อุบัติการณ์และรูปแบบการดื้อยาของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากฝูงไก่กระทงที่มีการเลี้ยงอย่างต่อเนื่อง

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OCCURRENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *CAMPYLOBACTER* SPP. ISOLATED FROM CONSECUTIVE BROILER FLOCKS

Miss Petcharatt Charununtakorn

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By	Miss Petcharatt Charununtakorn
Field of Study	Veterinary Public Health
Thesis Advisor	Taradon Luangtongkum, D.V.M., Ph.D.
Thesis Co-Advisor	Nipa Chokesajjawatee, Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Veterinary Science
(Professor Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Benjamas Patamalai, D.V.M., Ph.D.)
.....Thesis Advisor
(Taradon Luangtongkum, D.V.M., Ph.D.)
.....Thesis Co-Advisor
(Nipa Chokesajjawatee, Ph.D.)
.....Examiner
(Associate Professor Rungtip Chuanchuen, D.V.M., M.Sc., Ph.D.)
.....External Examiner
(Assistant Professor Prapansak Chaveerach, D.V.M., Ph.D.)

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจหาอุบัติการณ์ การคงอยู่ และรูปแบบการดื้อยาของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากฝูงไก่กระทงที่มีการเลี้ยงอย่างต่อเนื่อง โดยเก็บตัวอย่างจากฟาร์มไก่เนื้อ จำนวน 2 ฟาร์ม ที่เลี้ยงในระบบอุตสาหกรรมในเขตภาคตะวันออกของประเทศไทย จำนวน 3 รอบการผลิต โดยทำการเก็บตัวอย่างจากตัวไก่และสิ่งแวดล้อม 1,859 ตัวอย่าง ได้ทำการศึกษาลักษณะทางพันธุกรรมของเชื้อแคมไพโลแบคเตอร์ที่แยกได้โดยวิธี *flaA* short variable region (*flaA* SVR) sequencing และวิธี multilocus sequence typing (MLST) นอกจากนี้ยังได้ทำการทดสอบการดื้อต่อยาปฏิชีวนะ 5 ชนิด ด้วยวิธี agar dilution ผลการศึกษาพบเชื้อแคมไพโลแบคเตอร์ในไก่ของฟาร์ม A ในรอบการเลี้ยงที่ 1 (51.76%) และรอบการเลี้ยงที่ 2 (51.05%) และฟาร์ม B พบเชื้อแคมไพโลแบคเตอร์ ในรอบการเลี้ยงที่ 1 (25.29%) และรอบการเลี้ยงที่ 3 (39.42%) ผลการตรวจพันธุกรรมของเชื้อแคมไพลแบคเตอร์พบว่าเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากฟาร์ม A ในรอบการเลี้ยงที่ 1 มีความหลากหลายทางพันธุกรรมค่อนข้างมาก แต่เชื้อที่แยกได้ในรอบการเลี้ยงที่ 2 กลับพบลักษณะทางพันธุกรรมเพียงรูปแบบเดียว สำหรับฟาร์ม B เชื้อแคมไพโลแบคเตอร์ที่แยกได้จากทั้ง 2 รอบการผลิตจะพบลักษณะทางพันธุกรรมหนึ่งรูปแบบอันได้แก่ *flaA* SVR allele number 783 (ST-1232) นอกจากนี้ลักษณะทางพันธุกรรมของเชื้อที่เก็บมาจากสิ่งแวดล้อมยังพบในเชื้อที่แยกได้จากไก่อีกด้วย ผลการทดสอบการดื้อต่อยาปฏิชีวนะในการศึกษาครั้งนี้พบว่าเชื้อแคมไพโลแบคเตอร์ส่วนใหญ่ดื้อต่อ ciprofloxacin (94.86%) รองลงมา ได้แก่ การดื้อต่อ tetracycline (88.78%) และ ampicillin (56.07%) ในขณะที่การดื้อต่อ erythromycin และ gentamicin พบเพียงร้อยละ 7.01 และร้อยละ 6.07 ตามลำดับ รูปแบบของการดื้อต่อยาปฏิชีวนะที่พบมากที่สุดในการศึกษาครั้งนี้คือ CIP-TET-AMP (43.92%) และ CIP-TET (34.11%) การศึกษาครั้งนี้แสดงให้เห็นว่าระหว่างการเปลี่ยนฝูงไก่เชื้อแคมไพโลแบคเตอร์บางกลุ่มสามารถมีชีวิตรอด และคงอยู่ในสิ่งแวดล้อมของฟาร์ม จากนั้นจึงไปปนเปื้อนไก่ฝูงถัดไป นอกจากนี้การใช้ยาปฏิชีวนะเป็นประจำในฟาร์มอาจส่งผลให้เกิดการดื้อต่อยาปฏิชีวนะของเชื้อแคมไพโลแบคเตอร์ในอุตสาหกรรมการเลี้ยงไก่ได้ ดังนั้นจึงจำเป็นต้องมีการนำมาตรการการควบคุมเชื้อแคมไพโลแบคเตอร์ในฟาร์มมาใช้ โดยเฉพาะการทำความสะอาดและการฆ่าเชื้อเพื่อลดการปนเปื้อนของเชื้อแคมไพโลแบคเตอร์ระหว่างฝูงไก่เนื้อ นอกจากนั้นการใช้ยาปฏิชีวนะอย่างเหมาะสมในฟาร์มไก่เนื้อก็เป็นสิ่งที่ควรให้ความสำคัญด้วยเช่นกัน

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ปีการศึกษา	2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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PETCHARATT CHARUNUNTAKORN: OCCURRENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *CAMPYLOBACTER* SPP. ISOLATED FROM CONSECUTIVE BROILER FLOCKS. ADVISOR: TARADON LUANGTONGKUM, D.V.M., Ph.D., CO-ADVISOR: NIPA CHOKESAJJAWATEE, Ph.D., 82 pp.

The objectives of the present study were to examine the occurrence and persistence of *Campylobacter* in consecutive broiler flocks and to determine antimicrobial resistance patterns of *Campylobacter* isolated from conventional broilers reared consecutively. A total of 1,859 broiler and environmental samples were collected from 2 broiler farms located in the eastern part of Thailand for 3 production cycles. *Campylobacter* isolated strains were selected and genotyped by *flaA* short variable region (*flaA* SVR) sequencing and multilocus sequence typing (MLST). Furthermore, these *Campylobacter* isolates were tested for their antimicrobial resistance to 5 antimicrobial agents by the agar dilution method. The results showed that broilers in farm A and farm B were *Campylobacter* positive in the first (A1, 51.76%) and the second (A2, 51.05%) production cycles and in the first (B1, 25.29%) and the third (B3, 39.42%) production cycles, respectively. Although a high degree of genetic diversity was noticed in *Campylobacter* isolates from flock A1, only one genotype was found in flock A2. Unlike farm A, a single *Campylobacter* genotype, *flaA* SVR allele number 783 (ST-1232), was observed in farm B from both positive flocks. Moreover, the genotype that was present in environment was also detected in broilers. In the present study, the majority of *Campylobacter* isolates were resistant to ciprofloxacin (94.86%), followed by tetracycline (88.78%) and ampicillin (56.07%). In contrast, low rates of resistance were found for erythromycin (7.01%) and gentamicin (6.07%). The most common resistance patterns observed in this study were CIP-TET-AMP (43.92%) and CIP-TET (34.11%). Our findings suggested that certain clone of *Campylobacter* may survive and persist in the farm environment, then recontaminate the following flocks. In addition, the routine practice of antimicrobial usage as reported in this study may influence the occurrence of antimicrobial-resistant *Campylobacter* in conventional broiler production. Therefore, it is necessary to implement the control strategies, especially cleaning and disinfection measures, in order to reduce the contamination of *Campylobacter* between flocks. Moreover, the prudent use of antibiotics in broiler production should also be emphasized.

Department:	Veterinary Public Health	Student's Signature	.....
Field of Study:	Veterinary Public Health	Advisor's Signature	.....
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LIST OF ABBREVIATION

AMP	ampicillin
bp	base pair(s)
ºC	degree (s) Celsius
C.	*Campylobacter*
CFU	colony-forming unit
CIP	ciprofloxacin
CLSI	The Clinical and Laboratory Standards Institute
DNA	deoxyribonucleic acid(s)
dNTP	deoxyribonucleoside triphosphate(s)
EDTA	Ethylene diaminetetraacetic aci
EFSA	European Food Safety Authority
ERY	erythromycin
flaA SVR	flagellin A short variable region
GEN	gentamicin
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
MDR	multidrug resistance
MHA	Mueller Hinton agar
ml	milliliter (s)
MIC	minimal inhibitory concentrations
MLST	multilocus sequence typing
NARMS	The National Antimicrobial Resistance Monitoring System
OAE	Office of Agricultural Economics

PCR	polymerase chain reaction
rpm	round per minutes
TAE	Tris-Acetate EDTA
TET	tetracycline
U	unit
µl	microliter (s)

CHAPTER I
INTRODUCTION

Campylobacter is one of the major causes of gastroenteritis in human worldwide (Coker et al., 2002). In 25 member states in European Union, there were up to 214,268 confirmed cases of campylobacteriosis or 55.49 infection rate per 100,000 populations (EFSA, 2014). In Thailand, campylobacteriosis is commonly found in children younger than 12 years old (Bodhidatta et al., 2002). Outbreaks of *Campylobacter* infection worldwide are often found in patients with immunosuppression, children and elderly (Nachamkin et al., 1998; Humphrey et al., 2007). Most cases of human campylobacteriosis are caused by *Campylobacter jejuni* and *Campylobacter coli*. Although most *Campylobacter* infections are self-limiting, severe complications such as septicemia, acute inflammatory demyelinating polyneuropathy (Guillain-Barré Syndrome) and paralysis of eye muscles and absence of tendon reflexes (Miller Fisher Syndromes) can occur (Humphrey et al., 2007). Consumption of raw poultry meat is regarded as a major cause of foodborne campylobacteriosis. Since broilers can harbor a large number of *Campylobacter* in their gut, cross contamination of this organism to chicken meat during slaughtering process is likely to occur. To successfully reduce *Campylobacter* contamination in chicken meat, it is necessary to reduce the number of *Campylobacter*-colonized flocks.

During the last two decades, the incidence of antibiotic resistance in *Campylobacter* isolates from food animals and humans has increased rapidly,

particularly in countries with the widespread use of antibiotics in food animals (Silva et al., 2011). Inappropriate use of antibiotics on farms is considered one of the major causes of increasing antibiotic resistance in foodborne pathogens. A previous study reported that antimicrobial-resistant *Campylobacter* could displace antimicrobial-susceptible strains in the intestinal tract of poultry and the resistant strains could persist in the flock until slaughter age (Luangtongkum et al., 2008). Hence, the spread of antimicrobial-resistant *Campylobacter* strains during slaughtering process may occur. The restriction of antibiotic use is necessary because it does not only help decrease the incidence of antimicrobial-resistant *Campylobacter* at the farm level, but it also reduces the spread of antimicrobial-resistant *Campylobacter* from animals to humans.

In addition to being recognized as a major public health problem, *Campylobacter* also plays an important role in the country economics. Thailand is one of the major chicken meat exporters. In 2012, Thailand exported 492,542 tons of poultry products, which valued at 58,388 million baht (OAE, 2013). The export value has a tendency to increase due to the increased demand of raw chicken meat for consumption in EU and the abolition of import ban on Thai uncooked chicken meat (Globalmeatnews, 2012). Since *Campylobacter* might be used as a trade barrier, the contamination of this organism in chicken meat products must be as low as possible. To effectively control *Campylobacter* in poultry products, it is necessary to understand the ability of *Campylobacter* to survive and persist in food production chain, particularly at the farm level, which is the beginning process of the meat production.

Therefore, the present study was conducted to examine a potential carry-over of *Campylobacter* spp. in consecutive broiler flocks and to determine antibiotic resistance patterns of *Campylobacter* isolated from conventional broilers reared

consecutively. The information on the ecology and antibiotic resistance of *Campylobacter* gained from this study will allow a more complete understanding of the behavior of *Campylobacter* and can be used as a supporting data for development of *Campylobacter* control measures for Thai broiler production in the future.

CHAPTER II
LITERATURE REVIEW

2.1 Microbiology of *Campylobacter* spp.

The genus *Campylobacter* belongs to the family *Campylobacteraceae*. Currently, 18 species, 6 subspecies and 2 biovars of *Campylobacter* have been identified (Humphrey et al., 2007). The majority of *Campylobacter* species can motile by using a single flagellum. The detection of *Campylobacter* is quite limited by their fastidious growth characteristic (Linton et al., 1996). Suitable temperature for *Campylobacter* to grow is between 37°C and 42°C, with an optimum temperature at 41.5°C (Levin et al., 2007). Although *Campylobacter* is unable to grow below 30°C and above 55°C, it can be isolated from poultry carcass kept at 4°C or under frozen conditions (Simmons and Gibbs, 1979). These bacteria grow well under microaerobic condition with low oxygen tension (Garenaux et al., 2008). Generally, *Campylobacter* is a spirally curved rod, however, this bacterium can change to a coccoid form when it is exposed to unfavorable environments (Buck et al., 1983; Moran and Upton, 1987). In the coccoid stage, *Campylobacter* contains low levels of nucleic acids and peptides and lacks cellular integrity in order to survive longer (Moran and Upton, 1987; Beumer et al., 1992; Boucher et al., 1994). Although *Campylobacter* is ubiquitous in the environment, they are believed to be very fragile in conditions such as desiccation or higher oxygen tension (Fernandez et al., 1985; Silva et al., 2011). Among warm-blooded animals that can carry *Campylobacter*, poultry are considered as one of the most important reservoirs (Humphrey et al., 2007).

2.2 Colonization and transmission of *Campylobacter* in broilers

Campylobacter is commensal organism in chicken gut (Sahin et al., 2002; Newell and Fearnley, 2003; Dhillon et al., 2006). The bacterial cells are usually attached to the mucous layer of the crypts inside intestinal tracts of the host, especially in the lower intestines such as caecum and cloacal crypts, but sometimes they can be found in liver, spleen, small intestine and gall bladder (Beery et al., 1988; Meinersmann et al., 1991). The study of Stern et al. (1988) reported that the minimum dose of *Campylobacter jejuni* (*C. jejuni*) for colonization was as low as 35 CFU/bird via oral administration. *Campylobacter jejuni* is the main species found in broilers (Humphrey et al., 2007). Generally, broilers become colonized with *Campylobacter* at 2-3 weeks old. After colonization, *Campylobacter* can spread rapidly to other broilers in the flock and persist in broilers until they reach slaughter age (Jacobs-Reitsma et al., 1995; Berndtson et al., 1996; Gregory et al., 1997; Evans and Sayers, 2000; Shreeve et al., 2000). These findings suggested that young chickens might have maternal antibodies to protect themselves from *Campylobacter* colonization (Sahin et al., 2002).

Since *Campylobacter* has been found in various farm environments such as old litter, untreated drinking water, other farm animals, domestic pets, wildlife species, houseflies, insects, farm equipments, transport vehicles and farm workers, the horizontal transmission is considered a likely source of broiler infection (Sahin et al., 2002). The study of Humphrey et al. (1993) showed that *C. jejuni* is very sensitive to oxygen level and dry condition, so feed and new litter which have low moisture content should not be an important source of *Campylobacter*. Evidence for carry-over of *Campylobacter* during sequential production cycles was described in the studies of Petersen and Wedderkopp (2001) and Sahin et al. (2002) . The studies suggested that

Campylobacter jejuni may survive in rodents and insects which acted as reservoirs during cleaning and disinfection of a broiler house and then those pests may return to the broiler house in the next production cycle. In addition, Zweifel et al. (2008) revealed that the persistence and survival of *Campylobacter* in broiler houses or environments around the broiler house might be a risk factor for *Campylobacter* colonization in successive flocks. The study of Alter et al. (2011) reported that although a high diversity of *Campylobacter* genotypes was found in broiler farms, only one single genotype was present in several consecutive flocks.

