ความสัมพันธ์ทางพันธุกรรม และปัจจัยเสี่ยงที่เกี่ยวข้องกับเชื้อแคมไพโลแบคเตอร์ในห่วงโซ่การผลิตไก่เนื้อในประเทศไทย

นางสาวสกาวพร ประจันตะเสน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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GENETIC RELATEDNESS AND RISK FACTORS ASSOCIATED WITH CAMPYLOBACTER IN TH AI BROILER PRODUCTION CHAIN

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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สกาวพร ประจันตะเสน : ความสัมพันธ์ทางพันธุกรรม และปัจจัยเสี่ยงที่เกี่ยวข้องกับเชื้อแคมไพโลแบคเตอร์ในห่วงโซ่การผลิตไก่เนื้อในประเทศไทย (GENETIC RELATEDNESS AND RISK FACTORS ASSOCIATED WITH CAMPYLOBACTER IN THAI BROILER PRODUCTION CHAIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. น.สพ. ดร. ธราดล เหลืองทองคำ, หน้า.

แคมไพโลแบคเตอร์เป็นเชื้อแบคทีเรียก่อโรคอาหารเป็นพิษที่มีความสำคัญทางสาธารณสุขเป็นอย่างมาก การสัมผัสหรือรับประทานอาหารโดยเฉพาะอย่างยิ่งเนื้อไก่ที่ปนเปื้อนเชื้อจัดเป็นสาเหตุสำคัญของการติดเชื้อแคมไพโลแบคเตอร์ การศึกษาเชิงระบาดวิทยาของเชื้อแคมไพโลแบคเตอร์ในอุตสาหกรรมการผลิตเนื้อไก่จึงเป็นหนึ่งในแนวทางสำคัญที่อาจช่วยลดจำนวนผู้ป่วยโรคอาหารเป็นพิษจากเชื้อนี้ อย่างไรก็ตาม ในปัจจุบัน การศึกษาเกี่ยวกับเชื้อแคมไพโลแบคเตอร์ในประเทศไทยยังมีอยู่อย่างจำกัด ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อ 1) ศึกษาความชุกและปัจจัยเสี่ยงของการติดเชื้อแคมไพโลแบคเตอร์ในฝูงไก่เนื้อของประเทศไทย และ 2) วิเคราะห์ความสัมพันธ์ทางพันธุกรรมของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากกระบวนการผลิตเนื้อไก่ในประเทศไทย การศึกษานี้ได้ทำการเก็บตัวอย่างลำไส้และข้อมูลการเลี้ยงจากฝูงไก่เนื้อในเขตภาคกลางและภาคตะวันออกของประเทศไทยจำนวน 250 ฝูง ผลการเพาะเชื้อและข้อมูลการเลี้ยงจะถูกนำไปวิเคราะห์ด้วยวิธี logistic regression model (LRM) และ generalized estimating equations (GEE) เพื่อทำการศึกษาปัจจัยเสี่ยงที่เกี่ยวข้องกับการติดเชื้อแคมไพโลแบคเตอร์ในฝูงไก่เนื้อ นอกจากนี้ผู้วิจัยยังได้ทำการศึกษาความสัมพันธ์ทางพันธุกรรมของเชื้อแคมไพโลแบคเตอร์ตลอดวงจรการผลิตเนื้อไก่ทั้งหมด 5 แห่งและวิเคราะห์ลักษณะทางพันธุกรรมของเชื้อที่แยกได้ด้วยวิธี *flaA* SVR sequencing และ multilocus sequence typing ผลการศึกษาพบว่า จากฝูงไก่จำนวน 250 ฝูง มีฝูงไก่ที่ให้ผลบวกต่อเชื้อแคมไพโลแบคเตอร์จำนวน 119 ฝูง (47.60%; 95% CI 41.41 - 53.79%) โดยฝูงที่ติดเชื้อจะมีความชุกภายในฝูงค่อนข้างสูง (มากกว่า 75%) ในการศึกษาครั้งนี้พบว่าเชื้อ *C. jejuni* เป็นสายพันธุ์หลักที่พบ รองลงไปเป็น *C. coli* จากการวิเคราะห์ปัจจัยเสี่ยงที่เกี่ยวข้องกับการติดเชื้อแคมไพโลแบคเตอร์ในฝูงไก่เนื้อ พบว่าการมีประวัติการติดเชื้อแคมไพโลแบคเตอร์ในฝูงที่เลี้ยงก่อนหน้าเป็นปัจจัยเสี่ยงที่สำคัญที่สุดสำหรับการศึกษาครั้งนี้ จากการศึกษาลักษณะพันธุกรรมของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากวงจรการผลิตเนื้อไก่จำนวน 311 เชื้อ สามารถระบุลักษณะทางพันธุกรรมของเชื้อได้ทั้งหมด 29 แบบด้วยวิธี *flaA* SVR sequencing นอกจากนี้เชื้อบางส่วนที่ถูกนำมาวิเคราะห์ด้วยวิธี multilocus sequence typing สามารถแยกลักษณะทางพันธุกรรมได้ 17 แบบ โดย clonal complexes ที่พบส่วนใหญ่ได้แก่ CC-45 CC-353 CC-354 และ CC-574 โดยภาพรวม เชื้อที่แยกได้จากพ่อแม่พันธุ์มีความแตกต่างจากเชื้อที่พบในฝูงไก่เนื้อและโรงเชือด ในขณะที่เชื้อที่พบในฝูงไก่เนื้อมักมีความสัมพันธ์ทางพันธุกรรมใกล้เคียงกับเชื้อที่แยกได้จากอุปกรณ์ในโรงเชือดและเนื้อไก่ การศึกษานี้แสดงให้เห็นถึงความสำคัญของการจัดการฟาร์มและโรงเชือดอย่างถูกสุขลักษณะ รวมไปถึงการใช้ระบบความปลอดภัยทางชีวภาพ (biosecurity) ที่เข้มงวดในฟาร์มไก่เนื้อ เพื่อควบคุมและป้องกันการถ่ายทอดเชื้อแคมไพโลแบคเตอร์จากฟาร์มไปยังผู้บริโภค

ภาควิชา สัตวแพทยสาธารณสุข ลายมือชื่อนิสิต .....

