

A clinical report on prevalence of *Campylobacterspp.*  
colonization in broiler flocks at Chittagong



**A Clinical Report Submitted**

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## LIST OF ABBREVIATION AND SYMBOLS

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Abbreviations/Symbols	Elaborations
%	Percentage
+ve	Positive
-ve	Negative
<	Less than
≥	Greater than or equal
No.	Number
°C	Degree Celsius
°F	Degree Farenhight
BPW	Buffered Peptone Water
PBS	Phosphate Buffer Solution
et al.	And his associate
e.g.	Example
etc.	Et cetera
CMA	Chittagong Metropolitan Area
CVASU	Chittagong Veterinary and Animal Sciences University

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***PLAGIARISM CERTIFICATE***

*I, Md. Sirazul Islam, would like to strongly assure you that I have performed all works furnished here in this report. The information has been collected from different books, national and international journals, websites and references. All the references have been acknowledged duly.*

*Therefore, I reserve entire responsibility of this report.*

.....

**The Author**

**September, 2018**

## Abstract

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Campylobacteriosis remain as one of the major bacterial zoonotic diseases in humans. All commercially reared poultry species can carry the causative agent – *Campylobacter spp.*; the risk of transmission is greater from broiler chickens because of high level of consumption. Very few studies on *Campylobacter spp.* Colonization in broilers of Bangladesh was observed. Therefore a baseline survey was conducted during March 2018 to April 2018 to estimate the prevalence of *Campylobacter spp.* colonization and its associated risk factors in broilers of Chittagong. A total of 20 (randomly selected) broiler farms from Chittagong tested for *Campylobacter spp.* colonization. Data for risk factor analysis were gathered by a questionnaire. The sample material was comprised of five (5) cloacal swabs from five randomly selected broilers from each flock that were pooled into one for culturing in a selective media followed by incubation in CO<sub>2</sub> atmosphere. A total of 55% of the broiler flocks were found positive. Of that, 46% having flock size <2000, 71% having flock size ≥2000 (p-value 0.37). 67% positive farms were established before the year 2010 and 45% after 2010 (p-value 0.45). 20% farms were positive when single person entered into the house per day, 50% when 2 person entered per day, 100% when the number of person entered was >2 (p-value 0.05). Statistical analysis showed that less number of (50%) farms were positive when the age of the flocks were <2 weeks compared to >2 weeks age of the flocks (63%). Prevalence was lowest if the farm raised six flocks per house per year (0%) compared to eight flocks per house per year (63%) and ten flocks per house per year (50%). In this study, some of the risk factors did not show any significant association, might be because of less sample size (N=20). *Campylobacter spp.* does not spread from broiler to human only via consumption of meat but also handling of live broilers and during preparation of meat and meat products. As *Campylobacter spp.* can spread from broiler to human by several routes, control of infection in the primary broiler production is believed to have the greatest public health benefit. We have gathered evidence of presence of *Campylobacter spp.* colonization in broiler flocks at Chittagong through this baseline survey. Further extended study might provide useful information to formulate a national control program.

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**Keywords:** *Campylobacter spp.*, broiler meat, cloacal swab, risk factors, prevalence.

# Chapter I

## Introduction

*Campylobacter* is a gram-negative, non-spore forming, S-shaped or spiral bacteria. Typically they are microaerophilic with an exception of aerophilic and sometimes they can grow anaerobically (Konkel et al., 2001). Until 2009, 23 species under the genus *Campylobacter* have been identified (EFSA panel on biological hazards (BIOHAZ), 2011). The gastrointestinal (GI) tract of warm blooded animals is the most common reservoir of *Campylobacter spp.* The optimal growth temperature of thermophilic *Campylobacter* species (*Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis* and *Campylobacter helveticus*) range from 37°C to 42°C and they do not grow below 30 or above 46°C (Corry et al., 1995; Nachamkin et al., 1998). *Campylobacter* Does not multiply outside the hosts because they are very sensitive to different external physical conditions, e.g. temperature, UV ray, salt, desiccation and aerophilic environment (Wagenaar et al., 2006). But they can survive for a period of time outside the hosts in different environmental conditions, e.g. in dirty water and sewage, particularly if it is protected from dryness, which is a major threat to the organism (Jones, 2001; Nicholson et al., 2005).

Campylobacteriosis remain as one of the major bacterial zoonotic diseases in humans. The clinical form of *Campylobacter* infection in humans is known as Campylobacteriosis. It is mainly a food borne disease and chicken meat is regarded as the major source (Harris et al., 1986; Humphrey et al., 1993). All commercially reared poultry species can carry the causative agent – *Campylobacter spp.*; the risk of transmission is greater from broiler chickens because of high level of consumption. *Campylobacter* does not spread from broiler to human only via consumption of meat but also by handling of live birds (broiler and layer) and during the preparation of meat and meat products. It was estimated that 50-80 percent of the human case are attributed to transmission from a chicken reservoir as a whole (EFSA Panel on Biological Hazards (BIOHAZ), 2011), whereas 20-30 percent of this may be attributed to chicken meat. Implementation of control measures during handling and preparation of meat products might have a significant public health benefit.

Very few studies on *Campylobacter spp.* Colonization in broilers of Bangladesh was observed. Keeping in view the importance of broiler as a vital source of meat, and a potential zoonotic threat, the present baseline study was designed to conduct the following objectives:

1. To estimate the prevalence of *Campylobacter spp.* colonization in broiler flocks of Chittagong district.
2. To identify the risk factors associated with *Campylobacter spp.* colonization in broiler flocks of Chittagong district.

