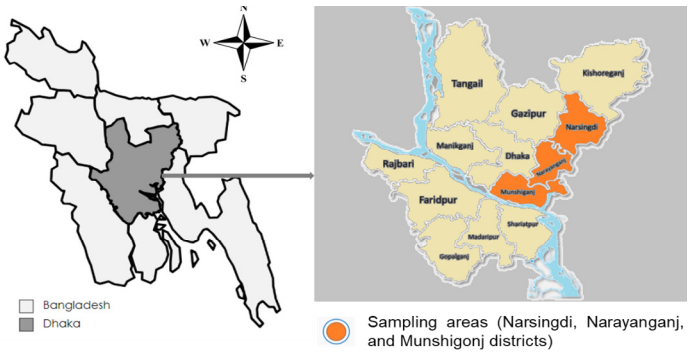

Figure 1: Study areas in Dhaka division (Munshigonj, Narayanganj, and Narsingdi districts) of Bangladesh.

Data Collection

Face-to-face interviews and on-site observations were utilized to gather epidemiological data at the farm using a pre-made structured questionnaire. The on-site observations were designed to minimize recall bias. Each time information was obtained from the farmers, it was cross-checked with the on-site observations. Before sampling the birds, the farmer was asked for their verbal consent as needed, and the questionnaire was used to record epidemiological data. The goal of the study and the process used to collect the sample were explained to the respondents. A farm was only added to the study if it received an affirmative response; it was not included otherwise. Data included "number of chicken", "number of shed", "water supply", "store of litter", "establishment of house", "person enters to shed", "flocks per shed", "litter amount", "use of distinct cloth to enter the shed", "use of separate footwear to enter the shed", "footbath facility", "floor type", "litter type", "flock size", "flock age", "number of dead birds per flock", "all-in all-out system", "broiler house empty for >14 days between flocks", "presence of rodents in the poultry house", and "elimination of dead birds every day".

Sample Collection from the Broiler Farms

Five birds were randomly sampled from each farm, and cloacal swabs were collected using sterile cotton swabs by inserting them into the bird's cloaca (Figure 2). Later, the cloacal swabs were combined and transferred using the same transport medium to the clinical pathology laboratory (CPL) of Chattogram Veterinary and Animal Sciences University (CVASU), maintaining a cool chain (4°C), in a falcon tube filled with buffered peptone water (BPW) (Oxoid Ltd, UK). It is noteworthy to emphasize that cloacal samples from each farm were pooled and analyzed for this study. We hypothesized that a higher rate of *Campylobacter* isolation would have resulted from screening cloacal samples separately. Samples were analyzed to identify *Campylobacter* spp. following the previous methods (Lund *et al*., 2003, 2004).

Figure 2: Collecting of cloacal samples from the live broiler in the farms.

Bacteriological Culture

Campylobacter was isolated and identified from broiler chicken cloacal swabs using standard bacteriological methods and molecular techniques. In a nutshell, 5-7% sheep blood and antibiotics were added to selective *Campylobacter* base agar (Oxoid Ltd., UK) before all samples were directly inoculated (Splittstoesser and Vanderzant, 1992). The plates were incubated in an anaerobic jar (Oxoid™ AnaeroJar™ 2.5L) under microaerophilic conditions with a CO_2 sachet (Thermo Scientific™ Oxoid Anaero Gen 2.5L sachet) (10% CO_2, 95% humidity) in 42° C for three days (Figure 3) (Splittstoesser and Vanderzant, 1992). After 72 hours, a distinct single colony (small, round, creamy-gray) was chosen from each plate. These colonies were then examined microscopically to observe the characteristic seagull appearance of *Campylobacter* spp. using Gram staining (Debruyne *et al*., 2008; Boyer *et al*., 2021) and were biochemically characterized using catalase and oxidase tests. After that, the isolates were kept at -80°C in brain heart infusion broth (Oxoid Ltd., UK) that contained 50% glycerol in order to undergo additional molecular validation.