สาขาวิชา สัตวแพทยสาธารณสุข ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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SAKAOPORN PRACHANTASENA: GENETIC RELATEDNESS AND RISK FACTORS ASSOCIATED WITH CAMPYLOBACTER IN THAI BROILER PRODUCTION CHAIN. ADVISOR: TARADON LUANGTONGKUM, D.V.M., Ph.D., pp.

Campylobacter is considered as the major foodborne bacterial pathogen worldwide. Consumption and handling of contaminated food, particularly poultry meat product, are the important cause of *Campylobacter* infection. To reduce the number of human cases, the epidemiology of *Campylobacter* in poultry must be better understood. In Thailand, only limited information on *Campylobacter* in chicken meat production has been reported. Therefore, the objectives of this study were 1) to determine the prevalence and risk factors associated with *Campylobacter* in Thai broiler flocks and 2) to investigate genetic relatedness of *Campylobacter* strains isolated from broiler production chain in Thailand. *Campylobacter* colonization status was identified in 250 broiler flocks which were mainly raised in central and eastern parts of Thailand. Moreover, farm and flock data was collected by structured questionnaires. To identify risk factors associated with *Campylobacter* colonization in broiler flocks, logistic regression model (LRM) and generalized estimating equations (GEE) were performed. The distribution and genetic relatedness of *Campylobacter* were determined in 5 broiler production chains. *flaA* SVR sequencing and multilocus sequence typing (MLST) were used as genotyping methods in this study. Of 250 examined broiler flocks, 119 flocks were tested positive for *Campylobacter* (47.60%; 95% CI 41.41 - 53.79%). Most positive flocks had high level of within-flock prevalence (>75%). *C. jejuni* was the predominant species observed in this study, followed by *C. coli*. For the risk factor analysis, the history of *Campylobacter* colonization in previous flocks was identified as the most important risk factor associated with *Campylobacter* colonization in examined broiler flocks. Amongst 311 *Campylobacter* isolates from breeders to slaughterhouses selected for genetic characterization, 29 *flaA* SVR alleles and 17 sequence types (STs) were identified. The common clonal complexes (CCs) found in this study were CC-45, CC-353, CC-354 and CC-574. Mostly, *C. jejuni* isolated from breeders were distantly related to those isolated from broilers and chicken carcasses, while *C. jejuni* isolates from the slaughterhouse environment and meat products were similar to those isolated from broilers. Our findings underline the importance of hygienic practices on farm and slaughterhouse as well as strict biosecurity as the effective tool for reducing the transmission of *Campylobacter* from chickens to humans.

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

bp	base pair(s)
ºC	degree(s) Celsius
C.	*Campylobacter*
DNA	deoxyribonucleic acid(s)
dNTP	deoxyribonucleoside triphosphate(s)
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
min	minute(s)
ml	millilitre(s)
PCR	polymerase chain reaction
spp.	species
U	unit

CHAPTER I
INTRODUCTION

Over the last decade, campylobacteriosis has been considered as the most prevalent bacterial gastrointestinal disease in humans worldwide, particularly in developed countries. *Campylobacter jejuni* is the most common species associated with human infection, followed by *Campylobacter coli*. In humans, ingestion of a small number of bacterial cells could cause mild to severe diarrhea, abdominal pain and fever (Humphrey, et al., 2007; Levin, 2007). Generally, this illness can be recovered without any treatments. However, serious complications (e.g., Guillain-Barré syndrome, Reiter's syndrome and Reactive arthritis) can sometimes occur (Schonberg-Norio, et al., 2010). Foods of animal origin, especially poultry and poultry products, are considered as the important sources of human infection.

The epidemiology of *Campylobacter* in broiler production chain has been investigated worldwide. In European member countries, 2.0 to 100.0% of chicken flocks were tested positive for *Campylobacter*, while the high prevalence of *Campylobacter* was reported in US broiler flocks (EFSA, 2011; Hiett, et al., 2002; Luangtongkum, et al., 2006). Similarly, a wide range of colonization rates was described in Asian countries such as China (77.80%), Vietnam (31.90%) and Japan (47.20%) (Carrique-Mas, et al., 2014; Chen, et al., 2010; Haruna, et al., 2012). In Thailand, the prevalence of *Campylobacter* in chickens was reported between 11.2 and 64.0 percent (Chokboonmongkol, et al., 2013; Padungtod and Kaneene, 2005;

Saengthongpinit, et al., 2010). To reduce the prevalence of *Campylobacter* in broiler chickens, studies on flock colonization should be conducted.

In chicken meat production chain, breeder and broiler flocks were generally colonized with high prevalence of *Campylobacter*, while this organism was rarely reported in hatchery (Newell and Fearnley, 2003). In addition, most of *Campylobacter* isolated from breeders were genetically unrelated to those of consecutive broiler flocks and meat products, while similarity between *Campylobacter* from broiler flocks and meat products was more common (O'Mahony, et al., 2011; Patriarchi, et al., 2011). These findings indicated that broilers are the primary source of *Campylobacter* contamination in chicken meat production chain.

To develop effective intervention measures for *Campylobacter* on broiler farms, risk factors associated with *Campylobacter* colonization during rearing period must be clarified. Age at slaughter, degree of biosecurity strictness, rodent infestation and presence of *Campylobacter* in previous batches were previously identified as the main risk factors associated with *Campylobacter* colonization in broilers (Barrios, et al., 2006; Bouwknegt, et al., 2004; Ellis-Iversen, et al., 2009; McDowell, et al., 2008). However, potential risk factors could vary between different areas.