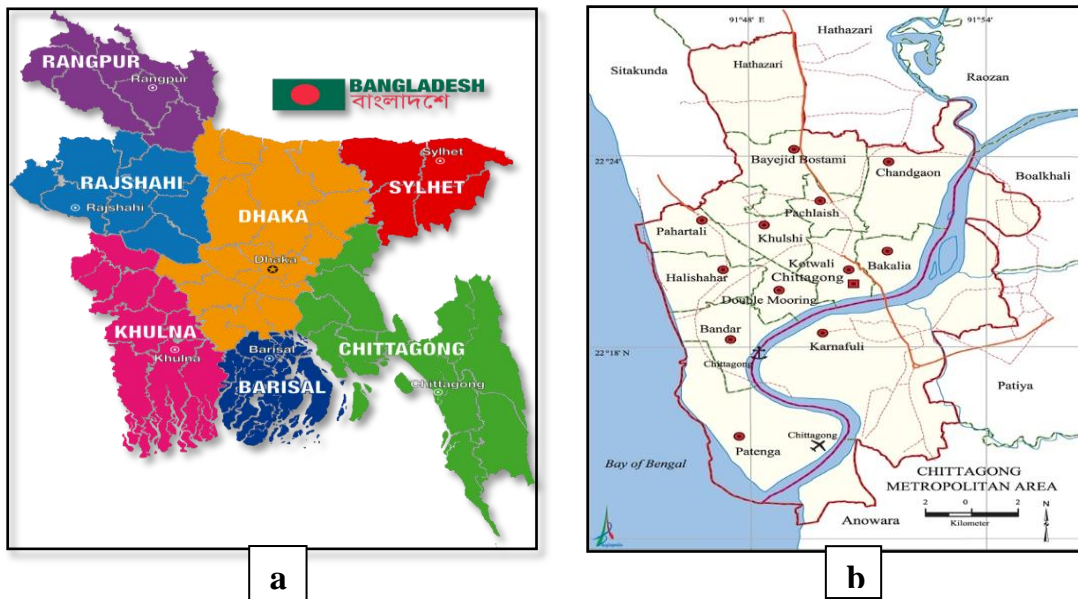


# Chapter II

## Materials and method

### 2.1 Study population and sample collection

The study was carried out for the periods of 2 months from 1<sup>st</sup> march, 2018 to 30th April, 2018. The samples were collected from twenty (N=20) randomly selected broiler farms in Hathazari and Rawzan upazilla under Chittagong district. Farm level epidemiological data were recorded using a structured questionnaire through face-to-face interview and by observation. About one hundred broiler chickens (N=100) were sampled during the study period. The sample material was comprised of five (5) cloacal swabs from five randomly selected broilers from each flock that were pooled into one 15 ml falcon tube containing 5 ml phosphate buffer solution (PBS). The samples were collected and transferred it to the Clinical Pathology Laboratory, CVASU to conduct laboratory diagnosis.