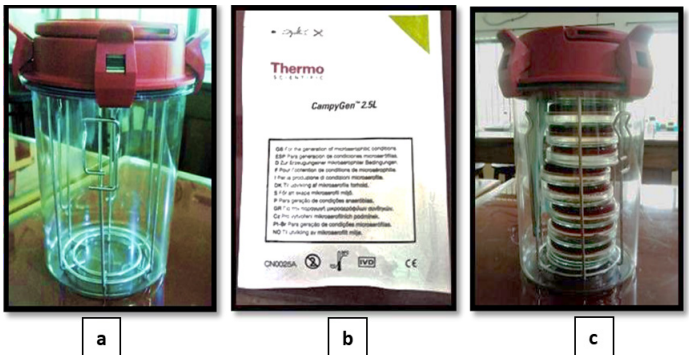

Figure 3: Anaerobic jar (a), CO_2 sachet (b), and anaerobic jar containing plate and sachet (c).

Molecular Identification of *Campylobacter* Spp.

Using the *lpx* gene primers listed in Table 1, a multiplex polymerase chain reaction (PCR) assay was performed for the final confirmation of the suspected isolates. The boiling method was utilized to extract DNA from the pure culture of *Campylobacter* spp. (Englen and Kelley, 2000). Briefly, from blood agar, a loop of fresh colonies (roughly three to four) was selected and moved to 1.5 ml Eppendorf tubes with 100µl de-ionized water inside. After that, the tubes were vortexed to create a uniform cell suspension. The lid of every tube was made with a ventilation hole. After that, the tubes were heated in a heat block (Major Science Company) to 99°C for 15 minutes. The tubes were immediately boiled and then left in the ice pack for five minutes. The bacterial cell wall broke down to release DNA due to the rapid cooling after the high-temperature boiling. Ultimately, the tubes containing the suspension underwent a 5-minute, 15,000 rpm centrifugation. Subsequently, a sterile Eppendorf tube was filled with 50 µl of the bacterial DNA-containing

Table 1: List of primers used for the identification of *Campylobacter* spp.

Gene	Primer Sequence	Product size (bp)	Reference
lpx	Forward primers:	331 (*Campylobacter jejuni*) and 391 (*Campylobacter coli*)	(Klena *et al*., 2004)
	lpxAC.coli (5'-AGACAAATAAGAGAGAATCAG-3');		
	lpxAC.jejuni (5'-ACAACT TGGTGACGATGTTGTA-3')		
	Reverse primer:		
	lpxARKK2m (5'CAATCATGDGCDATATGASAATAHGCCAT-3')		

supernatant from each tube, which was kept at -20°C until needed. The process of lpx gene amplification was used to find *Campylobacter* species. In summary, a 20-µl PCR tube held two microliters of the DNA template, ten microliters of a PCR master mix (Thermo Fisher Scientific, Singapore), one microliter of each forward and reverse primer, and six microliters of nuclease-free water for the amplification process. On a thermocycler (Applied Biosystem, 2720 thermal cycler, Singapore), PCR was conducted using the following protocol: five minutes of initial denaturation at 95°C; thirty-five minutes of denaturation at 94°C, one minute of annealing at 52°C, one minute of extension at 72°C; five minutes of final extension at 72°C, and finally an infinite period of time at 4°C. The PCR products were then kept at -20 °C until gel electrophoresis was carried out. Following electrophoresis on a 1.5% agarose gel stained with ethidium bromide, the amplified PCR products were seen. The identification of *Campylobacter* spp. was confirmed by the presence of 331-bp and/or 391-bp bands.