Besides risk factors mentioned above, *Campylobacter* colonization in broiler flocks reared in temperate zone was also different between seasons. In Nordic countries, the peak of colonization rate in chickens was observed in summer (Boysen,

et al., 2011; Jore, et al., 2010; Jorgensen, et al., 2011). Humidity, temperature, sunlight and rainfall were suggested as the climatic factors that facilitate the survival of *Campylobacter* during summer time in temperate zone (Bi et al., 2008; Lawes et al., 2012; Zweifel et al., 2008), while the effect of climatic factors on *Campylobacter* colonization in poultry has not been widely investigated in tropical region including Thailand.

To successfully reduce *Campylobacter* contamination in poultry meat products, control of *Campylobacter* at both farm and slaughter levels should be carried out. Since the information of *Campylobacter* in Thai chicken production is still limited, investigation on the epidemiology of this organism is necessary. Therefore, the objectives of this study were to determine the prevalence and risk factors associated with *Campylobacter* colonization in Thai broiler flocks and to investigate genetic relatedness of *Campylobacter* isolated from poultry production chain in Thailand.

CHAPTER II
LITERATURE REVIEW

2.1 General characteristics of *Campylobacter*

Campylobacter is gram negative, microaerophilic, non-spore forming bacteria. Generally, its appearance is described as gull wing-like shaped, S-shaped or spiral-shaped rod cell with 0.2 μm to 0.8 μm wide and 0.5 μm to 5 μm long. This organism can change into the coccoid form when exposing to the unpleasant condition or being in the stationary growth phase. With its unipolar or bipolar flagella, *Campylobacter* can exhibit darting or corkscrew-like movement (Levin, 2007). This fastidious bacterium needs to grow under microaerobic atmosphere which contains low level of oxygen (approximately 5%) (Silva et al., 2011). Generally, it unable to grow at temperature below 30°C and above 45°C, while its optimal temperature is between 37°C to 42°C. *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* are described as thermotolerant *Campylobacter* (Silva et al., 2011).

2.2 Ecological distribution and epidemiology of *Campylobacter*

Campylobacter can be extensively found in various sources, particularly in the intestinal tract of animals. Although *Campylobacter* lack of the ability to multiply when being outside the animal host, it can survived in several environment condition such as farm equipment, surface water and marine aquatic environment (Jokinen et

al., 2011). Domestic animal and wildlife were considered as the primary sources of *Campylobacter* shedding into environment.

2.2.1 Epidemiology of *Campylobacter* in human

Symptoms of foodborne campylobacteriosis in humans are characterized by watery diarrhea, abdominal cramp and fever (Schonberg-Norio et al., 2010). The infective dose of this foodborne pathogen is relatively low, which is approximately 500 - 800 cells of bacteria (Young et al., 2007). Once humans are infected with *Campylobacter*, symptoms usually develop within 24 - 72 hours (Zilbauer et al., 2008). Severity of the symptom depends on the virulence of the bacteria and susceptibility of the patients. Generally, this gastrointestinal disease is self-limiting and rarely required the antibiotic treatment. Although the illness could recovered spontaneously, several complications of *Campylobacter* infection including Guillain-Barré syndrome (GBS), reactive arthritis, post-infectious irritable bowel syndrome and potentially immunoproliferative small intestinal disease, could occur, particularly in elderly people (Baker et al., 2012). GBS which is an acute demyelination of the peripheral nerve causing acute flaccid paralysis was frequently reported (Zilbauer et al., 2008).

Campylobacter was the most common causative bacterial agent associated with human gastroenteritis in industrialized world (Scallan et al., 2011). *Campylobacter jejuni* was considered as the major cause of *Campylobacter* infection in human. The incidence rate of *C. jejuni* infection in EU member countries rose from

43.9 cases per 100,000 populations in 2008 to 45.6 per 100,000 populations in 2009 (EFSA, 2011a). In the United Kingdom, the number of *Campylobacter* infected cases increased significantly over the last 20 years from 33,280 cases in 1989 to 64,582 cases in 2011 (Nichols et al., 2012). In US, it was estimated that 2.4 million of *Campylobacter* infection cases were annually occurred. Unfortunately, incidence of this illness is not routinely recorded in developing countries. In Thailand, 28% of children admitted to hospitals with mucous bloody diarrhea between 1998 and 2000 were infected with *C. jejuni* (Bodhidatta et al., 2002). Moreover, this organism was reported as the major cause of diarrhea in people who travelled to Thailand (Serichantalergs et al., 2010).

Campylobacter infection was frequently associated with consumption of contaminated water and food, particularly food of animal origin. Unpasteurized milk was described as the cause of *Campylobacter* infection of several outbreaks. *Campylobacter* was isolated from various types of animal meat or aquatic product such as pork, lamb, beef, shell fish, etc. Handling, preparation or consumption of poultry meat was considered as the major cause of human infection (Hussain et al., 2007; Wilson et al., 2008).

2.2.2 Epidemiology of *Campylobacter* in domestic animals

Campylobacter is commonly found in intestinal tract of warm-blooded animals as the commensal organism, particularly in avian species. In some cases, the illness caused by *Campylobacter* was reported in young domestic animals such as

dog, cat and piglet (Newell and Fearnley, 2003). In other hand, asymptomatic infection frequently occurred in food animal, such as chicken, cattle and swine. High prevalence of *Campylobacter* was described in domestic poultry species which was considered as the significant source of *Campylobacter* transmission in human.

2.3 Prevalence of *Campylobacter* in commercial broilers

Campylobacter contamination in poultry meat production has been extensively studied. In Europe, the European Food Safety Authority (EFSA) reported that *Campylobacter* contamination in broiler carcasses in member countries, e.g., Germany, Greece, Netherland, Spain, Sweden and United Kingdom, ranged from 4.9% to 100% (EFSA, 2011). In the US, the prevalence of *Campylobacter* contamination on retail broiler meats varied among states ranging from 41.0% to 61.3% (Zhao et al., 2001; Williams and Oyarzabal, 2012). In Oceania, the prevalence of *Campylobacter* isolated from New Zealand retail chickens was 69.7% (NZFSA, 2011). In Asia, the rate of *Campylobacter* contamination in retail broiler meats was similar to those of other parts of the world (Luu et al., 2006; Suzuki and Yamamoto, 2009; Rahimi et al., 2010; Lay et al., 2011). In Vietnam, 31% of retail chickens were contaminated with *Campylobacter* (Luu et al., 2006), while 80.9% of broiler products of Cambodia were contaminated with this organism (Lay et al., 2011). In Thailand, *Campylobacter* contamination rate in retail broiler meats ranged from 15.0 to 90.6 percent. Saengthongpinit et al. (2010) and Meeyam et al. (2004) reported that 61.3% of retail

chickens in the central and 90.6% of chicken meats from fresh markets in the northern were contaminated with *Campylobacter*. In Bangkok, 15.0% of chicken meats from fresh markets and 35.0% of chicken meats from supermarkets were positive for *Campylobacter jejuni* (Vindigni et al., 2007).