Unlike the horizontal transmission, the vertical transmission is considered unlikely because several studies showed that progenies were often colonized with different *Campylobacter* strains from their parent flocks (Chuma et al., 1997; van de Giessen et al., 1998; Petersen et al., 2001). Though *Campylobacter* can be isolated from oviduct of laying hens, Camarda et al. (2000) suggested that oviduct colonization may result from ascending infection via the cloaca.

Flock thinning (partial depopulation) and animal reservoirs (wild birds and flies) were found to be risk factors for *Campylobacter* infection in broilers. Furthermore, the presence of other farm animals in adjacent area and a direct use of untreated water or rain water were also revealed in developed countries (Pacha et al., 1988; Schorr et al., 1994; Eberhart-Phillips et al., 1997; Hald et al., 2001; Hald et al., 2004; Zweifel et al., 2008). In Thailand, only limited information on the sources of *Campylobacter* infection in broiler flocks is available.

2.3 Genetic characterization of *Campylobacter* spp.

Several molecular typing techniques have been used for genetic characterization of *Campylobacter* including repetitive element sequence-based polymerase chain reaction technique (rep-PCR) (Versalovic et al., 1995; Hiett et al., 2006), *flaA*-restriction fragment length polymorphism (*flaA*-RFLP) (Nachamkin et al., 1993), pulsed-field gel electrophoresis (PFGE) (Yan et al., 1991), multilocus sequence typing (MLST) (Dingle et al., 2001), ribotyping, random amplified polymorphic DNA (RAPD) (Owen et al., 1993), amplified fragment length polymorphism (AFLP) (Duim et al., 1999), *flaA* short variable region (*flaA* SVR) sequencing (Meinersmann et al., 1997) and comparative genomic fingerprinting (CGF) method (Taboada et al., 2012). PFGE has been recognized as the gold standard for genotyping of clinically important bacteria including *Campylobacter* (Wassenaar and Newell, 2000). PFGE has a high discriminatory power and is a reproducible method. This method uses restriction enzyme to digest bacterial chromosomal DNA and then DNA fragments are separated by electrophoresis (Wassenaar and Newell, 2000). Even though PFGE provides a high discriminatory power, labor intensive and time consuming remain the disadvantages of this technique.

MLST is a high resolution genotyping method that is commonly used for epidemiological and population genetic studies of *Campylobacter jejuni* (Wilson et al., 2009). MLST is based on the sequence of internal fragment of 7 housekeeping genes. Although MLST has many advantages such as high discriminatory power and inter-laboratory reproducibility and typeability, it is quite expensive and elaborate. In addition to MLST, *flaA* SVR sequencing, which determines the sequence of a short variable region of flagellin A gene, is another typing method that is commonly used for molecular epidemiological studies in a wide range of organisms, including *C. jejuni*

(Meinersmann et al., 1997). This method is quite simple, rapid, inexpensive and reliable (Meinersmann et al., 2005; Wassenaar et al., 2009). Although MLST and *flaA* SVR sequencing yielded reliable results, the combination of MLST and *flaA* SVR sequencing provided a better discriminatory power for both longitudinal and cross-sectional studies of *Campylobacter* (Price et al., 2006).

2.4 Antibiotic resistance in *Campylobacter* spp.

Although *Campylobacter* infections in humans are usually mild or self-limiting without the need for antibiotic treatment; however, severe cases such as acute bloody diarrhea and acute neurological disease can occur in immunosuppressed persons, pregnant women, very young children and elderly (Nachamkin et al., 1998; Humphrey et al., 2007). Macrolides and fluoroquinolones are considered as drugs of choice for treatment of *Campylobacter* infection. Additionally, aminoglycosides can be used for treatment of serious cases of campylobacteriosis (Aarestrup and Engberg, 2001). Engberg et al. (2001) reported that most of *Campylobacter* strains isolated from patients in Denmark were resistant to fluoroquinolones and macrolides. In Thailand, the previous study by Padungtod et al. (2003) also reported a high proportion of fluoroquinolone-resistant *Campylobacter* isolates from chickens. In addition, Serichantalergs et al. (2007) showed that ciprofloxacin-resistant *Campylobacter* isolated from Thai children increased at a high level. The study of Alfredson and Korolik (2007) demonstrated that the incidence of fluoroquinolone resistance in *Campylobacter* isolated from Thailand was approximately 84% and the duration of illness was longer in patients infected with fluoroquinolone-resistant *Campylobacter* than those infected with susceptible *Campylobacter* strains. This study also showed

that the rates of fluoroquinolone-resistant *Campylobacter* in human and chicken isolates in Taiwan were 72% and 92%, respectively. Similarly, 99% of *Campylobacter* isolated from broilers in Spain were resistant to this antimicrobial. The high prevalence of fluoroquinolone-resistant *Campylobacter* in patients and animals is possibly due to the use of enrofloxacin, the second generation fluoroquinolones, in food animals (Silva et al., 2011).

A previous study in Thailand revealed that the rate of tetracycline-resistant *Campylobacter* was over 52% in cloacal swab samples and 81.3% in chicken meat samples collected from retail markets (Padungtod et al., 2006). Likewise, a study conducted in France also found the high rate of tetracycline-resistant *Campylobacter* in broilers ranging from 55-68% (Moore et al., 2006). In addition, Piddock et al. (2008) found that tetracycline-resistant strains were cross-resistant to at least one or more classes of antimicrobial such as quinolones or beta lactams. When antimicrobial resistance of *Campylobacter* isolates from conventional broiler production was compared to that of the isolates from organic broiler production, it was obvious that *Campylobacter* isolated from conventional broiler farms were more resistant to antibiotics than those isolates from organic broiler farms (Luangtongkum et al., 2006). Several studies showed that the prevalence of antimicrobial resistance, particularly fluoroquinolone resistance and tetracycline resistance, increased in *Campylobacter* isolated from food animals (Engberg et al., 2001; Luangtongkum et al., 2006; Moore et al., 2006). The study of Aarestrup et al. (1999) reported that usage of antimicrobial agents is the most important factor in the selection of resistance in bacteria. Since antibiotics are routinely used for treatment and prophylaxis, the development of

antibiotic resistance in commensal and pathogenic bacteria isolated from food animals is quite common in the modern intensive animal production system.

2.5 Studies of *Campylobacter* spp. in Thailand

In Thailand, the studies of *Campylobacter* in poultry production are limited. Only few studies have been established at the farm level. Previous report by Padungtod and Kaneene (2005) showed that the prevalence of *Campylobacter* at broiler farm was higher than 60%, while the prevalence of this organism at slaughterhouse was around 37%. Likewise, Meeyam et al. (2004) reported that 59.1% of the cloacal swab samples collected from broilers in Northern Thailand were positive for *Campylobacter.* Unlike the aforementioned studies, Chokboonmongkol et al. (2013) found that the prevalence of *Campylobacter* in broiler skin samples (51%) was higher than broiler caecal samples (11.2%). Even though *Campylobacter jejuni* was the most prevalent species in chickens at the farm level, *Campylobacter coli* was more prevalent in chicken meat collected from slaughterhouses and markets (Meeyam et al., 2004). Since *Campylobacter coli* was the predominant species isolated from slaughterhouse workers, it is possible that these workers might be the source of *Campylobacter coli* contamination in retail chicken meat. (Meeyam et al., 2004; Padungtod and Kaneene, 2005).

CHAPTER III

MATERIALS AND METHODS

3.1 Description of farm and flock characteristics

3.1.1 Farm management data

In this study, samples were collected from 2 conventional broiler farms, farm A and B, located in Chachoengsao and Prachinburi provinces. Criteria for farm selection are their location (high density poultry farm area), the history of *Campylobacter* positive in previous flocks and the cooperation of the farm owners. Both farms contain one broiler house with the flock size of 12,000 – 15,000 broilers per house. These broiler farms belong to the same integrated system and receive day old chicks from the same breeder farm and hatchery. For farm management practice, cleaning and disinfection procedures and hygiene practices were quite similar in both farms. Each broiler house had the anteroom for storing feed and equipment. This area was also used as hygiene barrier for changing and disinfecting boot. Water used in both broiler farms was not treated with chlorine. Rice husk was used as litter and disinfected before use. In both farms, broilers were raised for 30-35 days and then sent to the same slaughterhouse. After depopulation, the litter was immediately removed and equipment and broiler houses were cleaned and disinfected with Omnicide® (Conventry chemicals, United States). The broiler house remained empty for 10 to 20 days before introducing new birds to the farm. Although farm management in both broiler farms was quite similar, some practices were different. In farm A, the owner raised their own birds, while the owner of farm B employed a worker to raise their chickens. Additionally, in farm B, fence was also installed around the farm to separate

poultry area from living area. Generally, it is not overstated to conclude that the biosecurity of farm B was higher than that of farm A.

3.1.2 Antimicrobial usage data

In the broiler flocks from which the samples were collected, gentamicin was given to the birds at the hatchery via subcutaneous route for prevention of early mortality due to *E. coli* and *Pseudomonas* spp. infections. In addition to gentamicin, amoxicillin was also given to the birds at the age around 14 days via drinking water to relieve the symptoms of vaccination reaction. Narasin, nicarbazin and salinomysin were supplemented in broiler feed for prevention and control of necrotic enteritis until 7 days before slaughter. Likewise, tylosin and lincomycin were also used for treating mycoplasma infection in every flock. Although enrofloxacin was not used for therapeutic purposes in the flocks that we collected samples, it had been previously used in both farms.

3.2 Sample collection

A total of 1,859 samples were collected from cloaca and environments inside and around the broiler house during June to December, 2012. Each farm was examined consecutively for 3 production cycles. The number and type of samples were collected according to previous studies (Hook et al., 2005; Zweifel et al., 2008) with some modifications. The samples were collected sequentially from the downtime period to slaughtering.

During the downtime period, environmental samples including feeder, boots used in the broiler house, water from nipple, water inlet, litter, boot swab from

anteroom and boot swab inside and around the broiler house were collected before chicks arrival to examine for *Campylobacter* contamination in the house. During the rearing period, 30 cloacal swabs from 30 broilers as well as environmental samples were collected weekly. In addition, pests such as flies, darkling beetles and mice were collected as available. During slaughtering process, 30 intestines were randomly taken at the evisceration step. All samples were delivered to the laboratory for *Campylobacter* detection within 24 hours. A summary of samples collected in this study and the diagram of sample collection are shown in Table 1 and Figure 1, respectively.

Table 1. Types of sample collected in this study

Duration	Type of sample	Description of sample	No. of samples collected / flock	Sample collection frequency / flock
Downtime period	Feeder	Chick Tray (1 feeding pan/swab)	6	
		Automatic Pan (1 feeding pan/swab)	6	
	Boots	Boots used in a broiler house (1 pair of boot/sample)	2	Once
	Boot swab	Boot swab on pathway from an office to broiler house (1 pair of boot/sample)	1	
Rearing period	Boot swab	Boot swab from anteroom (1 pair of boot/sample)	1	
		Boot swab inside a broiler house (1 pair of boot/sample)	1	
		Boot swab around a broiler house (1 pair of boot/sample)	2	
	Litter	Husk (100 grams/sample)	3	Every week
	Water	Water inlet (1,000 ml of water before entry the house)	1	(5 weeks)
		Water from nipple (100 ml of water from 1 nipple/sample)	6	
	Day old chick	Meconium from box liner	10	
	Broiler	Cloacal swab	30	
	Pest	Flies, darkling beetles (10 pests/sample)	0-2	As available
Slaughterhouse	Intestine	Caeca	30	Once

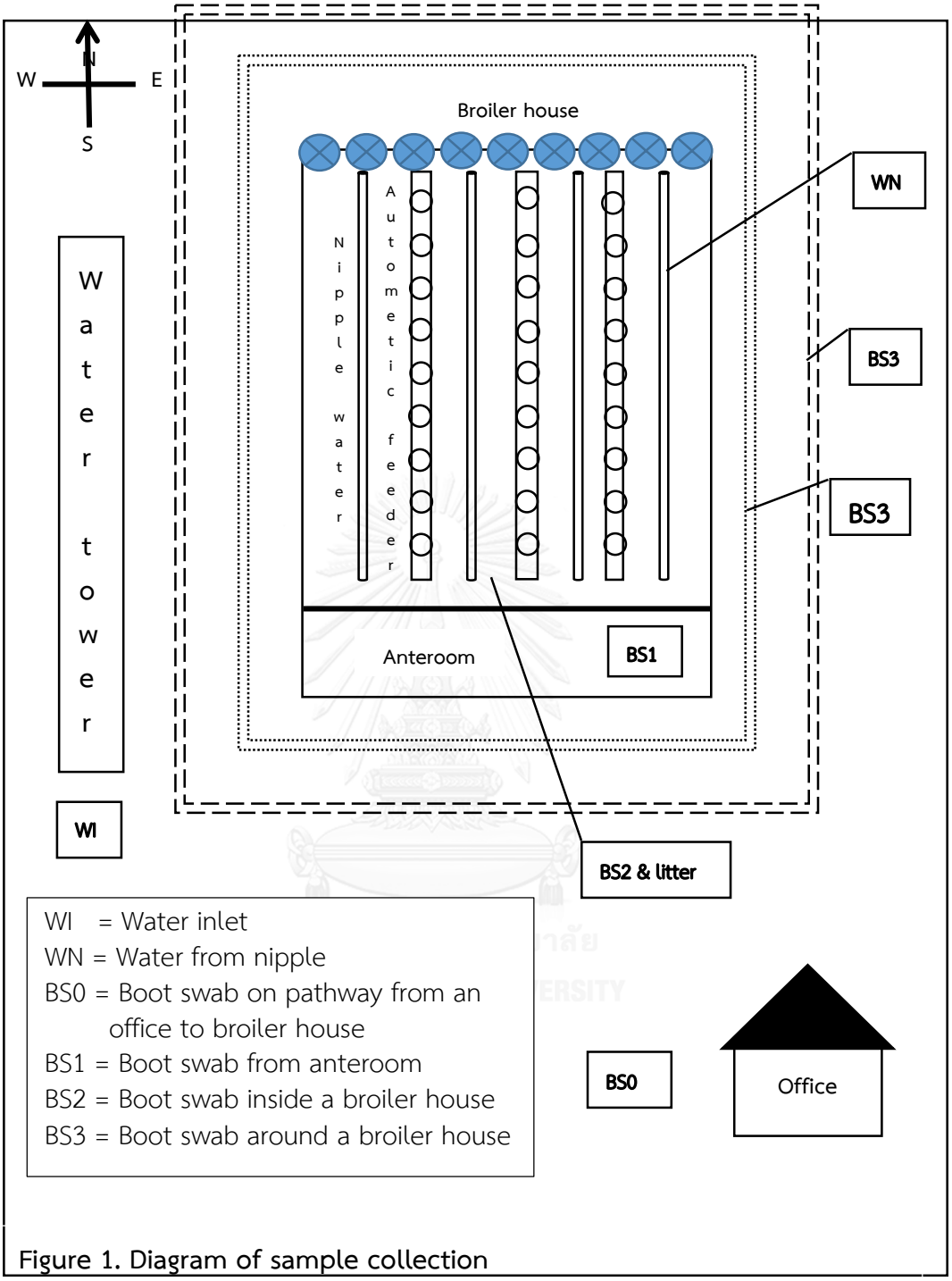

Figure 1. Diagram of sample collection

3.3 Isolation of *Campylobacter* spp.

Samples collected were divided into 2 categories including (i) cloacal swabs and caecal samples and (ii) environmental samples. Direct plating method and selective enrichment method were used for isolation of *Campylobacter* spp. from cloacal swabs and caecal samples and from environmental samples, respectively.