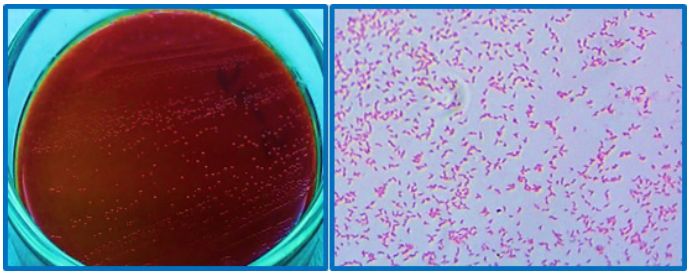

Figure 4: Cultural and staining properties of *Campylobacter* spp. (A) Cultural Response: Good-luxuriant growth of *Campylobacter* spp.; (B) Gram's staining of *Campylobacter* spp. isolate showing characteristic spiral, S-shaped bacteria.

Statistical Analysis

In the case of the cloacal samples, the farm served as the analysis's study unit. If a PCR test yielded a positive result for a combined farm sample, that farm was deemed positive. As a result, the dependent variable in our study was the binary outcome, which could be either positive or negative. The Microsoft Office Excel 2016 spreadsheet contained all of the data from the broiler farms across three distinct districts. The prevalence and 95% confidence intervals were determined using the modified Wald method in the QuickCalcs program on GraphPad. Univariable analysis was conducted using the $\chi 2$ test and univariable logistic regression models in STATA-IC 13 software (StataCorp) to assess the relationship between independent variables (risk factors/determinants) and the dependent variable (sample positive/negative). To control for confounding factors in the logistic regression models, we included potential confounders as covariates in the models. Cramer's V test, Spearman correlation coefficient, and Chi-square test were used to evaluate the correlation and multicollinearity in categorical and numerical variables. Variables with a significant association or a Spearman correlation coefficient above 0.4 were considered correlated. The significance level for the univariable model was set at a p-value ≤0.05.

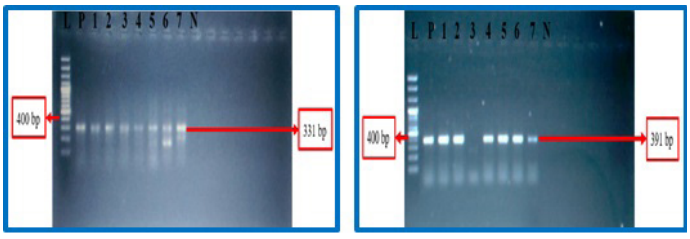

Figure 5: UV visualization of multiplex PCR of lipid A gene (*lpx*) (a) *C. jejuni* showing 331bp (b) *C. coli* showing 391bp.

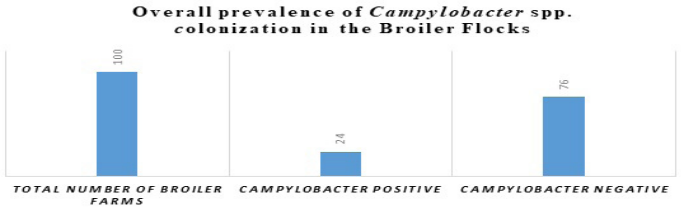

Figure 6: Overall prevalence of *Campylobacter* species in the study areas.

RESULTS AND DISCUSSION

Prevalence of *Campylobacter* Spp.

Campylobacter species were tentatively identified according to their characteristics cultural and staining properties (Figure 4). Molecular confirmation of the tentatively identified *Campylobacter* species (*C. jejuni* or *C. coli*) were done using the mPCR technique (Figure 5). Among the 100 broiler farms, 24.00% were infected with *Campylobacter* species (Figure 6). In terms of geographic location, farms of Narsingdi were contaminated with *Campylobacter* species (32.35%) followed by Narayanganj (27.78%), and Munshigonj (10.0%) (p>0.05) (Table 2).

Table 2: Geographic distribution pattern of *Campylobacter* species infected farms.

