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Full Length Research Paper

The presence of *Campylobacter jejuni* in broiler houses: Results of a longitudinal study

Yavuz Cokal[1]*, Vildan Caner[2], Aysin Sen[3], Cengiz Cetin[3] and Murat Telli[4]

[1]Bandirma Vocational School, Balikesir University, 10200, Bandirma, Balikesir, Turkey.
[2]Department of Medical Biology, School of Medicine, Pamukkale University, 20020, Kinikli, Denizli, Turkey.
[3]Department of Microbiology, Faculty of Veterinary Medicine, Uludag University, 16059, Gorukle, Bursa, Turkey.
[4]Department of Medical Microbiology, School of Medicine, Adnan Menderes University, 09100, Aytepe, Aydin, Turkey.

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In this study, the presence of *Campylobacter jejuni* in water lines of commercial broiler house and its role in the epidemiology of the infection of broiler flocks was investigated. The study was done in three sequential commercial broiler flocks previously known to be infected with *C. jejuni* in two poultry houses with different water sources. *C. jejuni* was identified in drinking water and drinking nipple swab samples in water-line samples from both houses. Fresh fecal dropping samples were taken from broiler flocks for determination of *C. jejuni*-carriage. Twenty and 130 *C. jejuni* isolates were recovered from water-line system and fecal dropping samples, respectively. A total of 150 *C. jejuni* isolates were genotyped by pulsed-field gel electrophoresis (PFGE) with *SmaI* digestion and 9 distinct PFGE patterns were identified. Six and 5 different PFGE types were identified in houses 1 and 2, respectively. *C. jejuni* isolates, recovered from water lines samples, were genotypically similar to the isolates from fresh fecal dropping in both houses. These results showed that *C. jejuni* water-line contamination was related to flock contamination and could help to continuously make it infected with *C. jejuni*.

Key words: *Campylobacter jejuni,* broiler, water, pulsed-field gel electrophoresis.

INTRODUCTION

Campylobacteriosis, a human enteric infection caused by thermophilic campylobacters, is a well established foodborne zoonotic disease (Humprey et al., 2007). The incidence of campylobacteriosis has markedly increased in many countries and *Campylobacter jejuni* is one of the most common cause of foodborne illness. *C. jejuni* can cause severe diseases in human, but it is an apparently commensal organism of the gastrointestinal tract of farm animals and many wild animals (Horrocks et al., 2009). Broiler chickens are frequently asymptomatic intestinal carriers of *C. jejuni*, although the seasonal differences in the carriage of the alimentary tract was reported (Wallace et al., 1997). The intestinal contents may leak on to the carcass during the slaughtering process (Keener et al., 2004) and it is well-known that the contaminated poultry meat is a major source of human campylobacteriosis (Wilson et al., 2008; Sheppard et al., 2009).

Preventing flock colonization is one of the most effective strategies to reduce *Campylobacter* infections in human at poultry industry level. Several epidemiological studies have examined the different routes of *Campylobacter* infection for broiler flocks such as carry-over from previously positive flock (Shreeve et al., 2002), vertical transmission from breeder hens (Cox et al., 2002) and horizontal transmission from the environmental source (Johnsen et al., 2006). *C. jejuni* can be often found in the broiler house environment (Hansson et al., 2007) and several risk factors can be linked to horizontal transmission of *Campylobacter* in broiler flocks, such as other farm animals on the farm, on-farm staff, insects, feed and water (Lehtola et al., 2006; Adkin et al., 2006; Bull et al., 2006; Hald et al., 2007).

C. jejuni is highly susceptible to environmental conditions, and its survival outside the normal host can be limited by environmental stress including nutritional

*Corresponding author. E-mail: yavuzcokal@yahoo.com. Tel: + 90 266 714 93 02. Fax: +90 266 714 93 04.

factors, temperature and oxygen tensions (Park, 2002). A number of studies conducted under the experimental conditions have reported that *C. jejuni* can be present in biofilms found in animal production watering systems and may play a role in the colonization of these animals (Reeser et al., 2007; Trachoo et al., 2002; Zimmer et al., 2003). The aim of this study is to investigate whether or not *C. jejuni* in water lines of commercial broiler house might play a role in the epidemiology of *C. jejuni* infection in broiler flocks in the field.

MATERIALS AND METHODS

Sampling

The sampling protocol for detection of *C. jejuni* in water line of broiler house had some minor modification to the protocol described by Zimmer et al. (2003). All samples were collected from two different houses that were previously known to be infected with *C. jejuni* (Cokal et al., 2009). The houses had a system of nipple drinkers, and the distance between both of them were approximately 25 km. Drinking water was supplied by groundwater in one house, while the municipal water system with polyvinly chloride (PVC) plastic pipe was the source of drinking water in the other. The samples were collected from three sequential flocks in both houses. House cleanout and disinfection procedures were performed before entering a new flock in both houses.