High prevalence of *Campylobacter* in broiler flocks has been reported in several countries, such as United Kingdom (75.3%), France (76.1%) and Spain (88.0%) (EFSA, 2011). Similarly, high colonization rate of *Campylobacter* in broiler batches was also reported in the US (87.5%) (Hiett et al., 2002b). In Asia, the prevalence of *Campylobacter* in broiler flocks varied from 11.2 to 83.3 percent (Meeyam et al., 2004; Ansari-Lari et al., 2011; Sasaki et al., 2011; Rejab et al., 2012). Study in Iran revealed that 76.0% of broiler flocks were colonized with *Campylobacter* (Ansari-Lari et al., 2011), whereas 43.5% of Japanese broiler flocks were *Campylobacter* positive (Sasaki et al., 2011). The prevalence of *Campylobacter* in Malaysian broiler flocks was relatively high (83.3%) (Meeyam et al., 2004; Rejab et al., 2012), while lower level of colonization rate was reported in Vietnam (31.90%) (Carrique-Mas et al., 2014). In Thailand, *Campylobacter* colonization rate in broilers was described ranging from 11.2 to 64.0 percent (Padungtod and Kaneene, 2005; Chokboonmongkol et al., 2013).

2.4 Distribution and molecular epidemiology of *Campylobacter* in broiler production chain

Although *Campylobacter* colonization in broilers possibly occur via either vertical transmission or horizontal transmission, several studies suggested that vertical transmission is not likely to be the main route of *Campylobacter* transmission in poultry (Pearson et al., 1996; Callicott et al., 2006; O'Mahony et al., 2011). Breeder flocks were found to be highly colonized with *Campylobacter*, but this organism was rarely recovered from fertile eggs (Sahin, 2003). Natural transmission of *Campylobacter* through the egg was rarely occurred due to the inability to penetrate the egg shell (Shanker et al., 1986; Sahin et al., 2003). Unlike vertical transmission, horizontal transmission seems to be more important for *Campylobacter* transmission in broiler production chain. Many studies suggested that potential origins of *Campylobacter* on broiler farms might be drinking water, farm workers, domesticated animals near the broiler farms, wild animals, insects, pests and organic matter from previous flock (Newell and Fearnley, 2003; Bates et al., 2004; Hald et al., 2004; Ridley et al., 2011). Hald and colleagues (2004) described the genetic similarity between isolated from flies around broiler houses and those from broilers. Likewise, Bull and colleagues (2006) found that genotype of *Campylobacter* isolated from environmental samples including feed, water, drinker and air was similar to *Campylobacter* strains from chickens. Similarly, Messens and colleagues (2009) revealed that *Campylobacter* isolated from nipple water had the same

genotypic pattern with the strains cultured from cecal samples of broilers. The carry-over of *Campylobacter* in positive flock transmitted to the new consecutive flock was proposed and proven by *fla* typing and pulsed-field gel electrophoresis (PFGE) in the study of Shreeve and colleagues (2002). In addition, some studies also reported that transport cages can be the source of *Campylobacter* contamination in broiler production (Hansson et al., 2005; Ellerbroek et al., 2010). Although many possible sources of *Campylobacter* on broiler farms were suggested in previous studies, the exact origins were still unclear (Messens et al., 2009).

Contamination of *Campylobacter* frequently reported in slaughterhouses environment and broiler meat products (Miwa et al., 2003; Takahashi et al., 2006; Melero et al., 2012). Intestinal content of chicken was considered as the initial sources of *Campylobacter* in slaughterhouses. During slaughtering process, *Campylobacter* can be recovered from scalding water, defeathering machines, chilling water and eviscerating tools (Miwa et al., 2003; Peyrat et al., 2008; Figueroa et al., 2009). Unsurprisingly, cross-contamination between broiler flocks usually arise from insufficient cleaning and disinfection procedure of processing plants (Peyrat et al., 2008). *Campylobacter* contamination in slaughterhouse can be reduced by proper hygienic operation or treatment of the carcasses. One of the effective measures to prevent and control of *Campylobacter* contamination is reducing the load of *Campylobacter* carried into slaughterhouses (Reich et al., 2008).

2.5 Molecular techniques for genetic characterization of *Campylobacter*

To reveal genetic diversity in epidemiological investigation of *Campylobacter*, genotyping methods were gradually developed for several decade, e.g., multilocus enzyme electrophoresis (MLEE), repetitive element sequence-based PCR (rep-PCR), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism analysis of the *flaA* gene (*flaA*-RFLP), sequencing of the short variable region of the *flaA* gene (*flaA* SVR sequencing), pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Repetitive element sequence-based PCR is PCR technique which identified bacterial genotype by targeting on repeated DNA sequences of bacteria such as repetitive extragenic palindromic elements, enterobacterial repetitive intergenic consensus elements and BOX elements (Giesendorf et al., 1994; Hiett et al., 2006; Patchanee et al., 2012). Restriction fragment length polymorphism of *flaA* gene or *flaA*-RFLP differentiates bacterial genotype by fragmenting on *flaA* gene which encodes the flagellin protein of *Campylobacter* (Harrington et al., 2003). This gene is also used for identify *Campylobacter* genotype by determining the sequence of short variable region of *flaA* gene or *flaA* SVR sequencing (Meinersmann et al., 1997b). Although pulse field gel electrophoresis or PFGE is the gold standard method for *Campylobacter* genotyping, determination of bacterial genotype by sequencing of house-keeping genes or MLST is becoming popular in the recent decade (Pittenger et al., 2009). With this method, the information of *Campylobacter* epidemiology in local and global

scale could be comparable via central internet database (Levesque et al., 2008; Colles and Maiden, 2012). However, to get the most reliable information, more than one genotyping techniques should be performed (O'Mahony et al., 2011).