Variable	Category	Positive (No.)	Prevalence (%)	p-value
Location	Munshigonj (30)	3	10	0.09
	Narayanganj (36)	10	27.78	
	Narsingdi (34)	11	32.35	
Total (100)		24	24	

Risk Factors of *Campylobacter* Spp. Colonization in Broiler Farms

"Number of chicken", "number of shed", "water supply", "store of litter", "establishment of house", "person enters to shed", "flocks per shed", "litter amount", "use of distinct cloth to enter the shed", "use of separate footwear to enter the shed", "footbath facility", "floor type", "litter type", "flock size", "flock age", "number of dead birds per flock", "all-in all-out system", "broiler house empty for >14 days between flocks", "presence of rodents in the poultry house", and "elimination of dead birds every day" were assessed as the probable risk factors of *Campylobacter* spp. colonization in broiler farms in the study areas. The findings of descriptive statistics of the above mentioned factors and univariate logistic regression analysis to evaluate the potential factors associated with *Campylobacter* spp. colonization in broiler farms are presented in Table 3 and 4.

Campylobacter spp. colonization was more prominent in farm supplied tube well water compared to deep tube well water (70.83% vs 9.21%) (p<0.001). Those farms allowed more than one person to enter into the bird's houses daily had higher colonization of *Campylobacter* spp. than those allowed only one person for it (10.00% vs 30.00%) (p=0.006). Those farms maintained the use of separate footwear to enter the shed had lower colonization of *Campylobacter* spp. than its counterpart 10.0% vs 30.0%) (p=0.032). Those farms practiced to left the broiler house empty for >14 days between flocks had lower colonization of *Campylobacter* spp. than those did not practice it (8.57% vs 32.31%) (p=0.008).

Univariate logistic regression analysis showed the similar findings regarding the potential risk factors for the colonization of *Campylobacter* spp in broiler farms in the study areas. The present study showed that the supply of tube well water was one of the risk factors of *Campylobacter* spp. colonization in broiler farms (95% CI: 7.4 – 77.47) (p<0.001). According to the current study, there was an increased chance of introducing *Campylobacter* spp. when multiple people entered the broiler house (95% CI: 1.42-9.91) (p=0.008). The present study explored that not using separate footwear while entering into the shed influenced the higher chance of *Campylobacter* spp. colonization (95% CI: 1.05-14.12) (p=0.041). The present study revealed that the majority farms did not kept 14 days gap between two batches which is also another source of colonization of *Campylobacter* spp. (95% CI: 1.4-18.54) (p=0.014). Furthermore, footbath facility and presence of rodents in the poultry house were revealed as factors associated with increased risk; however, rest of the variables had no influence on the colonization of *Campylobacter* spp. in broiler farm in the study areas.

Globally, Food safety is a major concern of public health irrespective of age, gender, socioeconomic status and occupation. *Campylobacter* is one of the widely recognized and significant food borne pathogen in both developed and developing countries. Thermophilic *Campylobacter* spp. have become the most frequent cause of bacterial gastroenteritis in human worldwide (Man, 2011). So far, 34 species and 14 subspecies of *Campylobacter* have been isolated, but *C. jejuni* and *C. coli* are most important from food safety point of view and causes gastroenteritis in domestic animal and human being (Blaser and Engberg, 2008). In the present study was designed to isolate and characterize *Campylobacter* spp. from chicken cloacal sample and find out the risk factors which mostly responsible for colonization. The study was carried out in three of Bangladesh's most important poultry districts, which supply city people with eggs and chicken meat. In this present study we observed the prevalence of *Campylobacter* spp. infection in broiler farms of Munshiganj 3 (10%), Narayanganj 10 (27.78%) and Narsindi 11 (32.35%) districts and evaluated their associated risk factors. The overall colonization of *Campylobacter* spp. from the Dhaka division (among all three districts) was 24% (95% CI: 16.02 – 33.57). Several studies conducted domestically and internationally have confirmed the general positivity status estimated in this study. Malik *et al.* (Malik *et al.*, 2014) reported that 32% of broiler flocks in India had positive *Campylobacter* status, while 29% and 21.5% positive status were observed in Pakistan. (Hussain *et al.*, 2007; Nisar *et al.*, 2018). However, in Bangladesh, colonization of *Campylobacter* spp. in the broiler farms has reported earlier by Hasan *et al.* (2020) and Logno *et al.* (2023). Hasan *et al.* (2020) reported 40.5% prevalence of *Campylobacter* infection in poultry flocks of Mymensingh and Gazipur, while Logno *et al.* (2023) showed that overall farm-level prevalence of *Campylobacter* in the broiler farm of Mirsharai, Chattogram was 45%. It is important to highlight that this study analyzed pooled cloacal samples from each farm. We speculated that if we had screened cloacal samples individually, the rate of *Campylobacter* isolation would have been higher. On the other hand, due to higher temperatures in Sri Lanka than in other regions of the Indian subcontinent, a comparatively higher prevalence of *Campylobacter* in broiler samples was reported to be 67% (Kottawatta *et al.*, 2017). Also, this finding is agreement with several previous studies from industrialized countries too, which have shown broiler flocks to be