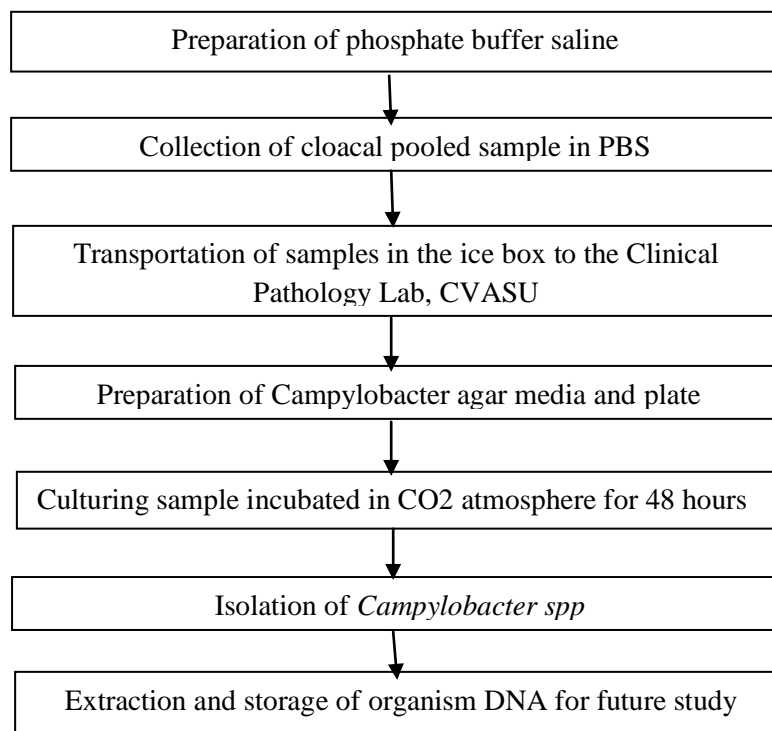


**Fig 2.1: Geographical location of sample collection site. (a) Map of Bangladesh; (b) Map of Chittagong district.**

## 2.2: Isolation and identification of *Campylobacter spp*:

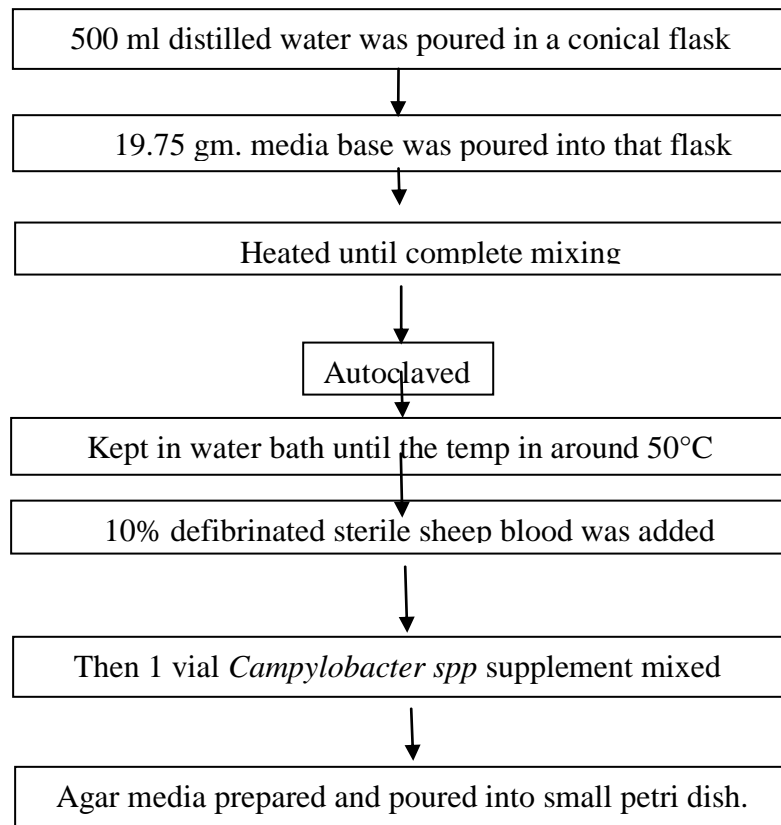
The falcon tube containing cloacal swab sample was carried in ice box to the Clinical Pathology Laboratory, CVASU for bacteriological study. Isolation and identification of the *Campylobacter* isolates were done based on by culturing in a selective media (Campylobacter agar plate) followed by 48 hours incubation in CO<sub>2</sub> atmosphere.

### EXPERIMENTAL DESIGN



**Fig. 2.2: Schematic illustration of experimental design.**

### 2.2.1: Preparation of *Campylobacter* agar plate:



**Fig: 2.2.1: Schematic illustration of *Campylobacter* agar plate preparation.**

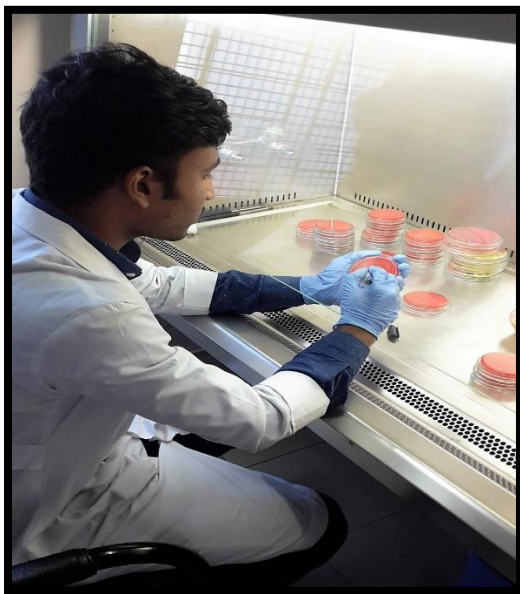


a



b

**Fig 2.2.2: (a) Preparation of *Campylobacter* agar media (b) *Campylobacter* agar plate.**

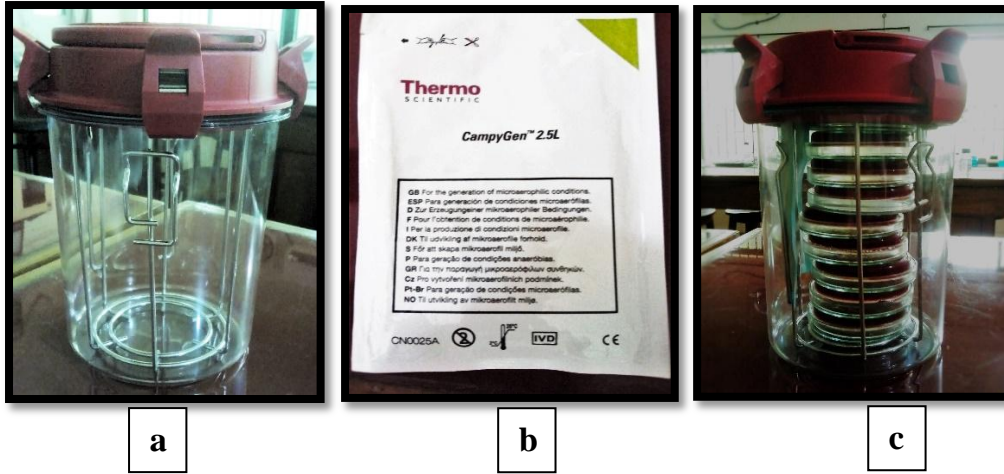


a



b

**Fig 2.2.3: (a) *Campylobacter* agar plate numbering for sample identification (b) streaking the sample in the *Campylobacter* agar plate.**