2.6 Distribution of *Campylobacter* sequence types in human and poultry sources

As one of the most reproducible genotyping techniques, MLST was widely applied for epidemiological investigations for *Campylobacter*. According to previous findings, *C. jejuni* population comprise of many clonal complexes which were distantly related to each other. In contrast, *C. coli* population could be divided only into three distinct clades (Colles and Maiden, 2012). ST-21 complex was extensively identified in wide-ranging sources, particularly in domestic animals and human. Moreover, this clonal complex was considered to be associated with human infection worldwide and has been reported as a common clonal complex in poultry. Similarly, ST-45 complex is known as one of the most common clonal complexes identified in human cases, various types of animal hosts and environmental samples. There is evidence indicating that members of the ST-45 complex were environmentally adapted strains, which can survive under unfavorable conditions better than other strains (Sheppard et al., 2007). Similar to the ST-45 complex, the ST-353 one was also mentioned as one of the common clonal complexes recovered from human cases

and poultry (Sheppard et al., 2009). ST-354 and ST-574 were reported as the predominant strains found in human and poultry samples of Thailand.

2.7 Risk factors associated with *Campylobacter* colonization in broiler flocks

To identify possible risk factors associated with *Campylobacter* colonization in broiler flocks, cross-sectional survey and cohort study was conducted in several studies (Barrios et al., 2006; Hansson et al., 2010; Agunos et al., 2014). Management in farms, such as partial depopulation, poor biosecurity, sanitary practices, age of birds and flock size, was identified to be the important risk factors for *Campylobacter* colonization in broiler farms (Lawes et al., 2012). Likewise, untreated drinking water use on broiler farms was revealed as a possible factor associated with *Campylobacter* colonization (Sasaki et al., 2011). In addition, the presence of animal reservoirs, e.g., insects, pests, domestic and wild animals on or near broiler farms was significantly associated with *Campylobacter* colonization in broilers (Lyngstad et al., 2008; McDowell et al., 2008; Ellis-Iversen et al., 2009). Hygiene barriers and pest interventions were investigated to be the protective factors of *Campylobacter* colonization in broiler flocks (Hald et al., 2000; Hald et al., 2007). These findings emphasized that farm management is involved with *Campylobacter* colonization in broiler flocks. Seasonality of *Campylobacter* prevalence in broiler production was reported by several publications, particularly in temperate zone. In northern hemisphere, number of *Campylobacter* colonization in broiler flocks was relatively

low, while prevalence of positive flock increased sharply in summer time. Similarly, *Campylobacter* contamination rate in retail chicken meat also exhibited in the seasonal pattern (Boysen et al., 2011).

2.8 Logistic regression model and generalized estimating equations

Identification of causal and disease relationship is always considered as the main objective in epidemiological study. To achieve the most accurate result, the statistical method that is the most appropriate for the characteristic of data is needed. As the most popular method among others in epidemiological investigation, logistic regression is the mathematical model that can be used to identify the association between multiple independent variables and a dichotomous dependent variable, such as disease (Hosmer and Lemeshow, 2000). The function of this model, called f(Y), is in the basic logistic regression formula as below:

$$f(Y) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k \qquad \text{Eq. 1}$$

Let Y = response variable or dependent variable, X = independent variable, $\beta_1, \beta_2, \ldots, \beta_k$ = regression parameters and α = intercept.

The output of logistic regression is the estimation of risk (probability) which is always ranging from 0 to 1 depending on the value of Y, while the value of Y could vary from $-\infty$ to ∞ (Figure 1). In term of epidemiological study, the prediction of probability gives the risk of the individual to get disease.

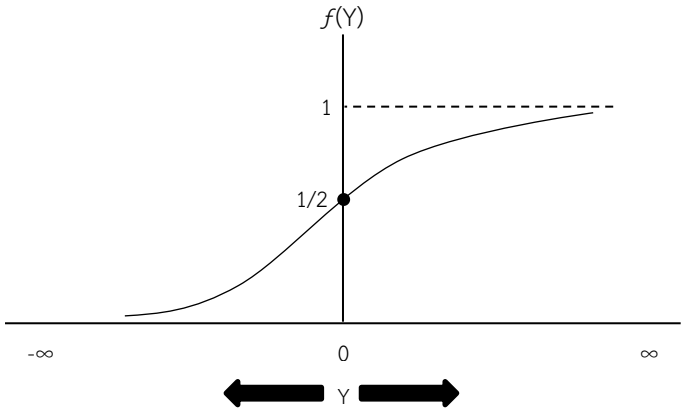


Figure 1 Range of function f(Y) depending on the value of Y

Similar to other statistical approaches, several assumptions are needed to be confirmed before operating the logistic regression model. If the assumption cannot be met in some cases, such as clustered or repeated data, alternative approaches should be carried out in order to avoid the incorrected conclusion. Generalized estimating equation (GEE) is known as the common technique used in large epidemiological studies because of its ability to handle many types of unmeasured data. This method is the extension of generalized linear models (GLMs) and classified as the semiparametric regression technique (Liang and Zeger, 1986). The basic formula of GEE (Eq. 2) is similar to GLM but full specification is not required.

$$P(Y) = \sum_{i=1}^{k} X_i^T \beta \qquad \text{Eq. 2}$$

Estimation of the parameter is commonly performed by quasi-likelihood equations without the assumption of normal distribution on dependent response. For this technique, multi-variables can be included within single analysis. To provide the correct estimation, choosing the right correlation structure is necessary. There are

four correlation structure that frequently be used; independence, exchangeable, autoregressive of first order and unstructured (Figure2).