Table 3: Frequency distribution (descriptive statistics) of different variables regarding farm and farmer demography and management practices variable category frequency percentage (N=100).

Variable	Category	Positive	Prevalence	Chi-square p-value
Number of Chicken	Min – 1000 (27)	5	18.52	0.563
	1001-1500 (41)	12	29.27	
	1501-max (32)	7	21.88	
Number of Shed	1 (82)	21	25.61	0.421
	2 – 4 (18)	3	16.67	
Water Supply	Deep Tube well (76)	7	9.21	<0.001
	Tube well (24)	17	70.83	
Store of Litter	Inside (10)	0	0	0.061
	Outside (90)	24	26.67	
Establishment of House	2017 and after (60)	11	18.33	0.104
	Before 2017 (40)	13	32.5	
Person enters to shed	1 (72)	12	16.67	0.006
	More than 1 (28)	12	42.86	
Flocks per Shed	9 (23)	4	17.39	0.398
	more than 9 (77)	20	25.97	
Litter amount	0 – 500 (57)	14	24.56	0.88
	501 – max (43)	10	23.26	
Use of distinct cloth to enter the shed	Yes (94)	23	24.47	0.664
	No (6)	1	16.67	
Use of separate footwear to enter the shed	Yes (30)	3	10	0.032
	No (70)	21	30	
Footbath facility	Yes (26)	3	11.54	0.084
	No (74)	21	28.38	
Floor Type	Bamboo (24)	3	12.5	0.302
	Mud (20)	5	25	
	Brick (56)	16	28.57	
Litter Type	Mixed (44)	9	20.45	0.462
	Saw dust (56)	15	26.79	
Flock Size	0-1000 (27)	5	18.52	0.563
	1001-1500 (41)	12	29.27	
	1501-max (32)	7	21.88	
Flock Age	21 (58)	14	24.14	0.970
	After 21 (42)	10	23.81	
Number of dead birds per flock	0 – 25 (50)	11	22	0.487
	26 – 50 (33)	7	21.21	
	more than 50 (17)	6	35.29	
All in all, out system	Yes (73)	17	23.29	0.784
	No (27)	7	25.93	
Broiler house empty for >14 days between flocks	Yes (35)	3	8.57	0.008
	No (65)	21	32.31	
Presence of rodents in the poultry house	Yes (56)	17	30.36	0.093
	No (44)	7	15.91	
Elimination of dead birds every day	Yes (58)	15	25.86	0.608
	No (42)	9	21.43	

Table 4: Univariable logistic regression analysis to evaluate potential factors associated with *Campylobacter* spp. (N=100) status of broiler farm.