Chlorine-based bleach were used as a water system sanitizer. The main periods of the sampling and the sample types are as follows: (i) Before the first flocks were placed at each house, 2 x 500 ml and 2 x 1 L water samples were taken from the PVC plastic pipe lines furnished already and used for transportation of drinking water from sources to houses; (ii) After the first flocks were placed at each house, five drinking water samples were taken weekly from different places for 3 weeks and 20 randomly selected fresh faecal droppings were weekly collected from each house with cotton swabs; (iii) Before the second flocks were placed at each house, four randomly swab samples of approximately 100 cm^2 area on the interior of the pipe were collected; (iv) After the second flocks were placed at each house, four randomly nipple pin swab samples were collected at 2, 4 and 6 weeks of age, and twenty randomly selected fresh faecal dropping materials were taken weekly at intervals from the house with sterile cotton swabs; (v) At the third flocks, placed in both houses, nipple pin swab samples and fresh faecal dropping materials were collected at weekly intervals, from 1 week of age until slaughter age. All the samples were immediately placed on ice to maintain a cool condition and transported immediately to the laboratory for bacteriological analysis.

Bacteriological and molecular analysis

Water samples were filtered through 0.22 µm membrane filters (Millipore, Bedford, USA), using membrane filtration system (Sartorius AG, Germany), and the filters were aseptically transferred into 50 ml Hunt enrichment broth (Hunt, 1992). Similarly, swab samples were aseptically transferred into 10 ml Hunt enrichment broth. The enrichment cultures were incubated microaerobically at 42°C for 48 h and then, the cultures were inoculated onto a modified charcoal cefoperazone deoxycholate agar (mCCDA) (CM739, Oxoid) with selective supplement (SR155, Oxoid). Fresh faecal materials were homogenized and cultured onto mCCDA. All plates were incubated microaerobically at 42°C for 48 h. Small, curved, catalase and oxidase-positive, gram negative bacilli were presumed to be *Campylobacter* spp. Conventional biochemical tests were used to identify the organism to species level. Real-time PCR analysis based on the *hip*O gene for confirmation was performed in the isolates with very weak activity and with negative activity by hippurate hydrolysis (Caner et al., 2008). *C. jejuni* isolates were frozen in Brucella broth supplemented with 7% lysed horse blood and 10% glycerol and stored at -80°C for further use.

Pulsed-field gel electrophoresis (PFGE) analysis

Molecular typing of *C. jejuni* isolates was performed by PFGE with a standardized PulseNet protocol (www.cdc.gov/pulsenet/protocols/campy_protocol.pdf). Briefly, agarose-embedded bacterial DNA were digested by *Sma*I enzyme. The digested DNA plugs were electrophoresed in a CHEF-DR II electrophoresis apparatus (Bio-Rad Laboratories, Hercules, CA, USA). The samples were then kept in a solution containing 5% µg/ml ethidium bromide for 30 min and the electrophoresis results were visualized under UV light. PFGE images were analyzed visually and the molecular patterns were grouped according to the criteria of Tenover et al. (1995).

RESULTS

Isolation of *C. jejuni*

Results of the isolation of *C. jejuni* from samples that are collected from three sequential broiler flocks in two houses are summarised in Table 1. In the first house, *C. jejuni* was isolated from samples including water from PVC pipe line, drinking water, nipple swab and fresh faecal dropping. However, in the second house, *C. jejuni* was isolated from drinking water, nipple swab and fresh faecal dropping samples.

PFGE types of *C. jejuni* isolates

The 150 *C. jejuni* isolates from houses 1 and 2 generated nine different genotypes by *Sma*I PFGE. These genotypes were assigned a letter from A to I. Six genotypes (A, B, D, F, H and I) were found in house 1, while 2 were infected with five genotypes (B, C, D, E and G) (Table 2). The most common genotypes were genotype B (2 water, 10 nipple swab and 58 fresh fecal dropping samples), genotype C (4 nipple swab and 16 fresh fecal dropping samples), genotype D (2 nipple swab and 12 fresh fecal dropping samples) and genotype F (14 fresh fecal dropping samples) (Figure 1).

In house 1, six genotypes were found in 78 *C. jejuni* isolates. The isolates of water origins were defined in genotypes A and B and they were of nipple pin origins in genotypes A, B and D. These strains were determined as closely related to the criteria reported by Tenover et al. (1995), and they were also recovered from fresh fecal samples of flocks. Genotype F isolates, which were of fecal origins were closely related to genotype B isolates which were of water and nipple pin origins. In addition, the isolates of genotypes H and I were only isolated from fresh fecal samples.