**Fig 2.2.4: (a) Anaerobic jar (b) CO<sub>2</sub> sachet (c) Anaerobic jar containing plate and sachet**

### 2.3 Statistical analysis

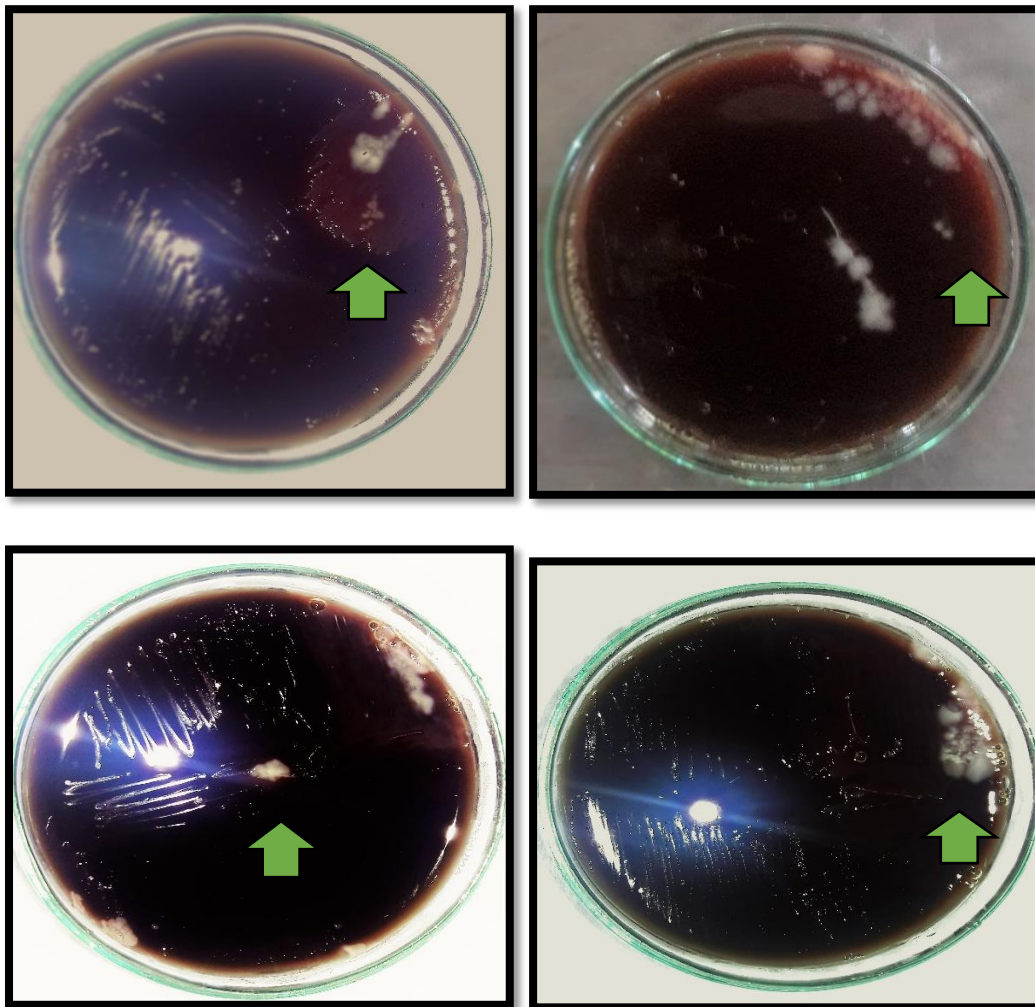
All data like production, number of houses, water supply, disposal system, farm establishment year, person entry in the farm, number of flocks per house per year, flock size, flock age, slaughter age, type of floor, type of litter materials, density etc. were collected on a questionnaire and were entered into MS excel (Microsoft office excel-2007, USA). Data management and data analysis were done by STATA version-13 (STATA Corporation, 4905, Lakeway River, College Station, Texas 77845, USA). The association of the outcome variable (presence of *Campylobacter spp* in sample) with different explanatory variables was evaluated by using chi-square ( $\chi^2$ ) test.  $P < 0.05$  set for significance.

## Chapter III

### Result

#### 3.1 Isolation and identification of *Campylobacter spp*:

For the isolation and identification of *Campylobacter spp*, each sample was cultured on *Campylobacter* selective culture media. Finally, good-luxuriant growth of *Campylobacter spp* observed under reduced oxygen (CO<sub>2</sub>) atmosphere after incubation at 37°C for 48 hour.



**Fig 3.1: Cultural Response: Good-luxuriant growth of *Campylobacter spp***

#### 3.2. Descriptive statistics of the sampled farm (Table 3.1)

Among 20 farms, 35% were large farm having more than 2000 broilers. Most of the farms (45%) were comprised of single house. In 50% farms, 2 persons enter into the bird's houses daily. 80% farms raise around 8 flocks per year per house and 70% farms follow 'all-in all-out' system. 90% sampled farms keep their house empty for 14 days before introduction of a new flock. 80% farm does not use a separate footwear for the houses.



### **3.3 Prevalence of *Campylobacter spp* colonization:**

A total number of 11 samples were found *Campylobacter spp* positive out of 20 pooled samples that makes farm level prevalence of *Campylobacter spp* colonization as 55% (95% CI: 31 to 76). Prevalence was greater in the farms of bigger flock size ( $\geq 2000$ ) than in smaller flock size (71% vs. 46%) (P-value 0.37). Farms established in 2010 or before had higher prevalence than in farms established in 2011 and after (67% vs. 45%) (P-value 0.45). Farms used rice husk and saw dust as litter had lower prevalence than in farms used only saw dust as litter (33% vs. 59%) (P-value 0.56). Flocks having birds of more than 2 weeks had greater prevalence than the flocks having 2 weeks of age or less (63% vs. 50%) (P-value 0.67). Farms having brick floor had higher PP than in mud floor (63% vs. 25%) (P-value 0.28). 20% farms were positive when single person entered into the house per day, 50% when 2 person entered per day, 100% when the number of person entered was  $>2$  (P-value 0.05). Prevalence was lowest if the farm raised six flocks per house per year (0%) compared to eight flocks per house per year (63%) and ten flocks per house per year (50%). In this study, some of the risk factors did not show any statistically significant association, might be because of low sample size (N=20) decreased the study power.