Variable	Category	Odds Ratio	95% CI	p-value
Number of Chicken	Min – 1000	Ref		
	1001-1500	1.82	0.56 – 5.93	0.32
	1501-max	1.23	0.34 – 4.44	0.75
Number of Shed	2 – 4	Ref		
	1	1.72	0.45 – 6.54	0.425
Water Supply	Deep Tube well	Ref		
	Tube well	23.94	7.4 – 77.47	<0.001
Establishment of House	2017 and after	Ref		
	Before 2017	2.14	0.85 – 5.44	0.108
Person enters to shed	1	Ref		
	More than 1	3.75	1.42 – 9.91	0.008
Flocks per Shed	9	Ref		
	more than 9	1.67	0.51 – 5.49	0.401
Litter amount	0 – 500	Ref		
	501 – max	0.93	0.37 – 2.36	0.88
Use of distinct cloth to enter the shed	Yes	Ref		
	No	0.62	0.07 – 5.56	0.667
Use of separate footwear to enter the shed	Yes	Ref		
	No	3.86	1.05 – 14.12	0.041
Footbath facility	Yes	Ref		
	No	3.04	0.82 – 11.2	0.095
Floor Type	Bamboo	Ref		
	Mud	2.33	0.48 – 11.3	0.292
	Brick	2.8	0.73 – 10.71	0.132
Litter Type	Mixed	Ref		
	Saw dust	1.42	0.55 – 3.65	0.463
Flock Size	0-1000	Ref		
	1001-1500	1.82	0.56 – 5.93	0.32
	1501-max	1.23	0.34 – 4.44	0.75
Flock Age	21	Ref		
	After 21	0.98	0.39 – 2.49	0.97
Number of dead birds per flock	0 – 25	Ref		
	26 – 50	0.95	0.33 – 2.78	0.932
	more than 50	1.93	0.58 – 6.41	0.281
All in all out system	Yes	Ref		
	No	1.15	0.42 – 3.19	0.784
Broiler house empty for >14 days between flocks	Yes	Ref		
	No	5.09	1.4 – 18.54	0.014
Presence of rodents in the poultry house	No	Ref		
	Yes	2.3	0.86 – 6.19	0.098
Elimination of dead birds every day	Yes	Ref		
	No	0.78	0.30 – 2.01	0.609

a significant reservoir of *Campylobacters* (Kapperud et al., 1993; Møller Nielsen et al., 1997). However, the variation in the prevalence of *Campylobacter* across different studies might be due to variation in the seasonal effects, farm management practices, rearing systems, biosecurity measures, hygiene standards and demographic factors (Cardinale *et al*., 2004; Guerin *et al*., 2007; Lyngstad *et al*., 2008; Näther *et al*., 2009; Sommer *et al*., 2013). Additionally, laboratory techniques, settings, and the expertise of technicians in preventing contamination are significant factors contributing to the variability in results (Rahimi and Ameri, 2011; Vinueza-Burgos *et al*., 2017).

The present study showed that the management related factors might be important drivers and increase the risk of *Campylobacter* spp. colonization. It is revealed that water supply, more than one person entering the house, use of separate foot wear to enter in to the shed, broiler house empty for >14 days between flocks were significantly associated with *Campylobacter* colonization in broiler farm while footbath facility and presence of rodents in the poultry house were appeared as factors associated with increased risk. Though the source of water supply had no influence on the colonization of *Campylobacter* spp. in broiler farm as reported by Näther *et al*., (2009). However, the present study observed that those farms supplied tube well water to their birds instead of deep tube well colonized more *Campylobacter* spp. This finding is in agreement with the finding of Logno *et al*. (2023). The reason might be due to the depth of underground water because the untreated ground water was identified as a risk factor for bacterial colonization (Sasaki *et al*., 2011).