3.3.1 Cloacal swabs and caecal samples

Cloacal swab samples and caecal contents were aseptically streaked onto modified Charcoal Cefoperazone Deoxycholate agar (mCCD agar) (Appendix A). All plates were incubated under microaerobic conditions containing approximately 10% CO_2 and 5% O_2 at 42°C for 44 ± 4 hours (Humphrey, 1989). Flat, grayish with metallic sheen colonies presumed to be *Campylobacter* were inspected after incubation. Presumptive *Campylobacter* colonies were subcultured onto blood agar and incubated under microaerobic atmosphere at 42°C for 44 ± 4 hours. Then, the pure isolated colonies were confirmed by multiplex PCR assay (Wang et al., 2002).

3.3.2 Environmental samples

Exeter enrichment broth supplemented with amphotericin B, trimethoprim, polymyxin B, cefoperazone, rifampicin, sodium pyruvate, sodium metabisulfite, iron sulfate and 5% of defibrinated sheep blood was used for isolation of *Campylobacter* from environmental samples (Appendix A). Samples were added to the broth at 1:9 (w/v) ratio and incubated at 37°C for 44 ± 4 hours under microaerobic conditions (Humphrey, 1989). One hundred microliters (µl) of the enriched broth were then plated onto mCCD agar and incubated under the same conditions as previously described.

Campylobacter suspected colonies were subcultured onto non-selective blood agar and incubated under microaerobic atmosphere at 42°C for 44 ± 4 hours. Then, the presumptive *Campylobacter* colonies were randomly selected and confirmed by multiplex-PCR assay (Wang et al., 2002).

3.4 Confirmation of *Campylobacter* spp. by multiplex PCR assay

3.4.1 DNA template preparation

DNA template was prepared by whole cell boiling method. Single colonies were collected and resuspended in 100 µl sterile distilled water. The suspension was heated in boiling water for 10 minutes and then centrifuged at 12,000 rpm for 5 minutes. Five microliters of the supernatant were used as DNA template in PCR mixture (Wang et al., 2002).

3.4.2 Primers and multiplex PCR conditions

Campylobacter species were identified by multiplex PCR according to the protocol previously published with some modifications (Wang et al., 2002). Briefly, each 25-µl PCR reaction mixture contained 1X PCR reaction buffer with Mg^{2+}, 0.4 mM of each deoxynucleoside triphosphates (dNTP), 10 pmol of each primer specific for *Campylobacter* spp., 1.25 U KapaTaq DNA polymerase (Kapabiosystems, Boston, MA, USA) and 5 µl of template DNA. The volume was adjusted to 25 µl with sterile distilled water. PCR amplification was conducted with the thermocycling conditions as follows: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 45

seconds with a final extension at 72°C for 7 minutes. Five microliters of each PCR product were separated by gel electrophoresis in a 1.2% agarose gel prepared with 1x TAE (40 mMTris acetate/1 mM EDTA, pH8) buffer. Gel was run for 30 minutes with a constant voltage of 90 V. Then, the gel was stained in a 0.5 µg/ml ethidium bromide solution for 10 minutes, destained in distilled water for 1 minute and photographed using a gel documentation (Viber Loumat, France). *C. jejuni* ATCC 33560, *C. coli* NTCT 11353, and *C. lari* ATCC 35223 were used as positive control strains for multiplex PCR. Primers used for species confirmation are shown in Table 2.

Table 2. Oligonucleotide primers used for *Campylobacter* identification

Species	Size (bp)	Target gene	GenBank accession no.	Primer	Sequence (5' to 3')
C. jejuni	323	*hipO*	Z36940	CJF	ACTTCTTTATTGCTTGCTGC
				CJR	GCCACAACAAGTAAAGAAGC
C. lari	251	*glyA*	AF136495	CLF	TAGAGAGATAGCAAAAGAGA
				CLR	TACACATAATAATCCCACCC
C. coli	126	*glyA*	AF136494	CCF	GTAAAACCAAAGCTTATCGTG
				CCR	TCCAGCAATGTGTGCAATG

3.5 Genetic characterization

3.5.1 Sequencing of *flaA* short variable region (*flaA* SVR)

One *Campylobacter* isolate from each positive sample was selected for genetic characterization by *flaA* SVR sequencing. The *flaA* gene amplification was performed using the protocol as previously described by Meinersmann et al. (1997). DNA

templates were prepared by whole cell boiling method as previously described. PCR reaction was performed in a 25 µl reaction mixture composed of 1X PCR buffer (Kapabiosystem, Boston, MA, USA), 0.4mM of dNTP and 1.25 U KapaTaq DNA polymerase (Kapabiosystem, Boston, MA, USA). DNA amplification was performed as follows: an initial denaturation at 94°C for 1 minute, followed by 35 cycles of denaturation at 92°C for 30 seconds, annealing at 55°C for 90 seconds and extension at 72°C for 2.5 minutes with a final extension at 72°C for 5 minutes. The 425-bp amplicon was examined by gel electrophoresis. Then, the PCR amplification product was purified by NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). The purified product was submitted for sequencing at 1st BASE Pte Ltd. (Gemini Singapore Science ParkII, Singapore). Nucleotide sequences were compared to the published sequences of *flaA* SVR allele using the online database (http://pubmlst.org/campylobacter/flaA). Primers used for *flaA* identification and sequencing are shown in Table 3.

Table 3. Oligonucleotide primers used for *flaA* identification

Primer	Sequence (5' to 3')	Size
FLA242FU	CTA TGG ATG AGC AAT T(AT)A AAA T	425 bp
FLA625RU	CAA G(AT)C CTG TTC C(AT)A CTG AAG	

3.5.2 Multilocus sequence typing (MLST)

C. jejuni isolates with the identical *flaA* SVR allele number that were originated from the same production cycle were selected for further genotyping using the MLST

protocol as described by Dingle et al. (2001) . Seven house-keeping genes including *asp*A (aspartase A), *gln*A (glutamine synthetase), *glt*A (citrate syntase), *gly*A (serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase) and *unc*A (ATP synthase **α** subunit) genes were amplified by PCR using the primers shown in Table 4. Each gene was amplified in 25 µl PCR reaction containing 5 µl of DNA template, 10 pmol of of the gene-specific primers, 1xPCR buffer, 1.5 mM of Mg^{2+}, 0.4 mM of dNTP and 1.25 U of KapaTaq DNA polymerase (Kapabiosystem, Boston, MA, USA). The thermal cycling was denaturation at 94°C for 2 minutes, annealing at 50°C for 1 minute and extension at 72°C for 1 minute for a total of 35 cycles. The size of the amplified fragments was visualized by gel electrophoresis and the PCR amplification products were purified using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel Duren, Germany). The purified products were submitted for sequencing at 1st BASE Pte Ltd. (Gemini Singapore Science ParkII, Singapore). The allele number and sequence type of the isolates were identified by submitting the sequences of the genes into the *C. jejuni* MLST database (http://pubmlst.org/campylobacter/MLST).

Table 4. Oligonucleotide primers for *Campylobacter* MLST

Locus	function	Primer sequences		Amplicon size (bp)
		Forward primer	Reverse primer	
aspA	Amplification	5'-AGT ACT AAT GAT GCT TAT CC-3	5'-ATT TCA TCA ATT TGT TCT TTG C-3'	899
	Sequencing	5'-CCA ACT GCA AGA TGC TGT ACC-3'	5'-TTA ATT TGC GGT AAT ACC ATC-3'	
glnA	Amplification	5'-TAG GAA CTT GGC ATC ATA TTA CC-3'	5'-TTG GAC GAG CTT CTA CTG GC-3'	1,262
	Sequencing	5'-CAT GCA ATC AAT GAA GAA AC-3'	5'-TTC CAT AAG CTC ATA TGA AC-3'	
gltA	Amplification	5'-GGG CTT GAC TTC TAC AGC TAC TTG-3'	5'-CCA AAT AAA GTT GTC TTG GAC GG-3'	1,012
	Sequencing	5'-GTG GCT ATC CTA TAG AGT GGC-3'	5'-CCA AAG CGC ACC AAT ACC TG-3'	
glyA	Amplification	5'-GAG TTA GAG CGT CAA TGT GAA GG-3'	5'-AAA CCT CTG GCA GTA AGG GC-3'	816
	Sequencing	5'-AGC TAA TCA AGG TGT TTA TGC GG-3'	5'-AGG TGA TTA TCC GTT CCA TCG C-3'	
pgm	Amplification	5'-TAC TAA TAA TAT CTT AGT AGG-3'	5'-CAC AAC ATT TTT CAT TTC TTT TTC-3'	1,150
	Sequencing	5'-GT TTT AGA TGT GGC TCA TG-3'	5'-TTC AGA ATA GCG AAA TAA GG-3'	
tkt	Amplification	5'-GCA AAC TCA GGA CAC CCA GG-3'	5'-AAA GCA TTG TTA ATG GCT GC-3'	1,102
	Sequencing	5'-GCT TAG CAG ATA TTT TAA GTG-3'	5'-ACT TCT TCA CCC AAA GGT GCG-3'	
uncA	Amplification	5'-ATG GAC TTA AGA ATA TTA TGG C-3'	5'-GCT AAG CGG AGA ATA AGG TGG-3'	1,120
	Sequencing	5'-TGT TGC AAT TGG TCA AAA GC-3'	5'-TGC CTC ATC TAA ATC ACT AGC-3'	

3.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Campylobacter* isolates was examined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2008). According to the CLSI guideline and the history of antimicrobial usage in the farm, five antimicrobial agents representing different antimicrobial groups, i.e. ampicillin, ciprofloxacin, erythromycin, gentamicin and tetracycline were tested in this study. All antimicrobial agents were purchased from Sigma Aldrich (Sigma, MO).The tested range of each antimicrobial agent was as follows: ampicillin (AMP, 0.008 to 512 µg/ml); ciprofloxacin (CIP, 0.008 to 512 µg/ml); erythromycin (ERY, 0.06 to 512 µg/ml); gentamicin (GEN, 0.06 to 128 µg/ml) and tetracycline (TET, 0.06 to 512 µg/ml). Two hundred and fourteen *Campylobacter* isolates from broilers and environments frozen at -80˚C were subcultured onto blood agar and incubated under microaerobic atmosphere at 42°C for 42 hours. After re-subcultured, the single colonies were suspended in 0.85% saline solution. Each *Campylobacter* suspension was adjusted to the turbidity equivalent to 0.5 McFarland standards (approximately 10^4 CFU per ml). These bacterial suspensions were inoculated onto a series of agar plates containing a two-fold dilution series of each antimicrobial and supplemented with 5% defribrinated sheep blood using a multipoint inoculator. The inoculated plates were allowed to dry at room temperature and then incubated under a microaerobic atmosphere at 42°C for 24 hours. After incubation, the minimal inhibitory concentration (MIC) was recorded. The resistance breakpoints for ciprofloxacin, erythromycin, gentamicin and tetracycline used by the U.S. National Antimicrobial Resistance Monitoring System (NARMS, 2011) and the resistance breakpoint for ampicillin used by the CLSI established guideline

(CLSI, 2008) were used as *Campylobacter* resistance breakpoints in the present study. The MIC breakpoints of each antimicrobial agent and the quality control range of *C. jejuni* 33560 used in this study are shown in Table 5. *C. jejuni* isolate that was resistant to three or more groups of antimicrobial agent was determined as multidrug resistance.

Table 5. The MIC quality control ranges and MIC breakpoints of antimicrobial agents used for antimicrobial susceptibility testing

Antimicrobial agents	MIC quality control range of *C. jejuni* ATCC 33560 (µg/ml)	MIC breakpoints (µg/ml)
Ampicillin	N/A	32
Ciprofloxacin	0.06 - 0.5	4
Erythromycin	1 - 4	32
Gentamicin	0.5 - 4	8
Tetracycline	1 - 4	16

CHAPTER IV

RESULTS

4.1 Occurrence of *Campylobacter* spp.

A total of 1,859 broiler and environmental samples were collected from three production cycles of two broiler farms (Farm A and B) located in the eastern part of Thailand. From the six broiler flocks sampling in this study, the broiler samples were positive in 4 out of 6 flocks, whereas the environmental samples were positive for all 6 flocks. From the samples tested in the present study, 338 samples (18.18%) were *Campylobacter* positive. Among the 338 *Campylobacter* isolates, 330 isolates were *C. jejuni* and 8 isolates were *C. coli*. Although all broiler samples were found to be positive with only *C. jejuni*, environmental samples were found to be positive for *C. jejuni* and *C. coli*. In farm A, 22.77% of the broilers and environmental samples were *Campylobacter* positive, while 14.36% of the samples collected from farm B were positive for *Campylobacter*. The occurrence of *Campylobacter* in both broilers and environmental samples in three production cycles of farm A was shown in Table 6. In the first production cycle (A1), 30% of the broilers became colonized with *Campylobacter* at week 2 and increased during the rearing period until reach 90% at week 5 and 100% at slaughter. However, in environmental samples, the positive samples were detected later than the broiler samples at week 3 (22%), and decreased to 5.56% at week 4, and no positive samples were detected at week 5. In contrast to the first flock, the environmental samples of the second flock (A2) were positive for *Campylobacter* before the broiler samples. Approximately 57% of the broilers in A2

were colonized with *Campylobacter* at week 3, increased to 80% at week 4, and 100% at week 5, but decreased to approximately 87% at slaughter. For the environmental samples, the positive samples were first detected at week 2 and remained positive until the end of the rearing period at week 5. In the third flock (A3), all broiler and environmental samples except 2 samples collected from farm environment in week 5 were negative for *Campylobacter.*

For farm B, the occurrence of *Campylobacter* in both broilers and environmental samples in three production cycles was shown in Table 7. Approximately 27% of the broilers in the first flock (B1) were *Campylobacter* positive at week 2. The colonization rate increased to 50% at week 3, but decreased to 3.33% at week 4. At week 5, the positive rate increased to 30% and then 100% at slaughter. Although the environmental samples of the B1 flock became positive at the same time as the broiler positive samples (at week 2), the positive rate decreased at week 3 and then negative throughout the rest of the rearing period. For the second flock (B2), only the environmental samples collected at week 2 and week 3 were *Campylobacter* positive, while the rest of environmental samples and all samples collected from broilers were negative for this organism. In the third flock (B3), all broilers were colonized with *Campylobacter* at week 4. Although the occurrence of *Campylobacter* decreased to approximately 73% at week 5, the colonization rate increased to 100% at slaughter. For the environmental samples, the samples became positive for *Campylobacter* at the same time as the broilers, at week 4.

The summary of *Campylobacter* positive environmental samples during the examined rearing periods from both farms were shown in Table 8. The environmental samples that were found positive were boot swabs, water and flies. Other environmental samples such as litter, chick trays, boots, automatic pans and darkling beetle were all negative. Notably, in contrast to the broiler samples in which only *C. jejuni* were found, *C. jejuni* and *C. coli* were isolated from environmental samples (water samples).