$$R(\rho) = \begin{bmatrix} 1 & 0 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & 0 \end{bmatrix}$$ independence

$$R(\rho) = \begin{bmatrix} 1 & \rho & \cdots & \rho \\ \rho & 1 & \cdots & \rho \\ \vdots & \vdots & \ddots & \vdots \\ \rho & \rho & \cdots & 1 \end{bmatrix}$$ exchangeable

$$R(\rho) = \begin{bmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{bmatrix}$$ autoregressive of first order

$$R(\rho) = \begin{bmatrix} 1 & \rho_{12} & \rho_{13} & \rho_{14} \\ \rho_{12} & 1 & \rho_{23} & \rho_{24} \\ \rho_{13} & \rho_{23} & 1 & \rho_{34} \\ \rho_{14} & \rho_{24} & \rho_{34} & 1 \end{bmatrix}$$ unstructured

Figure 2 Correlation structures that commonly used for GEE approach

CHAPTER III
MATERIALS AND METHODS

This study consisted of two major phases; 3.1) prevalence and risk factors associated with *Campylobacter* in broiler flocks, and 3.2) genetic relatedness of *Campylobacter* isolated from broiler production chain (Figure 3). For phase 1, *Campylobacter* colonization status of twenty broiler farms was investigated consecutively for two years. Criteria of farm selection were including farm location (within 4 - 5 hours distance to Bangkok), cooperation of farm owner and production capacity of broiler farms (approximately 5 production cycles per year). Amongst 20 selected farms, 6 broiler farms were selected to be the subject for longitudinal investigation throughout the broiler production chain. *Campylobacter* positive status of broiler flocks, farm location and willingness of farm owner to participate in the study were considered as the criteria of target farms in phase 2.

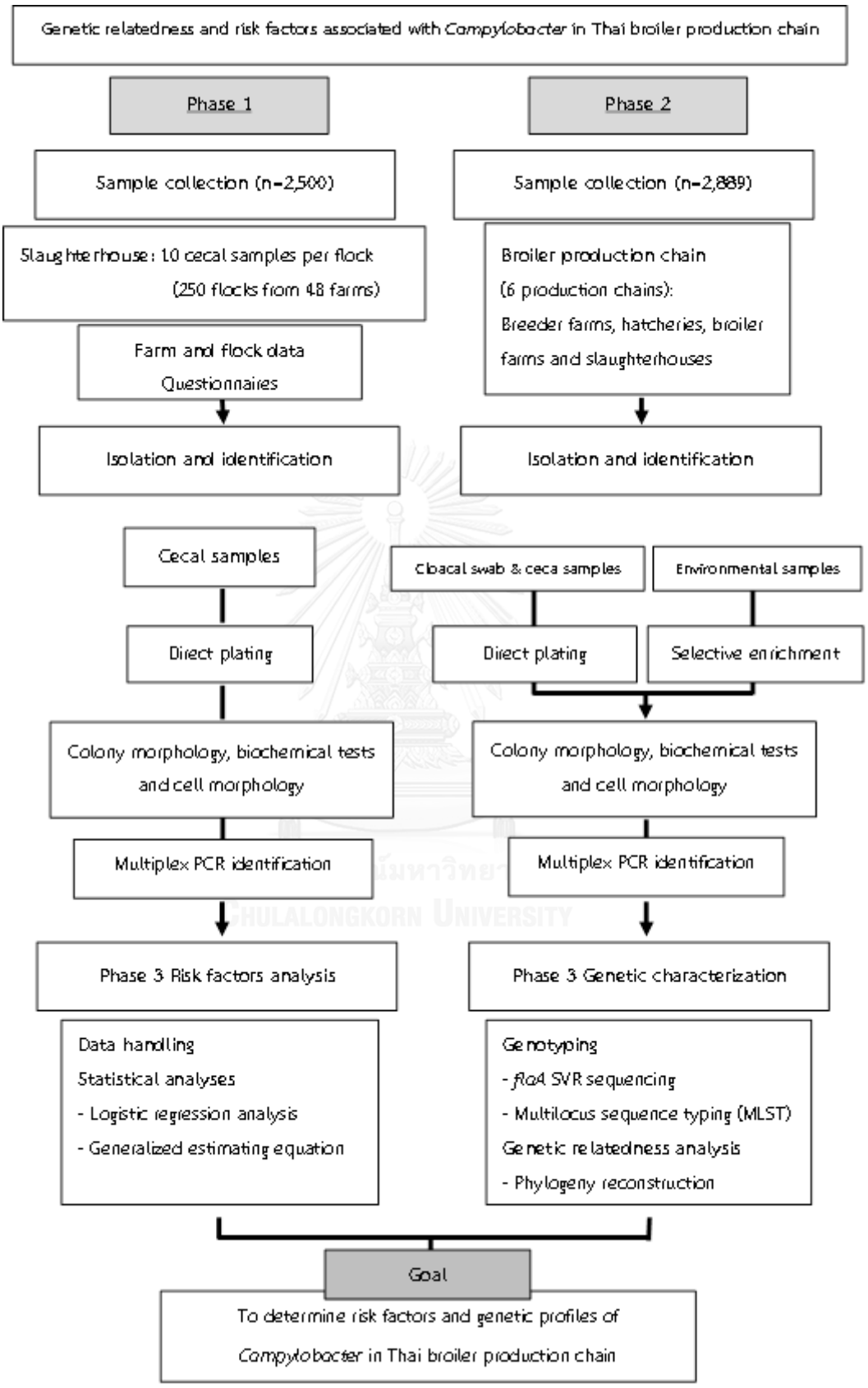


Figure 3 General outline of the study

3.1 Prevalence and risk factors associated with *Campylobacter* in broiler flocks

In this study, *Campylobacter* colonization status of 20 broiler flocks was determined and information of each broiler farm was also collected. Information will be analyzed by statistical method to identify risk factors associated with *Campylobacter* colonization in broiler flocks.