The present study reported that those farms allowed more than one persons to enter their shed or house were at higher risk of *Campylobacter* spp. colonization. This could be the result of the causative organism entering the sheds or farms through clothing, boots, hands, etc. It has been noted that human trafficking is a major conduit for the introduction of *Campylobacter* from outside environment through clothing, boots, and hands (Cardinale *et al*., 2004) particularly if proper biosecurity is not in place. The present study revealed that use of separate foot wear to enter in to the shed reduced the colonization of *Campylobacter* spp. in broiler farms. Previous study reported the similar finding (Logno *et al*., 2023). This might reduce the introduction of pathogens through the shoe used outside the farms. The present study showed that broiler house left empty for >14 days between flocks reduced the chance of colonization of *Campylobacter* spp. in broiler farms. Similar finding was observed by Hasan *et al*. (2020) and Lyngstad *et al*. (2008) who reported that shorter downtime increased the risk of *Campylobacter* colonization. This might allow the broiler shed for drying completely which helped to reduce the load of *Campylobacter* spp. in the farm environment that is needed to infect broilers. However, the present cross-sectional design limits the ability to infer causality between the identified risk factors and *Campylobacter* colonization. Therefore, this limitation suggests that the findings may be interpreted as associations rather than direct causes.

Footbath facility and rodent control measures reduced the introduction of *Campylobacter* spp. in the poultry farms (Hermans *et al*., 2011). Similar findings were reported in the present study though the data were not statically significant. There were several other such as number of chicken, number of shed, store of litter, establishment of house, flocks per shed, litter amount, use of distinct cloth to enter the shed, floor type, litter type, flock size, flock age, number of dead birds per flock, all-in all-out system, presence of rodents in the poultry house and elimination of dead birds every day which were predicted as risk factors; however, failed to find their significant association for the colonization of *Campylobacter* spp. in broiler farms in this study. This study not clarified the reason of the above mentioned non-significant findings. In line with previous research, our results highlight the critical role of biosecurity measures and effective farm management practices in preventing pathogen contamination in broilers. Given the identified significant risk factors association, we strongly recommend implementing motivational training programs for poultry farmers to ensure rigorous personal, environmental, and farm hygiene standards.

CONCLUSIONS AND RECOMMENDATIONS

Campylobacter spp. is a zoonotic pathogen that does not spread from broiler to human only via consumption of meat but also through the handling of live broilers and during the preparation of meat and meat products. The overall prevalence of *Campylobacter* spp. in the three selected poultry producing areas of Dhaka division of Bangladesh was 24.00%. A tended to be higher prevalence of *Campylobacter* spp. colonization was found in Narsindi district (32.35%) followed by Narayanganj (27.78%) and Munshiganj (10.00%). Water supply, more than one person entering the house, use of separate foot wear to enter in to the shed, and broiler house empty for >14 days between flocks were appeared as the significant risk factors for the colonization of *Campylobacter* spp. in broiler farms. Footbath facility and presence of rodents in the poultry house were the factors associated with increased risk for *Campylobacter* colonization. Therefore, the above mentioned biosecurity and management practices should be followed to prevent the *Campylobacter* colonization in the broiler farms.

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NOVELTY STATEMENT

This is the first molecular and epidemiological study on Campylobacter colonization in broiler farms in the Munshigonj, Narayanganj, and Narsingdi districts of Dhaka division, Bangladesh, which will help improve broiler management practices there.

AUTHOR'S CONTRIBUTIONS

Muhammad Al-Maruf, Mahfuzul Islam, and K. B. M. Saiful Islam: Designed and conceptualized the experiments.
Muhammad Al-Maruf, Mahfuzul Islam, Syidul Islam, Md. Sirazul Islam, Md. Roknuzzaman Khan, Md. Khairul Islam, and Md. Akib Zabed: Performed the sample collection and laboratory analysis.
Muhammad Al-Maruf, Mahfuzul Islam, Md. Rashedul Islam and K. B. M. Saiful Islam: Performed data checking.
Muhammad Al-Maruf, Mahfuzul Islam, and K. B. M. Saiful Islam: Performed statistical analysis.
Muhammad Al-Maruf and Mahfuzul Islam: Wrote the first draft of the manuscript which was revised by Muhammad Al-Maruf, Mahfuzul Islam, Md. Rashedul Islam and K. B. M. Saiful Islam.

All authors contributed to the final manuscript revision and approval.

Conflict of Interest

The authors have declared no conflict of interest.

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