Table 1. Results of the isolation of *C. jejuni* from the samples in two houses.

Sample		House 1				House 2			
		No. of samples	Number of *C. jejuni* isolate			No. of samples	Number of *C. jejuni* isolate		
			Flock				Flock		
			1	2	3		1	2	3
Water from PVC pipe line	500 ml	4	-	-	-	6	-	-	-
	1 L	4	1	-	-	6	-	-	-
Drinking water		20	-	1	-	15	-	1	-
Swab from inner surface of PVC pipe line		8	-	-	-	4	-	-	-
Nipple swab		36	1	5	2	36	-	7	2
Fresh faecal dropping		353	8	13	47	286	10	32	20

Table 2. Molecular typing of *C. jejuni* isolates by *Sma*I-PFGE.

Sample	House 1			House 2		
	Genotype (Number of isolates)			Genotype (Number of isolates)		
	Flock 1	Flock 2	Flock 3	Flock 1	Flock 2	Flock 3
Water	A (1)	B (1)	-	-	B (1)	-
Nipple swab	A (1)	B (5)	D (2)	-	B (3) C (4)	B (2)
Fresh faecal dropping	A (7) B (1)	B (6) F (1) I (6)	B (15) D (9) F (13) H (10)	B (7) D (3)	B (16) C (16)	B (13) E (2) G (5)

In house 2, five genotypes were found in 72 *C. jejuni* isolates. Genotypes B and C were observed in water isolate, nippel drinkers surface isolates and fresh fecal isolates, and the isolates were identified as closely related. The isolates which were only isolated from fresh fecal samples were typed in genotypes E and G in house 2.

DISCUSSION

C. jejuni is a significant organism for the poultry industry, because poultry products are important contributors in the epidemiology of human campylobacteriosis. Despite its fastidious nature and sensitivity to environmental stress, *C. jejuni* can survive in poultry production environment, and this may provide transmission of the bacteria to poultry and lead to human infections (Zimmer et al., 2003; Peyrat et al., 2008). This pathogen is also capable of surviving in water and biofilms (Buswell et al., 1998; Trachoo et al., 2002).

In addition, *C. jejuni* has been shown to colonize protozoa and survive longer than its planktonic counterpart in protozoan host (Axelsson-Olsson et al., 2005). In the present study, *C. jejuni* was isolated from drinking water samples in both houses. The drinking water was withdrawn from groundwater sources in house 1, while the city's water supply system was the source of drinking water in house 2.

The genotype of water isolates were also identical to the genotype of fresh fecal dropping isolates. Several epidemiological studies have investigated whether water source or drinking water play a role in the transmission of *Campylobacter* to poultry. Some studies reported that *Campylobacter* spp. were not isolated from water samples (Hansson et al., 2007; Patriarchi et al., 2009). However, in other studies, *Campylobacter* spp. were detected in drinking water of broiler flocks (Ogden et al., 2007; Sasaki et al., 2010). It was also reported that *C. jejuni* has been isolated from water biofilms in ground water (Stanley et al., 1998). In a study conducted by Bull et al. (2006), *Campylobacter* was found in water when the flock was positive.

C. jejuni can attach and form a biofilm on stainless steel, PVC, nitrocellulose membranes, glass filter fibers and glass (Gunther and Chen, 2009). PVC and stainless steel are commonly used materials in watering systems of poultry houses. It was reported that PVC pipe line and