### Overall Study of *Campylobacter spp* colonization in broiler flocks of Chittagong

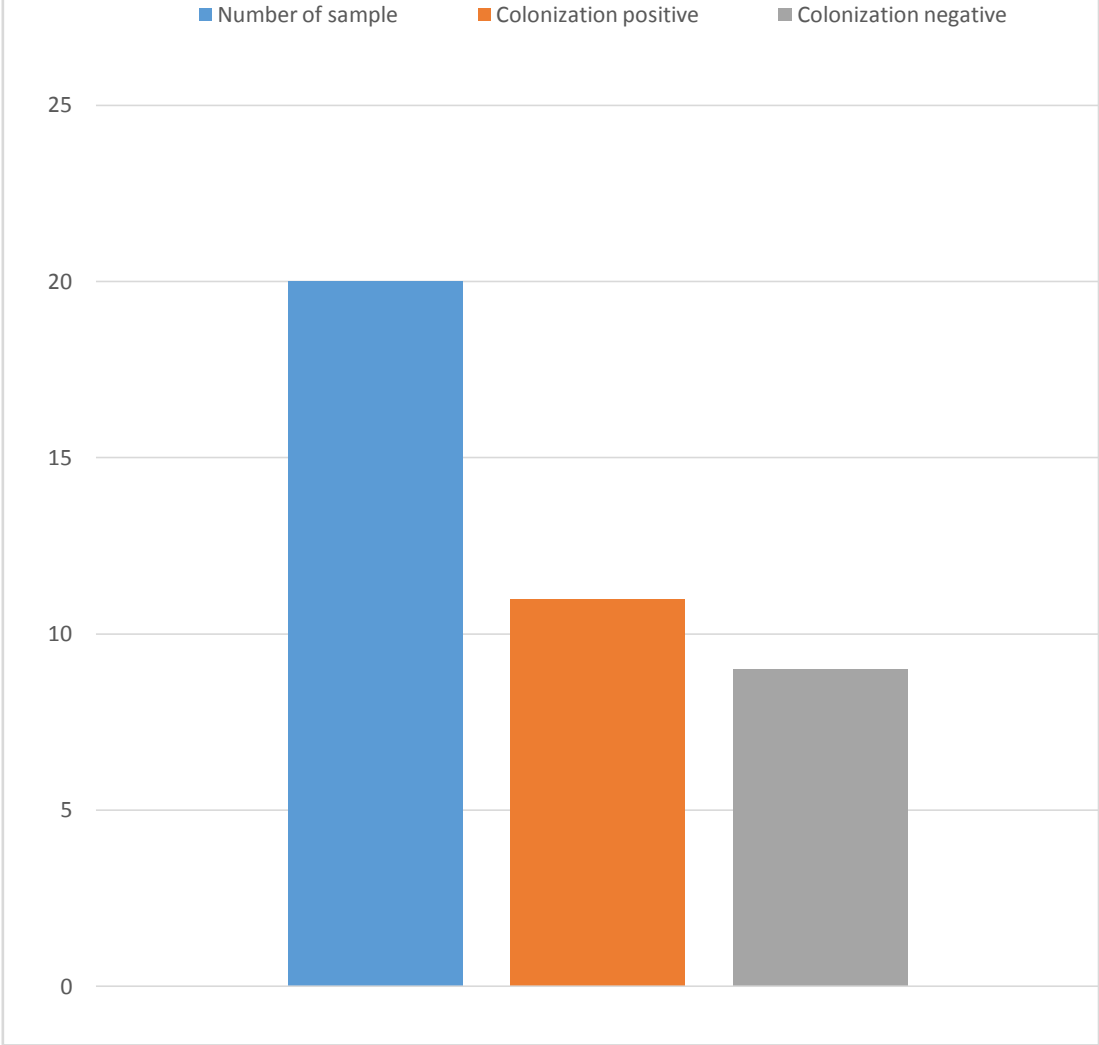


Figure 3.3: Graphical presentation of overall study.



**Table: 3.1: Frequency distribution (descriptive statistics) of different variables in the study area and farm**

<b>Variable</b>	<b>Category</b>	<b>Frequency</b>	<b>Percentage</b>
<b>Production size of the farm</b>	≤1000	6	30
	1001-2000	7	35
	>2000	7	35
<b>Number of houses</b>	1	9	45
	2	6	30
	>2	5	25
<b>Water supply</b>	Deep tube well	13	65
	Tube well	7	35
<b>Disposal system of dead birds</b>	Distant place	11	55
	Pit	9	45
<b>Establishment of farm</b>	Before and in 2010	9	45
	After 2010	11	55
<b>Number of person entry/day</b>	1	5	25
	2	10	50
	>2	5	25
<b>Number of flocks/house/year</b>	6	2	10
	8	16	80
	10	2	10
<b>Separate foot wear</b>	No	16	80
	Yes	4	20
<b>Type of floor</b>	Brick	16	80
	Mud	4	20
<b>Type of litter</b>	Rice husk and saw dust	3	15
	Saw dust	17	85
<b>Age of flock</b>	<2 weeks	12	60
	≥2 weeks	8	40
<b>Death of birds/flock</b>	0-50	8	40
	51-100	10	50
	101-200	1	5
	>200	1	5
<b>All-in all-out system</b>	No	6	30
	Yes	14	70
<b>House kept empty for 14 days before introduction</b>	No	2	10
	Yes	18	90
<b>Presence of rodents</b>	No	18	90
	Yes	2	10
<b>Campylobacter colonization</b>	Yes	11	55
	No	9	45

**Table 3.2: Association of different variables with the occurrence of *Campylobacter spp* colonization in broilers in the study area**