Table 6. Detection rate of *Campylobacter* in farm A

Age (week)	No. of positive samples / No. of samples tested (%)					
	1st production cycle (A1)		2nd production cycle (A2)		3rd production cycle (A3)	
	Broiler	Environment	Broiler	Environment	Broiler	Environment
Before chick arrival	N/A[a]	0/28 (0)	N/A	0/12 (0)	N/A	0/14 (0)
Arrival day	0/10 (0)	0/15 (0)	0/10 (0)	0/18 (0)	0/10 (0)	0/15 (0)
1	0/30 (0)	0/16 (0)	0/30 (0)	0/18 (0)	0/30 (0)	0/18 (0)
2	9/30 (30)	0/19 (0)	0/30 (0)	2/20 (10)	0/30 (0)	0/19 (0)
3	14/30 (46.67)	4/18 (22.22)	17/30 (56.67)	3/19 (15.8)	0/30 (0)	0/18 (0)
4	28/30 (93.33)	1/18 (5.56)	24/30 (80)	2/18 (11.11)	0/30 (0)	0/17 (0)
5	27/30 (90)	0/19 (0)	30/30 (100)	5/18 (27.78)	0/30 (0)	2/19 (10.53)
Slaughter	10/10 (100)	N/A	26 /30 (86.67)	N/A	0/30 (0)	N/A
Total	**88/170 (51.76)**	**5/133 (3.76)**	**97/190 (51.05)**	**12/123 (9.76)**	**0/190 (0)**	**2/120 (1.67)**

[a] N/A Not applicable

Table 7. Detection rate of *Campylobacter* in farm B

Age (week)	No. of positive samples / No. of samples tested (%)					
	1st production cycle (B1)		2nd production cycle (B2)		3rd production cycle (B3)	
	Broiler	Environment	Broiler	Environment	Broiler	Environment
Before chick arrival	N/A[a]	0/29 (0)	N/A	0/14 (0)	N/A	0/14 (0)
Arrival day	0/10 (0)	0/15 (0)	0/10 (0)	0/17 (0)	0/28 (0)	0/17 (0)
1	0/30 (0)	0/18 (0)	0/30 (0)	0/18 (0)	0/30 (0)	0/19 (0)
2	8/30 (26.67)	2/19 (10.53)	0/30 (0)	2/18 (11.11)	0/30 (0)	0/19 (0)
3	15/30 (50)	1/17 (5.88)	0/30 (0)	2/20 (10)	0/30 (0)	0/17 (0)
4	1/30 (3.33)	0/19 (0)	0/30 (0)	0/19 (0)	30/30 (100)	2/19 (10.53)
5	9/30 (30)	0/19 (0)	0/30 (0)	0/18 (0)	22/30 (73.33)	No sample
Slaughter	10/10 (100)	N/A	0/30 (0)	N/A	30/30 (100)	N/A
Total	**43/170 (25.29)**	**3/136 (2.21)**	**0/190 (0)**	**4/124 (3.23)**	**82/208 (39.42)**	**2/105 (1.91)**

[a] N/A Not applicable

Table 8. Summary of *Campylobacter* positive environmental samples during the examined rearing periods in farm A and B

Flock[a]	Type of positive samples[b]				
	Week 1	Week 2	Week 3	Week 4	Week 5
A1			BS0, BS2, BS3, Flies	BS3	
A2		WN1[c], NW2[c]	WN[c], BS2, BS3	BS1, BS3	WN[c], BS0, BS2, BS3, Flies
A3					WN[c], Flies
B1		BS2, WN	WN		
B2		WN1[c], WN2	WI[c], WN[c]		
B3				WN1, WN2	

[a] A1, farm A first production cycle; A2, farm A second production cycle; A3, farm A third production cycle; B1, farm B first production cycle; B2, farm B second production cycle; B3, farm B third production cycle

[b] BS0, boot swab on pathway from office to the broiler house; BS1, boot swab from anteroom; BS2, boot swab inside the broiler house; BS3, boot swab around the broiler house (outside); WN, water from nipple; WI, water inlet

[c] *Campylobater coli* positive samples

4.2 Genotypes of *Campylobacter jejuni* isolated from broiler farms

Among the 338 *Campylobacter* isolates, at least 40% of the isolates from each positive sample type were selected for genetic characterization. A total of 207 isolates were chosen and genotyped by *flaA* SVR sequencing technique. Additionally, 40 isolates were further characterized by MLST technique. The *flaA* SVR genotypes of *C. jejuni* isolated from Farm A and Farm B were shown in Table 9, 10 and 11, respectively.

In the first production cycle of farm A (A1), the most common genotype found in this flock was *flaA* SVR allele number 287, which was found in positive broilers from week 2 to week 5, and from boot swabs around the broiler house and flies in week 3 and week 4. All *C. jejuni* with *flaA* SVR allele number 287 from broilers and from flies were identified as MLST sequence type 5247 (ST-5247), whereas the isolate from boot swab in week 3 belonged to a different sequence type (ST-6995). The diversity of *C. jejuni* found in broilers was increased with broiler age i.e., from two genotypes in week 2 to three genotypes in week 4 and to seven genotypes in week 5. The second most prevalence genotype in the broilers of this flock was *flaA* SVR allele number 253. This genotype was found and persisted in the broilers from week 3 until slaughtered. Three isolates from this genotype were selected for MLST analysis and these isolates belonged to ST-1919. Interestingly, *flaA* SVR allele number 255 was found in boot swab on pathway from office to broiler house in week 3, then one week later, the same genotype was found in the broiler samples. Notably, one common *flaA* SVR genotype (genotype 45) was found in flock A1 (week 5) and the consecutive flock (A2) suggesting the possible carry-over of this *Campylobacter* genotype between the broiler flocks. In the second flock (A2), only one genotype, *flaA* SVR allele number 45 was found in both environments and broilers from week 3 to week 5 and in the broilers at slaughter.

The clonal identity of the *C. jejuni* isolates with *flaA* SVR genotype 45 from flock A1 and A2 was confirmed using MLST analysis. Notably, the *flaA* SVR allele number 45 isolates from flock A1 were ST-1919, but the same *flaA* SVR allele number 45 isolates from flock A2 were ST-2275. In contrast to A1, the allelic diversity of *C. jejuni* in flock A2 did not increase during the rearing period. For the third flock (A3), although *C. jejuni* (*flaA* SVR allele number 54) was found in fly sample collected in week 5, all broiler samples in this flock were *Campylobacter* negative (Table 9).

For farm B, the *flaA* SVR allele number 783 was the only genotype found in the first flock (B1) and the third flock (B3). In both flocks, the broiler and environmental samples were positive at the same sampling time. In flock B1, the majority of the *flaA* SVR genotype 783 isolates from broiler and environmental samples were identified by MLST as ST-1232. Only two isolates from week 5 and at slaughter were ST-5213. In the second flock (B2), although no *Campylobacter* was found in the broiler samples, *C. jejuni* isolate with *flaA* SVR genotype 45 (ST-2275) was isolated from nipple water in week 2. In flock B3, both broilers and nipple water became positive for *C. jejuni* in week 4 with a single genotype *flaA* SVR allele number 783 (ST-1232) (Table 10).

Table 9. Genotypes of *Campylobacter jejuni* isolated from broilers and environments during the rearing periods in farm A

Flocks[a]	Age (wk.)	*flaA* SVR genotype (n)	
		Broiler	Environment [c]
A1	1	N/A[b]	
	2	287 (5), 57 (2)	
	3	287 (11), 253 (3)	287(2), [BS3, flies] 255 (1),[BS0] 1239 (1) [BS2]
	4	287 (11), 253 (1), 255 (1)	287 (1)[BS3]
	5	18 (1), 45 (3), 253 (9), 255 (2), 287 (1), 854 (1), 1527 (2)	
	Slaughter	1527 (1), 253 (1), 783 (2)	
A2	1	N/A	
	2	N/A	
	3	45 (10)	45 (2) [BS2, BS3]
	4	45 (13)	45(2) [BS1, BS3]
	5	45 (16)	45 (3) [BS0, BS2, Flies]
	Slaughter	45 (16)	
A3	1	N/A	
	2	N/A	
	3	N/A	
	4	N/A	
	5	N/A	54 (1) [Flies]
	Slaughter	N/A	

[a] A1, farm A first production cycle; A2, farm A second production cycle; A3, farm A third production cycle

[b] N/A Not applicable

[C] BS0, boot swab on pathway from office to the broiler house; BS1, boot swab from anteroom; BS2, boot swab inside the broiler house; BS3, boot swab around the broiler house

Table 10. Genotypes of *Campylobacter jejuni* isolated from broilers and environments during the rearing periods in farm B

Flocks[a]	Age (wk.)	*flaA* SVR genotype (n)	
		Broiler	Environment [c]
B1	1	N/A[b]	
	2	783 (5)	783 (2) [BS2, NW]
	3	783 (13)	783 (1) [NW]
	4	783 (1)	
	5	783 (8)	
	Slaughter	783 (10)	
B2	1	N/A	
	2	N/A	45 (1) [NW]
	3	N/A	
	4	N/A	
	5	N/A	
	Slaughter	N/A	
B3	1	N/A	
	2	N/A	
	3	N/A	
	4	783 (9)	783 (2) [NW]
	5	783 (15)	
	Slaughter	783 (14)	

[a] B1, farm B first production cycle; B2, farm B second production cycle; B3, farm B third production cycle

[b] N/A Not applicable

[C] BS2, boot swab inside the broiler house; NW, water from nipple

Table 11. Comparison of *Campylobacter jejuni* genotypes characterized by both flaA SVR sequencing and MLST techniques

Flocks[a]	Genotype		
	flaA SVR	MLST	
		Sequence type	Clonal complex
A1	287	5247	ST 353 complex
		1919	ST 52 complex
		6995	ST 682 complex
	253	1919	ST 52 complex
	854	1919	ST 52 complex
	45	1919	ST 52 complex
A2	45	2275	ST 52 complex
B1	783	1232, 5213	ST 353 complex
B2	45	2275	ST 52 complex
B3	783	1232	ST 353 complex

[a] A1, farm A first production cycle; A2, farm A second production cycle; B1, farm B first production cycle; B2, farm B second production cycle; B3, farm B third production cycle

4.3 Antimicrobial resistance of *Campylobacter*

Distribution of antimicrobial resistance traits in *Campylobacter* isolates from both broilers and environmental samples from two conventional broiler farms (6 flocks) was shown in Table 12. Most *Campylobacter* isolates were resistant to ciprofloxacin (94.86%), followed by tetracycline (88.78%) and ampicillin (56.07%). On the other hand, the low rates of resistance were found for erythromycin (7.01%) and gentamicin (6.07%). Unlike *C. jejuni*, 100% of *C. coli* isolates in this study were resistant to all antimicrobial agents tested. The resistance rate of *Campylobacter* separated by location was shown in Figure 1. Approximately 94% and 97% of *Campylobacter* isolates from farm A and farm B were resistant to ciprofloxacin, respectively. Similar to ciprofloxacin resistance, a high prevalence of tetracycline resistance was observed in farm A (83.33%) and farm B (100%). Interestingly, although 74.31% of *Campylobacter* isolates from farm A were resistant to ampicillin, only 18.57% of the isolates from farm B were resistant to this antimicrobial agent. In terms of antimicrobial resistance patterns, the most common resistance pattern observed in farm A was CIP-TET-AMP resistance (63.57%), followed by CIP-TET resistance (13.95%), while the major resistance pattern among *C. jejuni* isolated from farm B was CIP-TET resistance (84.37%) (Table13).

In this study, we found that 110 out of 214 (51.4%) *Campylobacter* isolates from broilers and environments were resistant to three or more classes of antimicrobial agents. The occurrence of multidrug-resistant *Campylobacter* in farm A was higher than farm B. In farm A, the prevalence of multidrug-resistant *Campylobacter* was 67.36%, while the prevalence of multidrug-resistant *Campylobacter* in farm B was 18.57%. The most common multidrug resistance pattern observed in this study was CIP-TET-AMP

resistance (85.45%). Multidrug resistance patterns of *Campylobacter* isolated from broilers and environments in farm A and B were shown in Table 14.

Table 12. MICs distribution and antimicrobial resistance rates of *Campylobacter* isolated from broilers and environmental samples in farm A and B

Antimicrobial agents	*Campylobacter*[a]	No. of isolates inhibited at the following MIC (µg/ml)[b]															No. (%) Resistance
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512	
Ciprofloxacin	*C. jejuni*		3			4	4	24	47	111	8	2	3	1			195 (94.66)
	C. coli												4	4			8 (100)
Total			**3**			**4**	**4**	**24**	**47**	**111**	**8**	**2**	**7**	**5**			**203 (94.86)**
Erythromycin	*C. jejuni*	1	3	21	39	95	38	1	1			1	1	1	3	1	7 (3.4)
	C. coli											1		7			8 (100)
Total		**1**	**3**	**21**	**39**	**95**	**38**	**1**	**1**			**2**	**1**	**8**	**3**	**1**	**15 (7.01)**
Gentamicin	*C. jejuni*	3	35	78	74	11				4	1						5 (2.43)
	C. coli									1	7						8 (100)
Total		**3**	**35**	**78**	**74**	**11**				**5**	**8**						**13 (6.07)**
Tetracycline	*C. jejuni*					2	2	6	14	55	59	40	16	9	3		182 (88.35)
	C. coli														7	1	8 (100)
Total						**2**	**2**	**6**	**14**	**55**	**59**	**40**	**16**	**9**	**10**	**1**	**190 (88.78)**
Ampicillin	*C. jejuni*				2	5	11	48	19	9	35	64	11	1		1	112 (54.36)
	C. coli															8	8 (100)
Total					**2**	**5**	**11**	**48**	**19**	**9**	**35**	**64**	**11**	**1**		**9**	**120 (56.07)**

[a] *C. jejuni* (n = 206) and *C. coli* (n = 8), [b] The grey shading indicates resistant isolates.