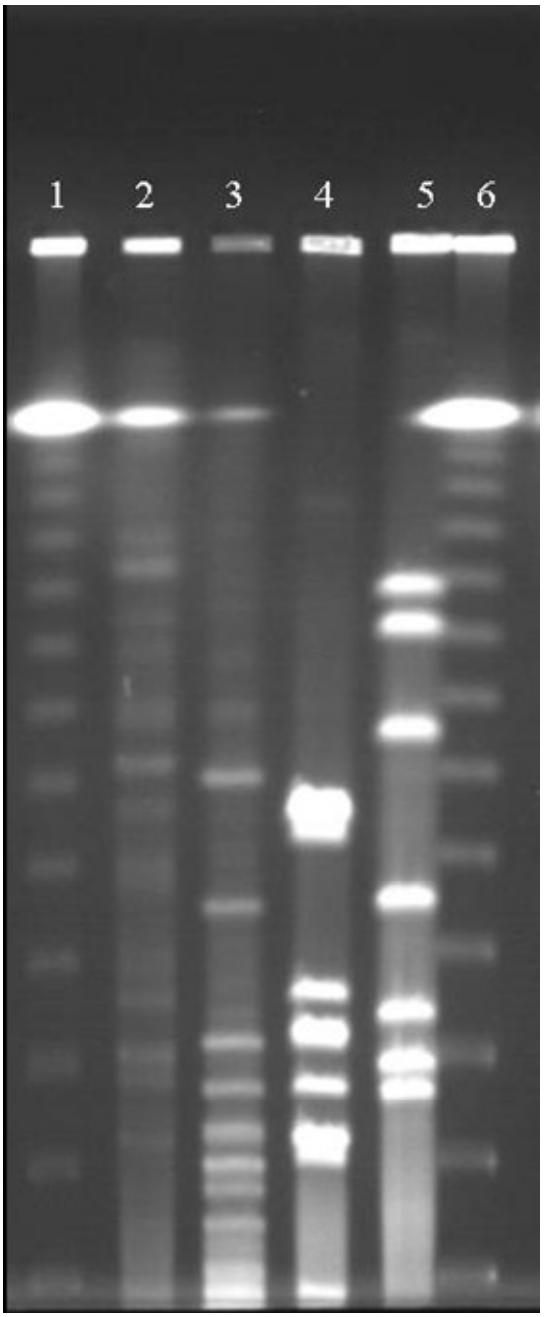

Figure 1. The most common genotypes by *Sma*I-PFGE. Lines 1 and 6: Molecular weight marker (BioRad, λ ladder); Line 2: Genotype B; Line 3: Genotype C; Line 4: Genotype D; Line 5: Genotype F.

nipple drinkers can harbour biofilms (Trachoo et al., 2002). Zimmer et al. (2003) showed that the presence of *C. jejuni* in biofilm was developed on drinking nipple surfaces by culture and immunofluorescence. In this study, *C. jejuni* were isolated from nipple drinkers surface samples using cultural method in both houses. It was also determined that the genotype of nipple swab isolates were identical to the genotype of fresh fecal dropping isolates. However, *C. jejuni* was not isolated from swab samples in the inner surface of PVC pipe line. The hybridization signals of the specific PNA probes for *C. jejuni* in some fresh PVC samples have indicated the presence of variable-but-nonculturable (VBNC) state of *C. jejuni* cells. The results are not shown here, because the fluorescence *in situ* hybridization analysis could be done only for a few samples (*C. jejuni*-specific PNA probes, a gift from Sven Poppert, Bernhard Nocht Institute, Germany). It was reported that VBNC *C. jejuni* cells have also been found in aqueous environments (Stern et al., 1994; Tholozan et al., 1999). Some studies have addressed the ability of the VBNC cells to remain infectious, and reported that the bacterium resuscitated after passing through the digestive system of animals (Cappelier et al., 1999; Baffone et al., 2006). It should also be noted that sampling from the PVC pipe line inner surface is very difficult under commercial poultry production conditions, and the sampling technique may affect the result.

In this study, the isolation of *C. jejuni*, attached to the surface of the nipple pipe, also suggested that the bacterium might form a biofilm or colonize a biofilm built by another microorganism. *C. jejuni* could survive and grow in biofilms in water distrubuting systems, but no pathogens, including *Vibrio chlorae* and *Salmonella enterica* serovar Typhi had such characteristics in the same systems (Rittmann, 2004; Lehtola et al., 2006).

Of the 150 *C. jejuni* isolates recovered, 9 different genotypes were identified by *Sma*I -generated PFGE. The most prevalent genotype detected was genotype B. It accounted for 46% of the isolates and was also isolated from the different samples collected from three sequential flocks in both houses. The companies, included in this study, were strictly adapted to the effective poultry cleanout before introducing the next flock, but *C. jejuni* was isolated from water in PVC pipe line in house 1 before entering a new flock. The genotype of this isolate was also identical to the genotype of fresh fecal dropping isolates in the same house. Typing of *C. jejuni* isolates recovered from both water and nipple drinker samples, and also fecal dropping samples in the same PFGE groups showed that this bacterium as a biofilm could persist in the water line system of the houses. However, further studies are needed before a clear conclusion can be given. There are some limitations to determine the source of persistence of the same strains because this study was conducted in the fields. Other sources of infection, such as litter, puddle, soil and wild birds also contributed to *C. jejuni* colonization of the flocks. Also, it has been observed that these flocks were colonized with more than one *C. jejuni* strain distinguishable by PFGE as reported by the other studies (Wassenaar et al., 1998; Höök et al., 2005).

In conclusion, the results of the bacteriological culture and the clonal relationships of the isolates suggest that

C. jejuni could survive in the water lines of poultry houses, and the presence of this bacterium in water lines of poultry houses may play an important role in the epidemiology of *C. jejuni,* which has a low infective dose.

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