<b>Variable</b>	<b>Category</b>	<b>Observation</b>	<b>Number positive (%)</b>	<b>P-value</b>

<b>Production size of the farm</b>	≤2000	13	6 (46)	0.37
	>2000	7	5 (71)	
<b>Number of houses</b>	1	9	5 (56)	1.00
	2	6	3 (50)	
	>2	5	3 (60)	
<b>Water supply</b>	Deep tube well	13	7 (54)	1.00
	Tube well	7	4 (57)	
<b>Disposal system of dead birds</b>	Distant place	11	4 (36)	0.09
	Pit	9	7 (78)	
	Before and in 2010	9	6 (67)	0.45
<b>Establishment of farm</b>	After 2010	11	5 (45)	
<b>Number of person entry/day</b>	1	5	1 (20)	0.05
	2	10	5 (50)	
	>2	5	5 (100)	
<b>Number of flocks/house/year</b>	6	2	0	0.42
	8	16	10 (63)	
	10	2	1 (50)	
<b>Separate foot wear</b>	No	16	10 (63)	0.28
	Yes	4	1 (25)	
<b>Type of floor</b>	Brick	16	10 (63)	0.28
	Mud	4	1 (25)	
<b>Type of litter</b>	Rice husk and saw dust	3	1 (33)	0.56
	Saw dust	17	10 (59)	
	<2 weeks	12	6 (50)	0.67
<b>Age of flock</b>	≥2 weeks	8	5 (63)	
	0-50	8	3 (38)	0.25
	51-100	10	7 (70)	
	101-200	1	1 (100)	
	>200	1	0	
<b>All-in all-out system</b>	No	6	3 (50)	1.00
	Yes	14	8 (57)	
<b>House kept empty for 14 days before introduction</b>	No	2	1 (50)	0.71
	Yes	18	10 (56)	
<b>Presence of rodents</b>	No	18	10 (56)	0.71
	Yes	2	1 (50)	

## Chapter IV

### Discussion

In this present study we estimate the prevalence and evaluated some risk factors of the occurrence in broiler flocks of Chittagong district extremely important zoonotic, food-borne pathogens *Campylobacter spp*, which worldwide infect millions of people each year. A variety of infection vehicles has been identified, but there is general agreement that contaminated broiler meat is the most important. The overall colonization of *Campylobacter spp* from Chittagong district was 55%. This finding is agreement with several previous studies from industrialized countries, which have shown broiler flocks to be a significant reservoir of Campylobacters (Kapperud et al., 1993; Humphrey, 1994; Jacobs-Reitsma et al., 1994; Stern et al., 1995; Berndtson et al., 1996; Nielsen et al., 1997). Different studies showed that *C. jejuni* is the predominant species in poultry (Oosterom et al., 1983b; Berndtson et al., 1996; Wallace et al., 1997; Nielsen et al., 1997). However we did not identify the isolates at species level and an extended study is in progress. The present study showed that the management related factors might be important drivers and increase the risk of *Campylobacter spp* colonization. It was revealed that older birds, more than one person entering the house and using older houses (established before 2010) were associated with positive campylobacter status. (Table-3.1)

The present study showed higher risk of *Campylobacter spp* colonization when more than one person entered the broiler house. Human traffic is an important route (via boots, hands, cloths) for introduction of *Campylobacter* from the external environment (Hald et al., 2000; Cardinale et al., 2004) particularly if proper biosecurity is not in place.

The number houses in the farm was not significantly associated with colonization in this study even though other studies (Bouwknegt et al., 2004, Refregier-Petton et al., 2001) identified it as a risk factor for broiler farms. More houses in the same premise might facilitate introduction of the organism from the environment.

The higher risk for chickens bred in a house built before 2010 could also be related to other factors e.g. type of ventilation system and temperature regulation system (cooling and heating). Old ventilation systems could be sub optimal in their abilities of evacuating damp and moist; this might facilitate campylobacter survival in the environmental materials.

Increased risk with increasing age of broilers has been documented previously (Bouwknegt et al., 2004; Barrios et al., 2006; EFSA, 2010). An extended time in the broiler house could be related with a higher risk of introduction of the organism from the environment around the house. Additional time before slaughter would also allow for cecal-colony concentrations to become detectable (Stern et al., 2001). Taking this association into account, a policy of slaughtering flocks at a younger age might lead to a reduction in the prevalence of *Campylobacter*.

The effect of increasing flock size on the odds of a flock being positive has been previously reported (Berndtson et al., 1996b), although other studies failed to find this association (Bouwknegt et al., 2004; Cardinale et al., 2004). In our study, a substantial rise of *Campylobacter spp* colonization in large flock size (e.g.  $\geq 2000$  birds) relative to the average size (e.g.  $< 2000$  birds) of broiler. This effect was independent of bird density, but could be due to bigger flocks offering more chances for introduction of *Campylobacter* because of increased personnel movements, or larger volume of water and air used (both potential carriers of the pathogen).

The number of per flocks per house per year directly related to prevalence of positive flocks. In our view when dry out period is maintained properly the prevalence of *Campylobacter spp* gradually decrease. Because it is unfavorable condition for *Campylobacter* to grow in dryness, which is a major threat to the organism (Jones, 2001; Nicholson et al., 2005). That's why more than 6 flocks per house per year has higher prevalence.

Among 55% positive farms, 46% having flock size  $\leq 2000$ , 71% having flock size  $> 2000$  (p-value 0.37) was identified as positive. Statistical analysis showed that less number of (50%) farms were positive when the age of the flocks was  $< 2$  weeks compared to  $> 2$  weeks age of the flocks (63%). Prevalence was lowest if the farm raised six flocks per house per year (0%) compared to eight flocks per house per year (63%) and ten flocks per house per year (50%).