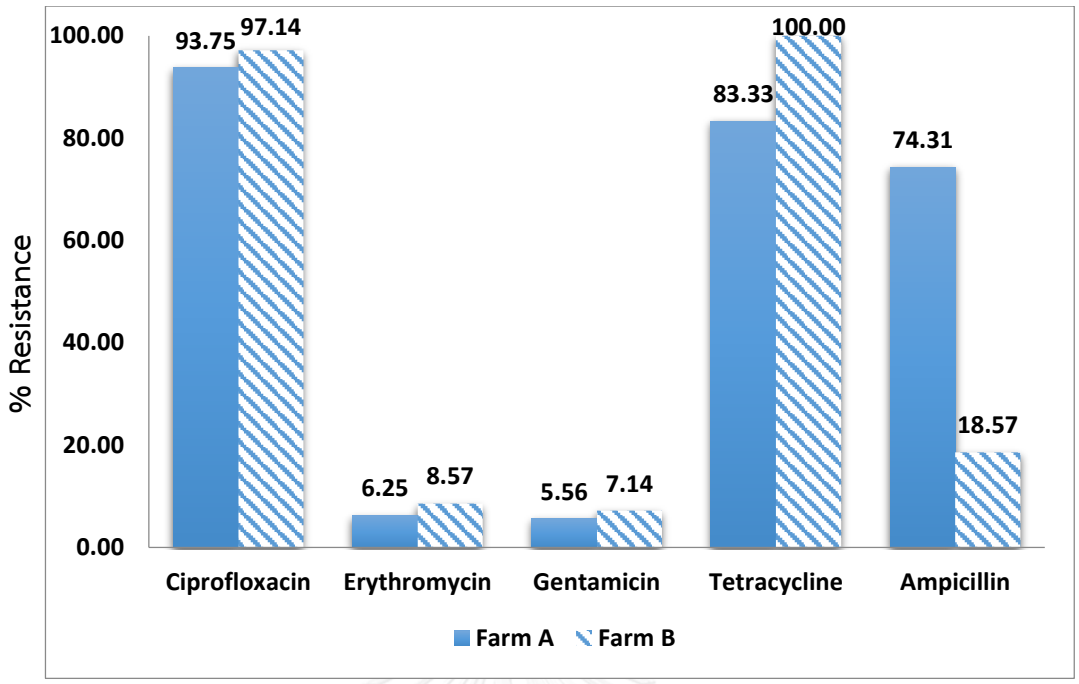

Figure 1. Comparison of antimicrobial resistance of *Campylobacter* spp. isolated from farm A and B

Table 13. Antimicrobial resistance patterns of *Campylobacter jejuni* isolated from cloaca and caeca of broilers in farm A and B

Age (week)	Farm A (n=129)		Farm B (n=64)	
	Antimicrobial resistance pattern[b] (n)		Antimicrobial resistance pattern (n)	
	1st production cycle	2nd production cycle	1st production cycle	3rd production cycle
1	N/A[a]	N/A	N/A	N/A
2	CIP-TET (5) CIP (1)	N/A	CIP-TET (4)	N/A
3	CIP-TET-AMP (4) CIP (4) CIP-TET (3) Pan-susceptible (1)	CIP-TET-AMP (5) CIP-ERY-TET-AMP (1) CIP-ERY-GEN-TET-AMP(1) CIP-AMP (1) TET-AMP (1)	CIP-TET (4) CIP-TET-AMP (3) CIP-ERY-TET-AMP(1) TET (1)	N/A
4	CIP-TET (9) CIP (6) CIP-TET-AMP (4) Pan-susceptible (1)	CIP-TET-AMP (15) TET-AMP (2) AMP (2) CIP (1) CIP-AMP (1)	Not available	CIP-TET (10)
5	CIP-TET-AMP (11) CIP (1)	CIP-TET-AMP (21) CIP-AMP (2) TET-AMP (2)	CIP-TET (5) CIP-TET-AMP (1)	CIP-TET (11) CIP-TET-AMP (1) CIP-ERY-GEN-TET-AMP (1)
Caeca	CIP-TET (1) CIP-TET-AMP (1)	CIP-TET-AMP (21) CIP-AMP (1)	CIP-TET (7) CIP-TET-AMP (1) TET (1)	CIP-TET (13)

[a] N/A Not applicable

[b] AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline

Table 14. Multidrug resistance patterns of *Campylobacter* strains isolated from broilers and environments in farm A and B

Flock[a]	Resistance pattern[b]	No. (%) of multidrug-resistant strains	
		C. jejuni	*C. coli*
A1	CIP-TET-AMP	21/21 (100)	0
A2	CIP-TET-AMP	65/70 (92.86)	4/4 (100)
	CIP-ERY-TET-AMP	2/70 (2.86)	0
	CIP-GEN-TET	1/70 (1.43)	0
	CIP-ERY-GEN-TET	1/70 (1.43)	0
	CIP-ERY-GEN-TET-AMP	1/70 (1.43)	0
A3	CIP-TET-AMP	1/1 (100)	0
	CIP-ERY-GEN-TET-AMP	0	1/1 (100)
B1	CIP-TET-AMP	5/6 (83.33)	0
	CIP-ERY-TET-AMP	1/6 (16.67)	0
B2	CIP-TET-AMP	1/1 (100)	0
	CIP-ERY-GEN-TET-AMP	0	3/3 (100)
B3	CIP-TET-AMP	1/3 (33.33)	0
	CIP-ERY-GEN-TET-AMP	2/3 (66.67)	0

[a] A1, farm A first production cycle; A2, farm A second production cycle; A3, farm A third production cycle; B1, farm B first production cycle; B2, farm B second production cycle; B3, farm B third production cycle

[b] AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline

CHAPTER V
DISCUSSION

This study provides information on occurrence, genetic profiles and antimicrobial resistance patterns of *Campylobacter* isolated from three consecutive broiler flocks of two commercial broiler farms in the eastern part of Thailand. Approximately 28% of broiler samples were *Campylobacter* positive, while 3.78% of environmental samples were contaminated with this organism. The explanation for the low detection rate in the environments is probably due to the stresses of the organism which reduced their viability and recovery rate in laboratory media as suggested by Ridley et al. (2008). The broilers became colonized with *Campylobacter* at 2-4 weeks of age and the *Campylobacter* spread rapidly amongst broilers within the flock and persisted until they were sent to slaughterhouse. Observations that *Campylobacter jejuni* colonization is usually absent in broilers less than 2-3 weeks of age were reported from several investigators (Evans and Sayers, 2000; Shreeve et al., 2000). In addition, Evan and Sayers (2000) suggested that the risk of infection was increased with broilers age. The studies of Sahin et al. (2001) and Sahin et al. (2002) showed that the age-related effect may result from maternal antibodies found in young chicks.

In all flocks studied, *Campylobacter* was not detected after cleaning and disinfection in the broiler house. Environmental samples around the broiler houses were also *Campylobacter* negative. This suggested that the cleaning and disinfection measures used in these broiler farms were effective and able to reduce the contamination of *Campylobacter* in the farm environments.

For farm A, in the first flock (A1), *C. jejuni* was first detected in broilers in week 2 after that, in week 3 *C. jejuni* was found in the environmental samples in boot swabs on pathway from office to broiler house, inside and outside broiler house and house flies. *flaA* SVR genotyping revealed the same genotype, *flaA* 287, from the broiler isolates and isolates collected from boot swabs outside the broiler house and house flies. MLST genotyping then confirmed that the isolate from flies was probably come from broilers since both fly and broiler isolates exhibited the same genotype (ST-5247). This result suggested that *Campylobacter* from broilers can persist in flies and can be later disseminated throughout the broiler flock. Possible role of flies as vehicles for transmitting *Campylobacter* among chickens were previously reported under experimental conditions (Shane et al., 1985) as well as in the broiler farm in which identical PFGE patterns among the fly isolates and the broiler isolates were found (Hald et al., 2004).

For the second flock of farm A (A2), we found *C. jejuni* in both broilers and boot swabs inside and outside the broiler house in week 3. Since both broilers and environments were positive at the same sampling time, we cannot conclude the direction of the *Campylobacter* transmission in this flock. In addition to *C. jejuni*, *C. coli* was also found in this flock. Although *C. coli* was first isolated from nipple water in week 2, this *C. coli* species was not detected in any of the broiler samples throughout the rearing period. Hence, water from nipple was not likely to be the source of *Campylobacter* infection in this broiler flock.

The potential for carry-over of *Campylobacter* to subsequent broiler flock reared in the same broiler house was investigated by *flaA* SVR sequencing and MLST technique. From *flaA* SVR sequencing, a high genetic diversity of *C. jejuni* in flock A1

with at least 10 *flaA* allele types was found. However, only one *flaA* SVR allele type, allele 45, was found in subsequent flock A2. This finding agrees with the study of Alter et al. (2011) that whereas the large number of *C. jejuni* were found during sampling period, only one genotype survived in the consecutive flock. A high diversity of *C. jejuni* in broilers was also reported by the studies of Petersen et al. (2001) and Hook et al. (2005). These studies suggested that the different clones of *C. jejuni* may originate from a variety of environments such as poor hygiene barriers around the broiler house and disturbance of insects and pest. In this study, *Campylobacter* may survive in environment such as house flies and other mammals, which acted as reservoirs during cleaning and disinfection of the broiler house. Then, those pests may return to the house in flock A2 and disseminated *Campylobacter* to the broilers again.

For farm B, in both positive flocks, B1 and B3, the broilers and environmental samples were found to be *Campylobacter* positive at the same week. Therefore, the direction of transmission cannot be concluded for these flocks. For possible carry-over of *Campylobacter* between consecutive flocks in farm B, a single *flaA* genotype, *flaA* SVR allele 783, was found in both B1 and B3 flocks. More detail analysis by MLST revealed that majority of the *flaA* 783 isolates from flock B1 belong to ST-1232 and the same genotype was also found in flock B3. This result suggested that this genotype may survive or persist in the farm environment and could return to colonize the broilers reared in the same house later.

In this study, some *C. jejuni* isolates were characterized by both *flaA* SVR and MLST techniques. Occasionally, *C. jejuni* isolates that had similar sequence type (ST), but different *flaA* SVR allele numbers were found, i.e. ST-1919 belonged to *flaA* SVR

allele number 45, 253 and 854. On the other hand, some *C. jejuni* isolates that had different STs, but similar *flaA* SVR allele number were also observed in the present study, i.e. *flaA* SVR allele number 45 isolated from flock A1 and A2 belonged to ST-1919 and ST-2275, respectively. This finding suggests that the combination of two molecular typing methods should be used for genetic characterization of *Campylobacter* in the epidemiological studies (Price et al., 2006).

The most common antimicrobial resistance rate observed in this study was ciprofloxacin resistance (94.86%), followed by tetracycline resistance (88.78%). Although this finding is in agreement with the previous studies (Mazi et al., 2008; Bardon et al., 2009), which also reported the high prevalence of ciprofloxacin and tetracycline resistance among *Campylobacter* isolates from chickens, our results are quite different from the results previously reported in Thailand where ciprofloxacin and tetracycline resistance in *Campylobacter* strains isolated from broiler chickens was 54% and 3%, respectively (Padungtod et al., 2006). The high rate of fluoroquinolone resistance in the present study is likely due to the use of this class of antimicrobials in previous broiler flocks for therapeutic purposes. It was previously shown that *Campylobacter* could rapidly develop resistance to fluoroquinolones after the birds were treated with enrofloxacin for 5 days and these fluoroquinolones-resistant *Campylobacter* isolates could persist over a long period after the treatment (McDermott et al., 2002). Likewise, the study of Aarestrup et al. (1999) also revealed that the occurrence of fluoroquinolone-resistant *Campylobacter* in poultry increased after the antimicrobials were introduced for veterinary uses. Fluoroquinolone-resistant strains were able to persist in the farm even though the drugs were no longer used for several years (Pedersen and Wedderkopp, 2003; Price et al., 2007).

In this study, tetracycline-resistant *Campylobacter* strains were found up to 88.78%. Although tetracycline has never been used in the broiler farms from which the samples were collected, the high prevalence of tetracycline-resistant *Campylobacter* strain was noticed in this study. Similar to our study, the previous studies showed that tetracycline resistance was common in *Campylobacter* isolates from conventional broiler farms (Cui et al., 2005; Luangtongkum et al., 2006). For organic broiler farms, approximately 60% of *Campylobacter* isolates were found to be resistant to tetracycline even though no antimicrobial agents including tetracycline were used in this operation system (Luangtongkum et al., 2006). The present study demonstrated that tetracycline-resistant *Campylobacter* can transmit and persist in the absence of antimicrobial selection pressure. The possible explanation of tetracycline resistance in these broiler farms may be the presence of plasmid-borne *tet* (O) gene that could be co-selected by other antibiotics and horizontally circulated among *Campylobacter* populations (Avrain et al., 2004).

In the present study, the resistance rates to erythromycin (7.01%) and gentamicin (6.07%) were low, which is similar to the previous study in Thailand showing that 5.8% and 0% of *Campylobacter* isolates from chickens were resistant to erythromycin and gentamicin, respectively (Padungtod et al., 2006). Although erythromycin was not used in broiler farms in the present study, tylosin and lincomycin were given to the broilers for the treatment of mycoplasma infection in the previous flocks (personnel communication). It was previously reported that the use of lincosamides could promote cross resistance to macrolides in *Campylobacter* isolates from broilers (Belanger and Shryock, 2007). Therefore, the low erythromycin resistance rate observed in this study is likely due to the previous use of macrolides and

lincosamides in these broiler farms. For gentamicin, the previous study of Chokboonmongkol et al. (2013) reported that no gentamicin resistance was observed in *Campylobacter* isolates from broilers. This result is quite similar to our finding, which revealed the low prevalence of gentamicin-resistant *Campylobacter*. Even though gentamicin was commonly used in broiler flocks, it was given to the birds at one day old to prevent the early mortality due to *E. coli* infection (Luangtongkum et al., 2006). Since *Campylobacter* was not found in day-old chicks and since this antimicrobial agent was given to the birds by subcutaneous injection, it is not surprising that the use of gentamicin had little impact on the development of gentamicin resistance in *Campylobacter.*

In Thailand, the study of Padungtod et al. (2006) reported that no ampicillin resistance was observed in *Campylobacter* isolates from chickens, while Chokboonmongkol et al. (2013) showed that 31.2% of *Campylobacter* isolates from broilers were resistant to ampicillin. Likewise, 23% of *C. jejuni* isolates from broilers in France were resistant to this antimicrobial agent (Avrain et al., 2003). In the present study, approximately 54% of *Campylobacter* isolates were resistant to ampicillin. This discrepancy is likely due to the differences in antibiotics use in broiler farms and geographical location. For these conventional broiler farms, amoxicillin was commonly used to relieve the symptoms of vaccination reaction via drinking water after the broilers were vaccinated with the combined vaccine against Newcastle disease and infectious bronchitis at the age around 14 days (base on the direct interviews with the farmers). Hence, this practice could encourage the development of ampicillin resistance in bacteria including *Campylobacter* which was commonly found in poultry intestinal tract.

Compared to *C. jejuni*, all *C. coli* isolated from environmental samples in the present study were resistant to all antimicrobial agents tested. This finding is in agreement with previous studies which also reported that *C. coli* from poultry were more frequently resistant to multiple antibiotics than *C. jejuni* (D'Lima C et al., 2007; Kim et al., 2008). Similarly, a study in Japan revealed that the prevalence of resistance to aminoglycosides, macrolides, tetracyclines and quinolones in *C. coli* from broilers was higher than that in *C. jejuni* (Ishihara et al., 2004). In conventional broiler flocks, antimicrobial agents were frequently used for treatment, prevention and control of diseases. The routine practice of antimicrobial usage in conventional broiler production may influence the development of antimicrobial resistance in commensal and pathogenic bacteria including *Campylobacter* as shown in the present study.

Conclusion and Suggestion

This study provides the information on the occurrence and potential carry-over of *Campylobacter* in consecutive broiler flocks and their antibiotic resistance patterns. Our results suggest that certain clones of *Campylobacter* may survive and persist in the environment around the broiler house, then recontaminate the next broiler flocks. In order to effectively control and reduce the occurrence of *Campylobacter* in broiler production, the control measures, such as treatment of water (chlorination), personnel hygiene (change cloths and boots before entering to the broiler house), cleaning and disinfection of broiler house and equipment, use of hygiene barriers (use of concrete or cleanable area around the broiler house), use of foot dip containing disinfectant and improvement of pest control should be enforced.

Furthermore, the high occurrence of antimicrobial resistance in *Campylobacter* from broilers found in the present study emphasizes the importance of prudent use of antibiotics in broiler production. To reduce the occurrence of antimicrobial resistance in foodborne pathogens, antimicrobial usage guidelines should be established for controlling overuse and inappropriate use of antibiotics in food animals.