3.1.1 Sample collection

Examined population in this study consisted of 250 broiler flocks from 48 broiler farms, which belong to two integrated poultry production companies; company A and B/C (Figure 4). To conduct the preliminary survey, *Campylobacter* colonization of 48 broiler farms were consecutively investigated for 2 production cycles. After that, 20 broiler farms were selected with the criteria of farm location (in central or eastern of Thailand), production capacity of the farm (approximately 5 production cycles per year) and cooperation of farmer to provide the farm data. Two-year sample collection was continuously conducted on 20 broiler farms in order to display the pattern of *Campylobacter* colonization throughout the year. Ten intact ceca per flock were collected in 2 participating slaughterhouses which were mainly located at the central and eastern part of Thailand. Chicken intestines were aseptically removed from the carcasses during the evisceration step, and then separately put into sterile plastic bag. Samples were kept on ice before laboratory process.

To obtain farm and flock-specific information, questionnaires (Appendix B) were constructed and modified according to previous studies. The structured questionnaires including farm management data (e.g., antibiotic usage, pest control and restriction of domestic animals), farm layout (e.g., house structure, house condition and feeding system), sanitary practice (e.g., carcasses disposal, frequency of boot dip disinfectant change and type of disinfectant used) and animal welfare practice (e.g., feed withdrawal period, flock density and light management) were used to obtain information from sampled broiler flocks. Data collection of examined flocks was performed by well-trained veterinarians or farm staffs.

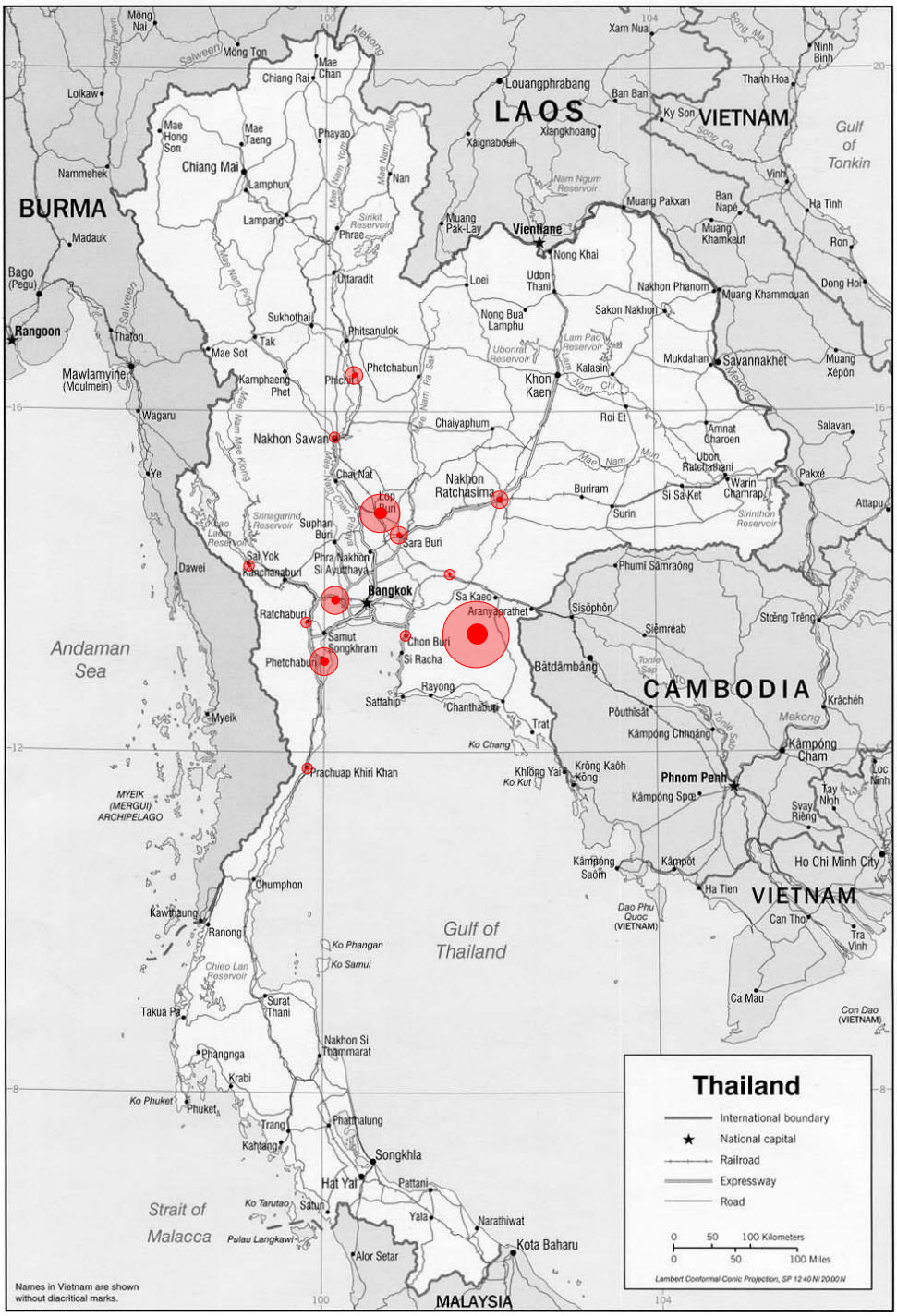


Figure 4 Location of target broiler farm participating in this study (red). The shape size indicates the number (density) of examined flocks located in each province (The picture was taken from www.bangkok-market.com)