Despite limitations, our study highlighted some potential risk factors for *Campylobacter spp* colonization in broiler flocks. Restricting the care taking of the broiler houses to a single person, putting effort into the cleaning and disinfection of houses properly and its surroundings between flocks adequately (and optimize the possibilities for doing so i.e. more frequent renovation of houses), would possibly reduce the prevalence of *Campylobacter* positive broiler flocks in Chittagong district.

Our results emphasize, like many studies conducted before, that biosecurity measures are of utter most importance in order to keep infections outside flocks of animals. Every action that could work as a vector for bringing *Campylobacter* into broiler house should therefore be restricted.

## **Chapter V**

### **Limitations**

The study was conducted in a small scale, area, short time period which might not be representative.

## **Chapter VI**

### **Conclusion**

The colonization of *Campylobacter spp* in cloacal samples of broiler chickens from field conditions indicates that the prevalence of the organism in broiler is common. These organisms act as reservoir for future Campylobacteriosis in humans. *Campylobacter* does not spread from broiler to human only via consumption of meat but also handling of live broilers and during preparation of meat and meat products. As *Campylobacter* can spread from broiler to human by several routes, control of infection in the primary broiler production is believed to have the greatest public health benefit. We have gathered evidence of presence of *Campylobacter spp.* colonization in broiler flocks at Chittagong through this baseline survey. Further extended study might provide useful information to formulate a national control program.

## Chapter VII

### References:

1. Adak, G. K., S. M. Meakins, H. Yip, B. A. Lopman, and S. J. O'Brian. 2005. Disease risks from foods, England and Wales, 1996–2000. *Emerg. Infect. Dis.* 11:365–372.
2. Aboaba, O. O., and S. I. Smith. 2005. Occurrence of *Campylobacter* species in poultry forms in Lagos area of Nigeria. *J. Environ. Biol.* 26:403–408.
3. Atterbury, R. J., E. Dillon, C. Swift, P. L. Connerton, J. A. Frost, C. E. R. Dodd, C. E. D. Rees, and I. F. Connerton. 2005. Correlation of *Campylobacter* bacteriophage with reduced presence of hosts in broiler chicken ceca. *Appl. Environ. Microbiol.* 71:4885–4887.
4. Axelsson-Olsson, D., J. Waldenstrom, T. Broman, B. Olsen, and M. Holmberg. 2005. Protozoan *Acanthamoeba polyphaga* as a potential reservoir for *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 71:987–992.
5. Barrios, P. R., J. Reiersen, R. Lowman, J. R. Bisailon, P. Michel, V. Fridriksdóttir, E. Gunnarsson, N. Stern, O. Berke, S. McEwen, and W. Martin. 2006. Risk factors for *Campylobacter spp.* colonization in broiler flocks in Iceland. *Prev. Vet. Med.* 74:264–278.
6. BISWAS, D., K. ITOH, AND C. SASAKAWA, 2000. Uptake pathways of clinical and healthy animal isolates of *Campylobacter jejuni* into INT-407 cells. *FEMS Immunol. Med. Microbiol.* 29:203-211.
7. BLASER, M. J., I. D. BERKOWITZ, F. M. LAFORCE, J. CRAVENS, L. B. RELLER, AND W. L. WANG, 1979. Experimental *Campylobacter enteritis*: clinical and epidemiological features. *Ann. Intern. Med.* 91:179-185.
8. BLASER, M. J., J. G. WELLS, R. A. FELDMAN, R. A. POLLARD, AND J. R. ALLEN, 1983. *Campylobacter enteritis* in the United States. A multicenter study. *Ann. Intern. Med.* 98:360-365.
9. Chowdhury S, Themudo GE, Sandberg M, Ersbøll AK. Spatio-temporal patterns of *Campylobacter spp* colonization in Danish broilers. *Epidemiology and infection* 2012.
10. Chowdhury S, Themudo GE, Sandberg M, Ersbøll AK. The effect of presence of infected neighbouring farms for the *Campylobacter* infection status in Danish broiler farms.
11. Chowdhury S, Themudo GE, Sandberg M, Ersbøll AK. Risk factors for the *Campylobacter* infection in Danish broiler chickens.
12. DE MELO, M. A., G. GABBIANI, AND J. C. PECHERE, 1989. Cellular events and intracellular survival of *Campylobacter jejuni* during infection of Hep-2 cells. *Infect. Immun.* 57:2214-2222.
13. Fernandez, H., and N. Torres. 2000. Occurrence of *Campylobacter jejuni* and *C. coli* in three groups of flocks of different geographic origin in southern Chile. *Arch. Med. Vet.* 32:241–244.
14. Heuer, O. E., K. Pedersen, J. S. Andersen, and M. Madsen. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Lett. Appl. Microbiol.* 33:269–274.