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APPENDIX

APPENDIX A

Culture media used for *Campylobacter* isolation

1. Exeter enrichment broth

Typical formula	**(mg/litre)**
amphotericin B	2
Cefoperazone	15
Iron sulfate	250
Polymyxin B	2500 IU
Rifampicin	5
Sodium metabisulfite	250
Sodium pyruvate	250
Trimethoprim	10
5% of defibrinated sheep blood	50

2. Nutrient broth no. 2 (CM0067; Oxoid)

Typical Formula	**(gm/litre)**
'Lab-Lemco' Powder	10.0
Peptone	10.0
Sodium chloride	5.0

pH 7.5 ± 0.2 @ 25°C

3. *Campylobacter* blood-free selective agar base (mCCDA) (CM0739; Oxoid)

Typical Formula	**(gm/litre)**
Nutrient Broth No.2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0

Sodium desoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0

pH 7.4 ± 0.2 @ 25°C

4. CCDA selective supplement

Antimicrobial agents	**mg/litre**
Cefoperazone	32
Amphotericin B	10

5. Mueller Hinton Agar (Difco™)

Typical formula	**gm/litre**
Beef Extract Powder	2.0
Acid Digest of Casein	17.5
Starch	1.5
Agar	17.0
5% of defibrinated sheep blood	50

pH 7.3 ± 0.1 @ 25°C

Illustration of *Campylobacter* isolation procedure (Selective enrichment method)

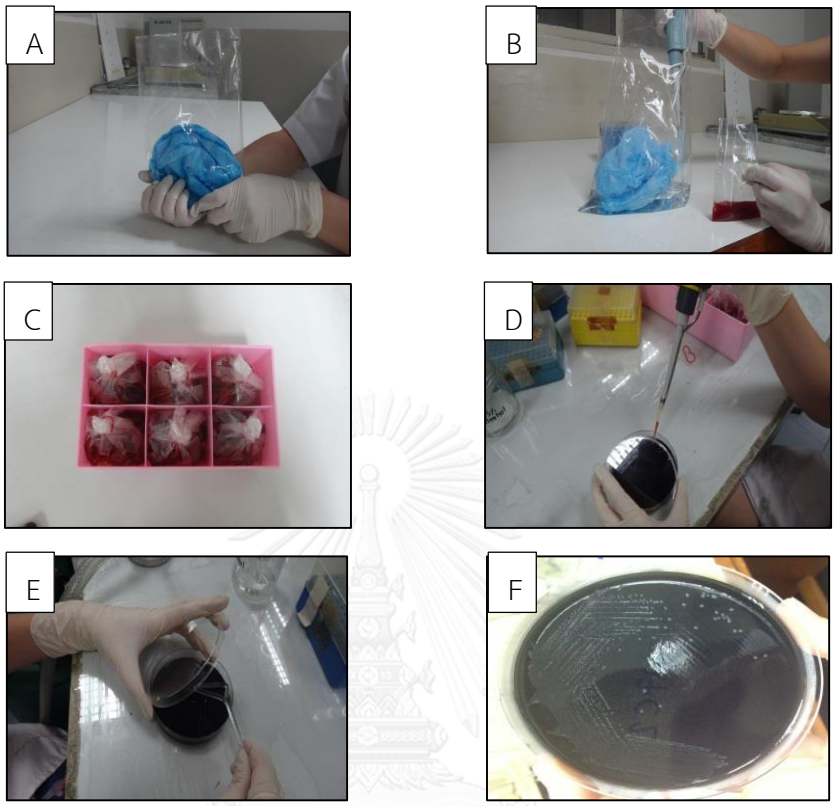

Illustration of *Campylobacter* isolation from environmental samples. A, environmental samples were mixed with NaCl; B, one ml of the mixture was transferred into 9 ml of Exeter enrichment broth; C, after inoculation, the enriched samples were incubated at 42 ℃ for 48 hours; D, one hundred µl of enriched samples were plated onto mCCDA plates; E, the enriched sample was spread and incubated at 42 ℃ for another 48 hours; F, suspected *Campylobacter* colonies on mCCDA plates were examined.

APPENDIX B

Diluents of antimicrobial agents used in this study

Antimicrobial agent	Diluent
Ampicillin	Distilled water
Ciprofloxacin	0.1N HCl and distilled water
Erythromycin	95% ethanol and distilled water
Gentamicin	Distilled water
Tetracycline	Distilled water

APPENDIX C

Table C-1 Genotypes of *Campylobacter* isolated from broilers and environments of farm A

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	*flaA* SVR allele no.	MLST (ST)
1	CSB14d011c1	*C. jejuni*	2	1	Cloacal swab	287	
2	CSB14d02c1	*C. jejuni*	2	1	Cloacal swab	287	
3	CSB14d03c1	*C. jejuni*	2	1	Cloacal swab	287	
4	CSB14d04c1	*C. jejuni*	2	1	Cloacal swab	287	
5	CSB14d05c1	*C. jejuni*	2	1	Cloacal swab	57	
6	CSB14d06c1	*C. jejuni*	2	1	Cloacal swab	287	5247
7	CSB14d08c1	*C. jejuni*	2	1	Cloacal swab	57	
8	CSB21d01c2	*C. jejuni*	3	1	Cloacal swab	287	
9	CSB21d02c2	*C. jejuni*	3	1	Cloacal swab	287	
10	CSB21d03c2	*C. jejuni*	3	1	Cloacal swab	287	
11	CSB21d04c2	*C. jejuni*	3	1	Cloacal swab	287	
12	CSB21d05c2	*C. jejuni*	3	1	Cloacal swab	287	
13	CSB21d06c2	*C. jejuni*	3	1	Cloacal swab	287	
14	CSB21d07c2	*C. jejuni*	3	1	Cloacal swab	253	
15	CSB21d08c2	*C. jejuni*	3	1	Cloacal swab	287	
16	CSB21d09c2	*C. jejuni*	3	1	Cloacal swab	287	5247
17	CSB21d10c2	*C. jejuni*	3	1	Cloacal swab	253	1919
18	CSB21d11c2	*C. jejuni*	3	1	Cloacal swab	287	
19	CSB21d12c2	*C. jejuni*	3	1	Cloacal swab	287	
20	CSB21d13c2	*C. jejuni*	3	1	Cloacal swab	253	1919
21	CSB21d14c2	*C. jejuni*	3	1	Cloacal swab	287	
22	BS0B21dc2	*C. jejuni*	3	1	BS0	255	
23	BS2B21dc2	*C. jejuni*	3	1	BS2	1239	
24	BS3B21dc2	*C. jejuni*	3	1	BS3	287	6995
25	FlyB21dc2	*C. jejuni*	3	1	Flies	287	5247
26	CSB28d02c5	*C. jejuni*	4	1	Cloacal swab	287	
27	CSB28d03c5	*C. jejuni*	4	1	Cloacal swab	287	
28	CSB28d05c5	*C. jejuni*	4	1	Cloacal swab	255	
29	CSB28d06c5	*C. jejuni*	4	1	Cloacal swab	253	1919
30	CSB28d07c5	*C. jejuni*	4	1	Cloacal swab	287	5247

*BS0, boot swab on pathway from office to the broiler house; BS2, boot swab inside the broiler house; BS3, boot swab around the broiler house

Table C-1 Genotypes of *Campylobacter* isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	*flaA* SVR allele no.	MLST (ST)
31	CSB28d08c5	*C. jejuni*	4	1	Cloacal swab	287	
32	CSB28d09c5	*C. jejuni*	4	1	Cloacal swab	287	
33	CSB28d10c5	*C. jejuni*	4	1	Cloacal swab	287	
34	CSB28d11c5	*C. jejuni*	4	1	Cloacal swab	287	
35	CSB28d12c5	*C. jejuni*	4	1	Cloacal swab	287	
36	CSB28d13c5	*C. jejuni*	4	1	Cloacal swab	287	
37	CSB28d14c5	*C. jejuni*	4	1	Cloacal swab	287	
38	CSB28d28c5	*C. jejuni*	4	1	Cloacal swab	287	
39	BS3B28dc5	*C. jejuni*	4	1	BS3	287	
40	CSB32d01c8	*C. jejuni*	5	1	Cloacal swab	253	
41	CSB32d02c8	*C. jejuni*	5	1	Cloacal swab	253	
42	CSB32d03c8	*C. jejuni*	5	1	Cloacal swab	255	
43	CSB32d04c8	*C. jejuni*	5	1	Cloacal swab	253	
44	CSB32d05c8	*C. jejuni*	5	1	Cloacal swab	253	
45	CSB32d06c8	*C. jejuni*	5	1	Cloacal swab	253	
46	CSB32d07c8	*C. jejuni*	5	1	Cloacal swab	255	
47	CSB32d08c8	*C. jejuni*	5	1	Cloacal swab	854	1919
48	CSB32d09c8	*C. jejuni*	5	1	Cloacal swab	287	
49	CSB32d10c8	*C. jejuni*	5	1	Cloacal swab	45	1919
50	CSB32d14c8	*C. jejuni*	5	1	Cloacal swab	253	
51	CSB32d15c8	*C. jejuni*	5	1	Cloacal swab	45	
52	CSB32d16c8	*C. jejuni*	5	1	Cloacal swab	18	
53	CSB32d18c8	*C. jejuni*	5	1	Cloacal swab	253	
54	CSB32d19c8	*C. jejuni*	5	1	Cloacal swab	1527	
55	CSB32d21c8	*C. jejuni*	5	1	Cloacal swab	253	
56	CSB32d22c8	*C. jejuni*	5	1	Cloacal swab	45	1919
57	CSB32d23c8	*C. jejuni*	5	1	Cloacal swab	1527	
58	CSB32d27c8	*C. jejuni*	5	1	Cloacal swab	253	
59	S5040_01C	*C. jejuni*	5	1	Caeca	1527	
60	S5040_04C	*C. jejuni*	5	1	Caeca	253	
61	S5040_05C	*C. jejuni*	5	1	Caeca	783	
62	S5040_10C	*C. jejuni*	5	1	Caeca	783	

*BS3, boot swab around the broiler house

Table C-1 Genotypes of *Campylobacter* isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	*flaA* SVR allele no.	MLST (ST)
63	D21CS01_S2	*C. jejuni*	3	2	Cloacal swab	45	2275
64	D21CS02_S2	*C. jejuni*	3	2	Cloacal swab	45	2275
65	D21CS03_S2	*C. jejuni*	3	2	Cloacal swab	45	
66	D21CS04_S2	*C. jejuni*	3	2	Cloacal swab	45	2275
67	D21CS05_S2	*C. jejuni*	3	2	Cloacal swab	45	
68	D21CS06_S2	*C. jejuni*	3	2	Cloacal swab	45	2275
69	D21CS08_S2	*C. jejuni*	3	2	Cloacal swab	45	2275
70	D21CS09_S2	*C. jejuni*	3	2	Cloacal swab	45	
71	D21CS10_S2	*C. jejuni*	3	2	Cloacal swab	45	
72	D21CS14_S2	*C. jejuni*	3	2	Cloacal swab	45	
73	D21BS2_S2	*C. jejuni*	3	2	BS2	45	
74	D21BS3_S2	*C. jejuni*	3	2	BS3	45	
75	D28CS03_S2	*C. jejuni*	4	2	Cloacal swab	45	
76	D28CS04_S2	*C. jejuni*	4	2	Cloacal swab	45	2275
77	D28CS05_S2	*C. jejuni*	4	2	Cloacal swab	45	2275
78	D28CS07_S2	*C. jejuni*	4	2	Cloacal swab	45	
79	D28CS09_S2	*C. jejuni*	4	2	Cloacal swab	45	
80	D28CS11_S2	*C. jejuni*	4	2	Cloacal swab	45	
81	D28CS15_S2	*C. jejuni*	4	2	Cloacal swab	45	
82	D28CS16_S2	*C. jejuni*	4	2	Cloacal swab	45	
83	D28CS17_S2	*C. jejuni*	4	2	Cloacal swab	45	
84	D28CS19_S2	*C. jejuni*	4	2	Cloacal swab	45	
85	D28CS20_S2	*C. jejuni*	4	2	Cloacal swab	45	
86	D28CS21_S2	*C. jejuni*	4	2	Cloacal swab	45	
87	D28CS22_S2	*C. jejuni*	4	2	Cloacal swab	45	
88	D28BS1_S2	*C. jejuni*	4	2	BS1	45	
89	D28BS3_S2	*C. jejuni*	4	2	BS3	45	
90	D33CS01_S2	*C. jejuni*	5	2	Cloacal swab	45	
91	D33CS02_S2	*C. jejuni*	5	2	Cloacal swab	45	
92	D33CS03_S2	*C. jejuni*	5	2	Cloacal swab	45	
93	D33CS04_S2	*C. jejuni*	5	2	Cloacal swab	45	

*BS1, boot swab from anteroom; BS2, boot swab inside the broiler house; BS3, boot swab around the broiler house

Table C-1 Genotypes of *Campylobacter* isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	*flaA* SVR allele no.	MLST (ST)
94	D33CS06_S2	*C. jejuni*	5	2	Cloacal swab	45	
95	D33CS11_S2	*C. jejuni*	5	2	Cloacal swab	45	
96	D33CS12_S2	*C. jejuni*	5	2	Cloacal swab	45	
97	D33CS14_S2	*C. jejuni*	5	2	Cloacal swab	45	
98	D33CS15_S2	*C. jejuni*	5	2	Cloacal swab	45	
99	D33CS16_S2	*C. jejuni*	5	2	Cloacal swab	45	
100	D33CS17_S2	*C. jejuni*	5	2	Cloacal swab	45	
101	D33CS18_S2	*C. jejuni*	5	2	Cloacal swab	45	
102	D33CS23_S2	*C. jejuni*	5	2	Cloacal swab	45	
103	D33CS24_S2	*C. jejuni*	5	2	Cloacal swab	45	
104	D33CS26_S2	*C. jejuni*	5	2	Cloacal swab	45	
105	D33BS0_S2	*C. jejuni*	5	2	BS0	45	2275
106	D33BS2_S2	*C. jejuni*	5	2	BS2	45	2275
108	D33FLY_S2	*C. jejuni*	5	2	Flies	45	
109	S6150_02C	*C. jejuni*	5	2	Caeca	45	
110	S6150_03C	*C. jejuni*	5	2	Caeca	45	
111	S6150_04C	*C. jejuni*	5	2	Caeca	45	
112	S6150_05C	*C. jejuni*	5	2	Caeca	45	2275
113	S6150_06C	*C. jejuni*	5	2	Caeca	45	
114	S6150_07C	*C. jejuni*	5	2	Caeca	45	
115	S6150_08C	*C. jejuni*	5	2	Caeca	45	
116	S6150_09C	*C. jejuni*	5	2	Caeca	45	
117	S6150_10C	*C. jejuni*	5	2	Caeca	45	
118	S6150_11C	*C. jejuni*	5	2	Caeca	45	
119	S6150_12C	*C. jejuni*	5	2	Caeca	45	
120	S6150_14C	*C. jejuni*	5	2	Caeca	45	
121	S6150_15C	*C. jejuni*	5	2	Caeca	45	
122	S6150_16C	*C. jejuni*	5	2	Caeca	45	
123	S6150_17C	*C. jejuni*	5	2	Caeca	45	
124	S6150_18C	*C. jejuni*	5	2	Caeca	45	
125	D34Fly_S3	*C. jejuni*	5	3	Flies	54	

*BS0, boot swab on pathway from office to the broiler house; BS2, boot swab inside the broiler

Table C-2 Genotypes of *Campylobacter* isolated from broilers and environments of farm B

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	*flaA* SVR allele no.	MLST (ST)
1	CSB15d01c3	*C. jejuni*	2	1	Cloacal swab	783	1232
2	CSB15d02c3	*C. jejuni*	2	1	Cloacal swab	783	1232
3	CSB15d03c3	*C. jejuni*	2	1	Cloacal swab	783	
4	CSB15d04c3	*C. jejuni*	2	1	Cloacal swab	783	
5	CSB15d06c3	*C. jejuni*	2	1	Cloacal swab	783	
6	BS2B15dc3	*C. jejuni*	2	1	BS2	783	1232
7	CWH3B15dc3	*C. jejuni*	2	1	Nipple water	783	1232
8	CSB21d01c6	*C. jejuni*	3	1	Cloacal swab	783	
9	CSB21d02c6	*C. jejuni*	3	1	Cloacal swab	783	
10	CSB21d05c6	*C. jejuni*	3	1	Cloacal swab	783	
11	CSB21d06c6	*C. jejuni*	3	1	Cloacal swab	783	1232
12	CSB21d07c6	*C. jejuni*	3	1	Cloacal swab	783	1232
13	CSB21d08c6	*C. jejuni*	3	1	Cloacal swab	783	
14	CSB21d09c6	*C. jejuni*	3	1	Cloacal swab	783	
15	CSB21d10c6	*C. jejuni*	3	1	Cloacal swab	783	
16	CSB21d11c6	*C. jejuni*	3	1	Cloacal swab	783	
17	CSB21d12c6	*C. jejuni*	3	1	Cloacal swab	783	
18	CSB21d13c6	*C. jejuni*	3	1	Cloacal swab	783	
19	CSB21d14c6	*C. jejuni*	3	1	Cloacal swab	783	
20	CSB21d15c6	*C. jejuni*	3	1	Cloacal swab	783	
21	CWH2B21dc6	*C. jejuni*	3	1	Nipple water	783	
22	CSB28d02c9	*C. jejuni*	4	1	Cloacal swab	783	1232
23	CSB32d01c10	*C. jejuni*	5	1	Cloacal swab	783	
24	CSB32d02c10	*C. jejuni*	5	1	Cloacal swab	783	1232
25	CSB32d03c10	*C. jejuni*	5	1	Cloacal swab	783	1232
26	CSB32d04c10	*C. jejuni*	5	1	Cloacal swab	783	
27	CSB32d05c10	*C. jejuni*	5	1	Cloacal swab	783	5213
28	CSB32d06c10	*C. jejuni*	5	1	Cloacal swab	783	
29	CSB32d07c10	*C. jejuni*	5	1	Cloacal swab	783	1232
30	CSB32d08c10	*C. jejuni*	5	1	Cloacal swab	783	
31	CSB32d09c10	*C. jejuni*	5	1	Cloacal swab	48	2131

*BS2, boot swab inside the broiler house

Table C-2 Genotypes of *Campylobacter* isolated from broilers and environments of farm B (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	*flaA* SVR allele no.	MLST (ST)
32	S534_01C	*C. jejuni*	5	1	Caeca	783	5213
33	S534_02C	*C. jejuni*	5	1	Caeca	783	
34	S534_03C	*C. jejuni*	5	1	Caeca	783	
35	S534_04C	*C. jejuni*	5	1	Caeca	783	
36	S534_05C	*C. jejuni*	5	1	Caeca	783	
37	S534_06C	*C. jejuni*	5	1	Caeca	783	
38	S534_07C	*C. jejuni*	5	1	Caeca	783	
39	S534_08C	*C. jejuni*	5	1	Caeca	783	
40	S534_09C	*C. jejuni*	5	1	Caeca	783	1232
41	S534_10C	*C. jejuni*	5	1	Caeca	783	
42	D14CWH2_K2	*C. jejuni*	2	2	Nipple water	45	2275
43	D28CS002_K3	*C. jejuni*	4	3	Cloacal swab	783	
44	D28CS003_K3	*C. jejuni*	4	3	Cloacal swab	783	1232
45	D28CS004_K3	*C. jejuni*	4	3	Cloacal swab	783	
46	D28CS005_K3	*C. jejuni*	4	3	Cloacal swab	783	
47	D28CS007_K3	*C. jejuni*	4	3	Cloacal swab	783	
48	D28CS010_K3	*C. jejuni*	4	3	Cloacal swab	783	
49	D28CS016_K3	*C. jejuni*	4	3	Cloacal swab	783	
50	D28CS018_K3	*C. jejuni*	4	3	Cloacal swab	783	
51	D28CS022_K3	*C. jejuni*	4	3	Cloacal swab	783	
52	D28CWH1_K3	*C. jejuni*	4	3	Nipple water	783	1232
53	D28CWH4_K3	*C. jejuni*	4	3	Nipple water	783	1232
54	D30CS001_K3	*C. jejuni*	5	3	Cloacal swab	783	
55	D30CS005_K3	*C. jejuni*	5	3	Cloacal swab	783	
56	D30CS006_K3	*C. jejuni*	5	3	Cloacal swab	783	
57	D30CS007_K3	*C. jejuni*	5	3	Cloacal swab	783	
58	D30CS008_K3	*C. jejuni*	5	3	Cloacal swab	783	
59	D30CS009_K3	*C. jejuni*	5	3	Cloacal swab	783	
60	D30CS010_K3	*C. jejuni*	5	3	Cloacal swab	783	
61	D30CS011_K3	*C. jejuni*	5	3	Cloacal swab	783	
62	D30CS012_K3	*C. jejuni*	5	3	Cloacal swab	783	1232

Table C-2 Genotypes of *Campylobacter* isolated from broilers and environments of farm B (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	*flaA* SVR allele no.	MLST (ST)
63	D30CS014_K3	*C. jejuni*	5	3	Cloacal swab	783	
64	D30CS015_K3	*C. jejuni*	5	3	Cloacal swab	783	
65	D30CS018_K3	*C. jejuni*	5	3	Cloacal swab	783	
66	D30CS019_K3	*C. jejuni*	5	3	Cloacal swab	783	
67	D30CS021_K3	*C. jejuni*	5	3	Cloacal swab	783	
68	D30CS022_K3	*C. jejuni*	5	3	Cloacal swab	783	
69	S736_01C	*C. jejuni*	5	3	Caeca	783	
70	S736_02C	*C. jejuni*	5	3	Caeca	783	
71	S736_06C	*C. jejuni*	5	3	Caeca	783	1232
72	S736_08C	*C. jejuni*	5	3	Caeca	783	
73	S736_09C	*C. jejuni*	5	3	Caeca	783	
74	S736_10C	*C. jejuni*	5	3	Caeca	783	
75	S736_11C	*C. jejuni*	5	3	Caeca	783	
76	S736_13C	*C. jejuni*	5	3	Caeca	783	
77	S736_16C	*C. jejuni*	5	3	Caeca	783	
78	S736_17C	*C. jejuni*	5	3	Caeca	783	
79	S736_21C	*C. jejuni*	5	3	Caeca	783	
80	S736_24C	*C. jejuni*	5	3	Caeca	783	
81	S736_27C	*C. jejuni*	5	3	Caeca	783	
82	S736_30C	*C. jejuni*	5	3	Caeca	783	

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
1	CSB14d011c1	*C. jejuni*	2	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
2	CSB14d02c1	*C. jejuni*	2	1	Cloacal swab	4	4	0.25	0.125	4	CIP
3	CSB14d03c1	*C. jejuni*	2	1	Cloacal swab	4	4	0.5	0.125	256	CIP-TET
4	CSB14d05c1	*C. jejuni*	2	1	Cloacal swab	8	32	0.25	1	128	CIP-TET
5	CSB14d06c1	*C. jejuni*	2	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
6	CSB14d08c1	*C. jejuni*	2	1	Cloacal swab	16	32	1	1	128	CIP-TET
7	CSB21d02c2	*C. jejuni*	3	1	Cloacal swab	128	32	1	1	128	CIP-TET-AMP
8	CSB21d03c2	*C. jejuni*	3	1	Cloacal swab	2	16	0.25	0.125	4	CIP
9	CSB21d05c2	*C. jejuni*	3	1	Cloacal swab	4	8	0.5	0.5	4	CIP
10	CSB21d06c2	*C. jejuni*	3	1	Cloacal swab	4	8	0.5	0.5	8	CIP
11	CSB21d07c2	*C. jejuni*	3	1	Cloacal swab	64	16	0.5	0.25	128	CIP-TET-AMP
12	CSB21d08c2	*C. jejuni*	3	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
13	CSB21d09c2	*C. jejuni*	3	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
14	CSB21d10c2	*C. jejuni*	3	1	Cloacal swab	32	16	1	0.5	128	CIP-TET-AMP
15	CSB21d11c2	*C. jejuni*	3	1	Cloacal swab	2	8	1	1	8	CIP
16	CSB21d12c2	*C. jejuni*	3	1	Cloacal swab	4	8	1	1	32	CIP-TET
17	CSB21d13c2	*C. jejuni*	3	1	Cloacal swab	32	16	0.25	0.5	128	CIP-TET-AMP
18	CSB21d14c2	*C. jejuni*	3	1	Cloacal swab	2	2	0.25	0.125	1	S
19	BS0B21dc2	*C. jejuni*	3	1	BS0	64	8	0.5	0.25	64	CIP-TET-AMP
20	FlyB21dc2	*C. jejuni*	3	1	Flies	4	4	0.25	0.25	1	CIP

* BS0, boot swab on pathway from office to the broiler house

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
21	CSB28d01c5	*C. jejuni*	4	1	Cloacal swab	2	16	4	1	128	CIP-TET
22	CSB28d02c5	*C. jejuni*	4	1	Cloacal swab	4	4	0.5	0.125	8	CIP
23	CSB28d03c5	*C. jejuni*	4	1	Cloacal swab	4	8	1	0.5	8	CIP
24	CSB28d04c5	*C. jejuni*	4	1	Cloacal swab	16	16	1	0.125	32	CIP-TET
25	CSB28d05c5	*C. jejuni*	4	1	Cloacal swab	32	4	0.5	0.125	128	CIP-TET-AMP
26	CSB28d06c5	*C. jejuni*	4	1	Cloacal swab	64	16	1	0.5	128	CIP-TET-AMP
27	CSB28d08c5	*C. jejuni*	4	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
28	CSB28d09c5	*C. jejuni*	4	1	Cloacal swab	2	4	0.5	0.125	8	CIP
29	CSB28d10c5	*C. jejuni*	4	1	Cloacal swab	8	8	1	0.5	16	CIP-TET
30	CSB28d11c5	*C. jejuni*	4	1	Cloacal swab	32	8	1	0.5	64	CIP-TET-AMP
31	CSB28d12c5	*C. jejuni*	4	1	Cloacal swab	4	8	1	0.5	8	CIP
32	CSB28d14c5	*C. jejuni*	4	1	Cloacal swab	4	8	1	0.5	16	CIP-TET
33	CSB28d15c5	*C. jejuni*	4	1	Cloacal swab	16	16	2	0.5	512	CIP-TET
34	CSB28d16c5	*C. jejuni*	4	1	Cloacal swab	8	8	1	0.5	8	CIP
35	CSB28d17c5	*C. jejuni*	4	1	Cloacal swab	8	16	1	0.5	128	CIP-TET
36	CSB28d18c5	*C. jejuni*	4	1	Cloacal swab	4	4	0.5	0.5	32	CIP-TET
37	CSB28d19c5	*C. jejuni*	4	1	Cloacal swab	16	4	0.5	0.125	32	CIP-TET
38	CSB28d20c5	*C. jejuni*	4	1	Cloacal swab	1	4	0.5	0.125	8	CIP
39	CSB28d24c5	*C. jejuni*	4	1	Cloacal swab	32	8	0.5	0.125	128	CIP-TET-AMP
40	CSB28d25c5	*C. jejuni*	4	1	Cloacal swab	4	2	0.25	0.125	4	S
41	BS3B28dc5	*C. jejuni*	4	1	BS3	4	8	1	0.5	2	CIP

BS3, boot swab around the broiler house

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (μg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
42	CSB32d03c8	*C. jejuni*	5	1	Cloacal swab	8	16	2	0.25	4	CIP
43	CSB32d04c8	*C. jejuni*	5	1	Cloacal swab	32	16	1	0.5	64	CIP-TET-AMP
44	CSB32d06c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.5	256	CIP-TET-AMP
45	CSB32d07c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.5	256	CIP-TET-AMP
46	CSB32d08c8	*C. jejuni*	5	1	Cloacal swab	32	8	0.5	0.125	128	CIP-TET-AMP
47	CSB32d10c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.25	256	CIP-TET-AMP
48	CSB32d14c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.25	128	CIP-TET-AMP
49	CSB32d15c8	*C. jejuni*	5	1	Cloacal swab	32	4	0.25	0.125	32	CIP-TET-AMP
50	CSB32d18c8	*C. jejuni*	5	1	Cloacal swab	64	8	0.5	0.125	64	CIP-TET-AMP
51	CSB32d22c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.25	256	CIP-TET-AMP
52	CSB32d23c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.5	256	CIP-TET-AMP
53	CSB32d27c8	*C. jejuni*	5	1	Cloacal swab	64	16	1	0.5	256	CIP-TET-AMP
54	S5040_01C	*C. jejuni*	5	1	Caeca	64	8	0.25	0.25	32	CIP-TET-AMP
55	S5040_10C	*C. jejuni*	5	1	Caeca	4	16	0.5	0.5	32	CIP-TET
56	D14CWH2_S2	*C. coli*	2	2	Nipple water	>512	256	256	32	512	CIP-ERY-GEN-TET-AMP
57	D14CWH3_S2	*C. coli*	2	2	Nipple water	>512	256	256	32	512	CIP-ERY-GEN-TET-AMP
58	D21CS01_S2	*C. jejuni*	3	2	Cloacal swab	256	8	1	0.25	64	CIP-TET-AMP
59	D21CS02_S2	*C. jejuni*	3	2	Cloacal swab	64	1	1	0.25	16	TET-AMP
60	D21CS03_S2	*C. jejuni*	3	2	Cloacal swab	32	8	1	0.5	32	CIP-TET-AMP
61	D21CS04_S2	*C. jejuni*	3	2	Cloacal swab	32	4	0.5	0.25	8	CIP-AMP
62	D21CS05_S2	*C. jejuni*	3	2	Cloacal swab	64	8	1	0.5	64	CIP-TET-AMP

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
63	D21CS06_S2	*C. jejuni*	3	2	Cloacal swab	64	64	64	0.5	512	CIP-ERY-TET-AMP
64	D21CS07_S2	*C. jejuni*	3	2	Cloacal swab	64	16	1	0.5	32	CIP-TET-AMP
65	D21CS08_S2	*C. jejuni*	3	2	Cloacal swab	64	32	512	16	256	CIP-ERY-GEN-TET-AMP
66	D21CS10_S2	*C. jejuni*	3	2	Cloacal swab	64	16	1	0.5	64	CIP-TET-AMP
67	D21CWH4_S2	*C. coli*	3	2	Nipple water	>512	256	256	32	512	CIP-ERY-GEN-TET-AMP
68	D21BS2_S2	*C. jejuni*	3	2	BS2	32	8	1	0.25	16	CIP-TET-AMP
69	D21BS3_S2	*C. jejuni*	3	2	BS3	32	8	0.5	0.25	16	CIP-TET-AMP
70	D28CS02_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	16	CIP-TET-AMP
71	D28CS03_S2	*C. jejuni*	4	2	Cloacal swab	32	4	0.5	0.25	8	CIP-AMP
72	D28CS04_S2	*C. jejuni*	4	2	Cloacal swab	32	0.125	0.5	0.25	8	AMP
73	D28CS05_S2	*C. jejuni*	4	2	Cloacal swab	8	4	0.5	0.25	2	CIP
74	D28CS06_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
75	D28CS07_S2	*C. jejuni*	4	2	Cloacal swab	64	0.125	1	0.25	8	AMP
76	D28CS09_S2	*C. jejuni*	4	2	Cloacal swab	128	0.125	1	0.25	32	TET-AMP
77	D28CS11_S2	*C. jejuni*	4	2	Cloacal swab	32	8	1	0.5	32	CIP-TET-AMP
78	D28CS12_S2	*C. jejuni*	4	2	Cloacal swab	64	16	1	0.25	32	CIP-TET-AMP
79	D28CS13_S2	*C. jejuni*	4	2	Cloacal swab	32	4	1	0.06	16	CIP-TET-AMP
80	D28CS14_S2	*C. jejuni*	4	2	Cloacal swab	64	16	1	0.5	64	CIP-TET-AMP
81	D28CS15_S2	*C. jejuni*	4	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
82	D28CS16_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	64	CIP-TET-AMP
83	D28CS17_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	64	CIP-TET-AMP