15. Jacobs-Reitsma, W., C. Becht, T. De Vries, J. Van der Plas, B. Duim, and J. Wagenaar. 2001. No evidence for vertical transmission of *Campylobacter* in a study on Dutch breeder and broiler farms. *Int. J. Med. Microbiol.* 291:39.
16. KONKEL, M. E., M. D. CORWIN, L. A. JOENS, AND W. CIEPLAK, 1992. Factors that influence the interaction of *Campylobacter jejuni* with cultured mammalian cells. *J. Med. Microbiol.* 37:30-37.
17. KULKARNI, S. P., S. LEVER, J. M. J. LOGAN, A. J. LAWSON, J. STANLEY AND M. S. SHAFI. 2003. Detection of *Campylobacter* species: a comparison of culture and polymerase chain reaction based methods. *J. Clin. Pathol.* 55:749-53.
18. Kapperud, G., E. Skjerve, L. Vik, K. Hauge, A. Lysaker, I. Aalmen, S. Ostroff, and M. Potter. 1993. Epidemiological investigation of risk factors for *Campylobacter* colonisation in Norwegian broiler flocks. *Epidemiol. Infect.* 111:245-255.
19. OELSCHLAEGGER, T. A., P. GUERRY, AND D. J. KOPECKO, 1993. Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. *Proc. Natl. Acad. Sci. USA.* 90:6884-6888.
20. PEI, Z., CH. BURUCOA, B. GRIGNON, S. BAQAR, Z. Z. HUANG, D. J. KEPECKO, A. L. BOURGEOIS, J. L. FAUCHERE, AND M. J. BLASER, 1998. Mutation in the *peb1A* locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. *Infect. Immun.* 66:938-943.
21. PADUNGTOD P., D. WILSON, J. BELL, J. KANEENE, R. HANSON, AND J. E. LINZ, 2002. Identification of *Campylobacter jejuni* isolates from cloacal and carcass swabs of chickens in Thailand by a 5' nuclease fluorogenic polymerase chain reaction assay. *J. Food Prot.* 65:1712-1716.
22. Pearson, A. D., M. Greenwood, T. D. Healing, D. Rollins, M. Shahamat, J. Donaldson, and R. R. Colwell. 1993. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 59:987-996
23. Van de Giessen, A. W., J. J. H. C. Tilburg, W. S. Ritmeester, and J. van der Plas. 1998. Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. *Epidemiol. Infect.* 121:57-66.
24. Wieland, B., G. Regula, J. Danuser, M. Wittwer, A. P. Burnens, T. M. Wassenaar, and K. D. Stärk. 2005. *Campylobacter spp.* in dogs and cats in Switzerland: Risk factor analysis and molecular characterization with AFLP. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 52:183-189.
25. World Health Organization. 2002. The increasing incidence of human campylobacteriosis. Report and proceedings of a W.H.O. consultation of experts, Copenhagen, Denmark, 21-25 November 2000. W.H.O./CDS/ CSR/APH publication 2001.7. World Health Organization, Geneva, Switzerland.

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*Author*

*September, 2018*

# Appendix-1

## Questionnaire on broiler flocks rearing system

**Study area:** Chittagong (Rawzan/Hathazari/Patiya/ -----)

**Date:**

**Name of the farm:**

**Longitude:**

**Latitude:**

**Farm ID:**

**Sample code:**

### Owner's information

Name of the owner:

Contact number:

### Farm information

1. Number of chicken production of the farm:  
a. 1000    b. 2000    c. more than 2000
2. Number of houses in the farm:  
a. 1    b. 2    c. more than 2
3. Water supply of the farm:  
a. Deep tube well    b. tube well    c. pond    d. others
4. What is the disposal system of dead birds?  
Ans:
5. How do you store litter materials?  
Ans:

### House information:

1. In which year house was established? Ans:
2. What is the length of house (in feet)? Ans:
3. What is the width of the house (in feet)? Ans:
4. Number of person enter into the house:  
a. 1    b. 2    c. more than 2
5. Number of Flocks per house per year:  
a. 4    b. 6    c. 8    d. 10
6. Litter amount(kg):  
a. 200-600    b. >600-800    c. >800

### Observational checklist:

7. Is there any kinds of fly net?    a. Present    b. absent
8. Use of any distinct cloth to enter the house:  
a. Yes    b. no

9. Use of separate foot wear to enter the house:
  - a. Yes
  - b. no
10. Foot bath facility in the house:
  - a. Yes
  - b. no
11. Type of floor:
  - a. Mud
  - b. Bamboo
  - c. Wood
  - d. Tin
  - e. Brick
  - f. Others
12. Litter type:
  - a. rice husk
  - b. saw dust
  - c. both a & b
  - d. others
13. Type of cooling system during summer season:
  - a. Fan
  - b. water sparkling
  - c. others

**Flock information:**

- Density of broiler per square meter of the house( 1 square meter= 10.764 square feet)  
(1 meter=3 feet 3.37 inches):
- Flock size:
- Flock age:
- Average slaughter age of the bird:
  - a. <35 days
  - b. >35 days
- Number of dead birds per flock:
  - a. 0-50
  - b. 50-100
  - c. 100-200
  - d. more than 200
- Season of the sample collection:
  - a. Summer
  - b. Autumn
  - c. Spring
  - d. Winter
- Number of day old chicks per meter square house area:
- Presence of infected neighboring broiler farms? (2km, 30 days before and 14 days after Sample collection):
  - a. presence
  - b. absent
- Practice of 'all in all out' system: yes/no
- Disinfection of farm before restock: yes/no
- Broiler house empty for >14 days between flocks: yes/no
- Presence of rodents in the poultry house: yes/no
- Elimination of dead birds every day: yes/no

## Appendix-2

### Peptone water

<i>Composition</i>	<i>Gm./Liter</i>
<i>Peptone</i>	10.0
<i>Sodium chloride</i>	5.0
<i>Disodium phosphate</i>	3.5
<i>Potassium dihydrogen phosphate</i>	1.5

### Campylobacter Agar Base Media

<i>Composition</i>	<i>Gm. / Liter</i>
<i>Proteose peptone</i>	15.0
<i>Liver digest</i>	2.5
<i>Yeast extract</i>	5.0
<i>Sodium chloride</i>	5.0
<i>Agar</i>	12.0

## Biography:



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<b>No. of publication</b>	No
<b>Research interest</b>	PCR based isolation and identification of Virus