* BS2, boot swab inside the broiler house; BS3, boot swab around the broiler house

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
84	D28CS18_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
85	D28CS19_S2	*C. jejuni*	4	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
86	D28CS20_S2	*C. jejuni*	4	2	Cloacal swab	128	1	1	0.25	32	TET-AMP
87	D28CS21_S2	*C. jejuni*	4	2	Cloacal swab	128	16	1	0.25	64	CIP-TET-AMP
88	D28CS22_S2	*C. jejuni*	4	2	Cloacal swab	128	16	1	0.25	32	CIP-TET-AMP
89	D28CS23_S2	*C. jejuni*	4	2	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
90	D28CS24_S2	*C. jejuni*	4	2	Cloacal swab	64	16	1	0.25	64	CIP-TET-AMP
91	D28BS1_S2	*C. jejuni*	4	2	BS1	32	8	1	1	32	CIP-TET-AMP
92	D28BS3_S2	*C. jejuni*	4	2	BS3	32	8	>512	0.5	16	CIP-ERY-TET-AMP
93	D33CS02_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.25	64	CIP-TET-AMP
94	D33CS03_S2	*C. jejuni*	5	2	Cloacal swab	128	16	1	0.25	64	CIP-TET-AMP
95	D33CS04_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
96	D33CS05_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.25	32	CIP-TET-AMP
97	D33CS06_S2	*C. jejuni*	5	2	Cloacal swab	64	64	0.125	0.25	8	CIP-AMP
98	D33CS09_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.5	64	CIP-TET-AMP
99	D33CS11_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.25	64	CIP-TET-AMP
100	D33CS12_S2	C. jejuni	5	2	Cloacal swab	128	16	1	0.25	32	CIP-TET-AMP
101	D33CS14_S2	C. jejuni	5	2	Cloacal swab	64	16	1	0.5	32	CIP-TET-AMP
102	D33CS15_S2	*C. jejuni*	5	2	Cloacal swab	128	1	1	0.25	16	TET-AMP
103	D33CS16_S2	*C. jejuni*	5	2	Cloacal swab	128	1	1	0.25	16	TET-AMP
104	D33CS17_S2	*C. jejuni*	5	2	Cloacal swab	64	4	0.5	0.25	8	CIP-AMP

* BS1, boot swab from anteroom; BS3, boot swab around the broiler house

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample*	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
105	D33CS18_S2	*C. jejuni*	5	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
106	D33CS19_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.5	32	CIP-TET-AMP
107	D33CS20_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.25	32	CIP-TET-AMP
108	D33CS21_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.25	16	CIP-TET-AMP
109	D33CS22_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
110	D33CS23_S2	*C. jejuni*	5	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
111	D33CS24_S2	*C. jejuni*	5	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
112	D33CS25_S2	*C. jejuni*	5	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
113	D33CS26_S2	*C. jejuni*	5	2	Cloacal swab	64	16	2	0.5	16	CIP-TET-AMP
114	D33CS27_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.5	64	CIP-TET-AMP
115	D33CS28_S2	*C. jejuni*	5	2	Cloacal swab	128	16	2	1	64	CIP-TET-AMP
116	D33CS29_S2	*C. jejuni*	5	2	Cloacal swab	64	16	1	0.25	64	CIP-TET-AMP
117	D33CS30_S2	*C. jejuni*	5	2	Cloacal swab	32	16	1	0.06	16	CIP-TET-AMP
118	D33BS0_S2	*C. jejuni*	5	2	BS0	0.5	4	1	16	16	CIP-GEN-TET
119	D33CWH3_S2	*C. coli*	5	2	Nipple water	>512	256	256	16	>512	CIP-ERY-GEN-TET-AMP
120	D33BS2_S2	*C. jejuni*	5	2	BS2	0.5	128	512	16	64	CIP-ERY-GEN-TET
121	S6150_01C	*C. jejuni*	5	2	Caeca	32	4	0.5	0.25	4	CIP-AMP
122	S6150_02C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
123	S6150_03C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
124	S6150_04C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
125	S6150_05C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP

* BS0, boot swab on pathway from office to the broiler house; BS2, boot swab inside the broiler house

Table C-3 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm A (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (μg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
126	S6150_06C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
127	S6150_07C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
128	S6150_08C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
129	S6150_09C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
130	S6150_11C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
131	S6150_13C	*C. jejuni*	5	2	Caeca	128	4	1	0.25	16	CIP-TET-AMP
132	S6150_14C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
133	S6150_15C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
134	S6150_16C	*C. jejuni*	5	2	Caeca	32	8	0.5	0.125	16	CIP-TET-AMP
135	S6150_17C	*C. jejuni*	5	2	Caeca	32	8	0.5	0.125	64	CIP-TET-AMP
136	S6150_18C	*C. jejuni*	5	2	Caeca	64	16	2	0.5	16	CIP-TET-AMP
137	S6150_19C	*C. jejuni*	5	2	Caeca	64	16	2	0.25	32	CIP-TET-AMP
138	S6150_21C	*C. jejuni*	5	2	Caeca	64	16	1	0.25	16	CIP-TET-AMP
139	S6150_22C	*C. jejuni*	5	2	Caeca	64	16	1	0.25	32	CIP-TET-AMP
140	S6150_23C	*C. jejuni*	5	2	Caeca	64	16	1	0.25	32	CIP-TET-AMP
141	S6150_24C	*C. jejuni*	5	2	Caeca	64	8	1	0.25	16	CIP-TET-AMP
142	S6150_26C	*C. jejuni*	5	2	Caeca	32	8	0.5	0.125	16	CIP-TET-AMP
143	D34CWH2_S3	*C. coli*	5	3	Nipple water	>512	128	256	32	512	CIP-ERY-GEN-TET-AMP
144	D34Fly_S3	*C. jejuni*	5	3	Flies	64	8	0.5	0.25	32	CIP-TET-AMP

Table C-4 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm B

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (μg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
1	CSB15d01c3	*C. jejuni*	2	1	Cloacal swab	4	16	0.25	0.5	32	CIP-TET
2	CSB15d02c3	*C. jejuni*	2	1	Cloacal swab	16	8	0.25	0.125	32	CIP-TET
3	CSB15d03c3	*C. jejuni*	2	1	Cloacal swab	16	8	0.25	0.25	32	CIP-TET
4	CSB15d04c3	*C. jejuni*	2	1	Cloacal swab	2	4	0.25	0.125	32	CIP-TET
5	CSB21d02c6	*C. jejuni*	3	1	Cloacal swab	8	8	0.5	0.125	64	CIP-TET
6	CSB21d03c6	*C. jejuni*	3	1	Cloacal swab	32	16	1	0.25	32	CIP-TET-AMP
7	CSB21d06c6	*C. jejuni*	3	1	Cloacal swab	64	32	2	1	128	CIP-TET-AMP
8	CSB21d07c6	*C. jejuni*	3	1	Cloacal swab	1	4	0.125	0.06	32	CIP-TET
9	CSB21d09c6	*C. jejuni*	3	1	Cloacal swab	1	4	0.25	0.5	32	CIP-TET
10	CSB21d10c6	*C. jejuni*	3	1	Cloacal swab	64	128	128	0.25	64	CIP-ERY-TET-AMP
11	CSB21d11c6	*C. jejuni*	3	1	Cloacal swab	2	2	0.06	0.25	64	TET
12	CSB21d12c6	*C. jejuni*	3	1	Cloacal swab	64	32	2	1	128	CIP-TET-AMP
13	CSB21d14c6	*C. jejuni*	3	1	Cloacal swab	4	16	0.25	0.25	64	CIP-TET
14	CSB32d01c10	*C. jejuni*	5	1	Cloacal swab	4	16	0.25	0.25	64	CIP-TET
15	CSB32d02c10	*C. jejuni*	5	1	Cloacal swab	2	8	0.125	0.125	32	CIP-TET
16	CSB32d05c10	*C. jejuni*	5	1	Cloacal swab	1	16	0.25	0.5	64	CIP-TET
17	CSB32d06c10	*C. jejuni*	5	1	Cloacal swab	64	16	0.25	0.5	64	CIP-TET-AMP
18	CSB32d08c10	*C. jejuni*	5	1	Cloacal swab	4	8	0.5	0.125	64	CIP-TET
19	CSB32d09c10	*C. jejuni*	5	1	Cloacal swab	4	8	0.5	0.125	64	CIP-TET
20	S534_01C	*C. jejuni*	5	1	Caeca	16	8	0.5	0.5	32	CIP-TET
21	S534_02C	*C. jejuni*	5	1	Caeca	4	16	1	0.25	32	CIP-TET

Table C-4 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm B (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
22	S534_03C	*C. jejuni*	5	1	Caeca	4	8	0.5	0.125	32	CIP-TET
23	S534_04C	*C. jejuni*	5	1	Caeca	8	16	1	0.25	32	CIP-TET
24	S534_06C	*C. jejuni*	5	1	Caeca	2	2	0.25	0.125	32	TET
25	S534_07C	*C. jejuni*	5	1	Caeca	1	4	0.25	0.5	64	CIP-TET
26	S534_08C	*C. jejuni*	5	1	Caeca	4	16	1	0.125	64	CIP-TET
27	S534_09C	*C. jejuni*	5	1	Caeca	8	16	1	0.5	32	CIP-TET
28	S534_10C	*C. jejuni*	5	1	Caeca	64	32	8	1	256	CIP-TET-AMP
29	D14CWH2_K2	*C. jejuni*	2	2	Nipple water	64	16	2	0.25	16	CIP-TET-AMP
30	D14CWH6_K2	*C. coli*	2	2	Nipple water	>512	128	256	32	512	CIP-ERY-GEN-TET-AMP
31	D21CWHM_K2	*C. coli*	3	2	Water inlet	>512	128	256	32	512	CIP-ERY-GEN-TET-AMP
32	D21CWH6_K2	*C. coli*	3	2	Nipple water	>512	128	64	32	512	CIP-ERY-GEN-TET-AMP
33	D28CS002_K3	*C. jejuni*	4	3	Cloacal swab	4	16	2	0.5	16	CIP-TET
34	D28CS003_K3	*C. jejuni*	4	3	Cloacal swab	4	16	2	0.5	16	CIP-TET
35	D28CS004_K3	*C. jejuni*	4	3	Cloacal swab	4	16	2	0.25	16	CIP-TET
36	D28CS005_K3	*C. jejuni*	4	3	Cloacal swab	4	16	0.5	0.25	32	CIP-TET
37	D28CS007_K3	*C. jejuni*	4	3	Cloacal swab	4	16	0.5	0.125	64	CIP-TET
38	D28CS010_K3	*C. jejuni*	4	3	Cloacal swab	4	16	2	0.125	16	CIP-TET
39	D28CS016_K3	*C. jejuni*	4	3	Cloacal swab	4	16	1	0.25	16	CIP-TET
40	D28CS019_K3	*C. jejuni*	4	3	Cloacal swab	4	16	1	0.125	64	CIP-TET
41	D28CS026_K3	*C. jejuni*	4	3	Cloacal swab	4	16	1	0.125	32	CIP-TET
42	D28CS029_K3	*C. jejuni*	4	3	Cloacal swab	8	16	2	0.25	64	CIP-TET

Table C-4 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm B (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
43	D28CWH1_K3	*C. jejuni*	4	3	Nipple water	>512	256	256	16	512	CIP-ERY-GEN-TET-AMP
44	D28CWH4_K3	*C. jejuni*	4	3	Nipple water	4	8	0.5	0.5	32	CIP-TET
45	D30CS005_K3	*C. jejuni*	5	3	Cloacal swab	4	16	2	0.5	16	CIP-TET
46	D30CS008_K3	*C. jejuni*	5	3	Cloacal swab	4	16	2	0.5	16	CIP-TET
47	D30CS009_K3	*C. jejuni*	5	3	Cloacal swab	64	128	512	32	128	CIP-ERY-GEN-TET-AMP
48	D30CS012_K3	*C. jejuni*	5	3	Cloacal swab	4	8	0.25	0.25	64	CIP-TET
49	D30CS013_K3	*C. jejuni*	5	3	Cloacal swab	16	16	1	0.125	64	CIP-TET
50	D30CS014_K3	*C. jejuni*	5	3	Cloacal swab	4	16	2	0.25	16	CIP-TET
51	D30CS015_K3	*C. jejuni*	5	3	Cloacal swab	4	8	0.5	0.125	64	CIP-TET
52	D30CS016_K3	*C. jejuni*	5	3	Cloacal swab	4	16	1	0.125	64	CIP-TET
53	D30CS018_K3	*C. jejuni*	5	3	Cloacal swab	2	16	2	0.25	16	CIP-TET
54	D30CS019_K3	*C. jejuni*	5	3	Cloacal swab	2	16	2	0.5	16	CIP-TET
55	D30CS020_K3	*C. jejuni*	5	3	Cloacal swab	64	16	1	0.5	32	CIP-TET-AMP
56	D30CS021_K3	*C. jejuni*	5	3	Cloacal swab	4	16	2	0.25	16	CIP-TET
57	D30CS022_K3	*C. jejuni*	5	3	Cloacal swab	4	16	2	0.25	16	CIP-TET
58	S736_01C	*C. jejuni*	5	3	Caeca	8	16	1	0.5	32	CIP-TET
59	S736_02C	*C. jejuni*	5	3	Caeca	8	16	1	0.5	64	CIP-TET
60	S736_06C	*C. jejuni*	5	3	Caeca	8	16	0.5	0.25	32	CIP-TET
61	S736_08C	*C. jejuni*	5	3	Caeca	8	16	1	0.5	32	CIP-TET
62	S736_11C	*C. jejuni*	5	3	Caeca	4	8	1	0.25	32	CIP-TET
63	S736_13C	*C. jejuni*	5	3	Caeca	8	16	1	0.25	32	CIP-TET

Table C-4 Antimicrobial susceptibility of *Campylobacter* spp. isolated from broilers and environments of farm B (continued)

No.	Strain ID	Species	Age (week)	Production cycle	Type of sample	MICs (µg/ml)					Resistance pattern
						AMP	CIP	ERY	GEN	TET	
64	S736_16C	*C. jejuni*	5	3	Caeca	4	16	0.5	0.25	32	CIP-TET
65	S736_17C	*C. jejuni*	5	3	Caeca	8	32	1	0.25	32	CIP-TET
66	S736_19C	*C. jejuni*	5	3	Caeca	4	16	0.5	0.125	32	CIP-TET
67	S736_21C	*C. jejuni*	5	3	Caeca	16	16	1	0.25	32	CIP-TET
68	S736_24C	*C. jejuni*	5	3	Caeca	8	4	0.5	0.25	32	CIP-TET
69	S736_27C	*C. jejuni*	5	3	Caeca	8	4	1	0.25	32	CIP-TET
70	S736_30C	*C. jejuni*	5	3	Caeca	8	16	1	0.25	32	CIP-TET

VITA

Miss Petcharatt Charununtakorn was born on May 18, 1983 in Buriram, Thailand. She got the Degree of Veterinary Sciences (D.V.M.) from the Faculty of Veterinary Medicine, Khonkaen University, Khonkaen, Thailand in 2008. Later, she enrolled the Master of Science Program in the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University since academic year 2012.