3.1.2 Isolation and identification of *Campylobacter*

Campylobacter isolation in cecal samples was performed by the direct plating method according to the previous published protocol (Hook et al., 2005). Ceca were aseptically incised, then cecal content were directly streaked onto Modified Charcoal-Cefoperazone-Deoxycholate agar or mCCDA (CM0739; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) supplemented with *Campylobacter* selective supplement (SR0155; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). For bacterial enumeration, cecal content was diluted in normal saline solution and inoculate onto mCCDA. The inoculated plates were incubated at 42°C for 48 hours under microaerobic conditions (5% of oxygen, 10% of carbon dioxide and 85% of nitrogen). Suspected *Campylobacter* colonies (greyish, metallic sheen, flat and moist) were primarily confirmed by their cell morphology according to the ISO 10272-1: 2006 standard. *Campylobacter* species were identified by multiplex-PCR method according to the previous published protocols as in Table 1 (Linton et al., 1996; Wang et al., 2002). *Campylobacter* suspected colonies were suspended in 100 µl of distilled water (Hyclone®, Thermo Scientific, Utah, USA). Cell mixtures were heated at 100 °C for 10 minutes and centrifuged to separate cell debris. The supernatant was used as DNA template in PCR reaction (25 µl) containing 2.5 µl of 10x reaction buffer; 200 µM of deoxynucleoside triphosphate; 0.5 µM of *C. jejuni* and *C. coli* primers; 25 ng of DNA template; and 0.625 U of Takara Ex Taq TM (Takara Bio Inc., Japan). PCR was performed in a thermocycler (Biometra GmbH, Germany) under the conditions as

follows: 30 cycles of denaturation at 94 °C for 1 minute, amplification at 58 °C for 1 minute and extension at 72 °C for 1 minute. PCR products were examined on 1.5% agarose gel in 1xTris-acetate-EDTA (TAE) buffer and visualized by ultraviolet transilluminator. *Campylobacter* positive status of broiler flock was determined by the presence of *Campylobacter* colonies in cecal samples.

Table 1 Set of primers for *Campylobacter* identification

Primer	Sequence (5'-3')	Size (bp)
16SF	GGA TGA CAC TTT TCG GAG C	816
16SR	CAT TGT AGC ACG TGT GTC	
CJF	ACT TCT TTA TTG CTT GCT GC	323
CJR	GCC ACA ACA AGT AAA GAA GC	
CCF	GTA AAA CCA AAG CTT ATC GTG	126
CCR	TCC AGC AAT GTG TGC AAT G	

3.1.3 Risk factors analysis

Data obtained from broiler farms was verified and entered into a Microsoft Excel database (Microsoft Corporation). Frequency, mean, standard error and confident interval were calculated using online GraphPad Prism® (GraphPad Software, Inc.). Possible explanatory variables were combined with *Campylobacter* colonization status of broiler flocks to identify the risk factors associated with *Campylobacter*

colonization in broiler flock. The data was grouped into 2 groups by season i.e., wet season (May to October) and dry season (November to April). The difference of *Campylobacter* prevalence between seasons was determined by chi-square test. In addition, the records of climatic factors (i.e., rainfall, ambient temperature and relative humidity) during the rearing period of each examined flocks was obtained from Thai Meteorological Department (TMD).

Since broiler farms were investigated continuously for 2 years in order to describe the pattern of *Campylobacter* colonization throughout the year, the response data in this study should be clustered by farm. However, there is still unclear whether the response variables of these flocks are related to each other since broiler flocks from each production cycles were not actually the same individual. Thus, to identify risk factors associated with *Campylobacter* in broiler flocks, logistic regression model (for independent data) and generalized estimating equation (for repeated data) were performed (Liang and Zeger, 1986; Hosmer and Lemeshow, 2000). Statistical analysis procedures were carried out using SAS version 9.0 (SAS institute Inc., Cary, NC). *Campylobacter* colonization status (positive or negative) was considered as the dependence or response variable (Y) which was defined as follows: Y=1 if *Campylobacter* positive and Y=0 if *Campylobacter* negative. Similarly, independence variables (farm and flock characteristic data) were defined as X=1 if the factor was found and X=0 if the factor was do not found. The general forms of those models are described as below (Eq. 3 and 4):

$$f(Y) = \log(\frac{p_{ij}}{1-p_{ij}}) \quad \text{Eq. 3}$$

Let $\beta_1, \beta_2, \ldots, \beta_k$ are regression parameters or estimating values, and α is the intercept of prediction model as below:

$$f(Y) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k \quad \text{Eq. 4}$$

For logistic regression model (proc LOGISTIC), univariate analysis was used as a screening method for measuring associations between explanatory variables and *Campylobacter* colonization of broiler flocks. In univariate procedure of logistic regression model, Wald test and chi-square test were conducted in order to crudely estimate the statistical association between exposure variables and *Campylobacter* colonization status. The significant exposure variables, which were reasonable in biological or statistical aspect, were further tested by multivariable analysis. In multivariable analysis, stepwise selection was used to complete the model developing process. Test for multicollinearity among variables was performed to confirm that variables in the model are not related to each other. Null hypothesis of the model was tested by likelihood ratio statistic. Hosmer and Lemeshow goodness of fit test was performed to determine how well the model fits to the studied data.

For GEE analysis, repeated statement (**repeated subject**=*subject-effect*/**options**) in **GENMOD** procedure was used for activating the GEE command to measure the correlation between *Campylobacter* colonization in broilers and the variable. In this study, the working correlation structure was specified through the **type = EXCH** option for an exchangeable structure. In addition, **within=within-**

subject option is used to specified an ordering for unequally spaced repeated measures or repeated measures with missing time points. To calculate odd ratio estimation of independent variables, '**estimate**' command were applied. Similar to logistic regression model, the formula used to transform the coefficient value into odd ratio is display as follow:

$$OR = e^{b} \text{ or } OR = \exp(\beta) \quad \text{Eq. 5}$$

Univariate analysis was applied to all independent factors to screen only the factors possibly associated with dependent variable ($p \leq 0.05$). Then, GEE model with multiple factors was performed. Stepwise backward elimination procedure was manually conducted until the most suitable model is achieved. Test for multicollinearity among variables was applied to confirm whether variables in the model are not related to each other. Goodness-of-fit test was determined by Pearson chi-square/DF and mean deviance. Finally, two types of model based estimators (i.e., Model-based and Empirical variance estimators) were used to determine how well the GEE model correctly fit to the studied data.

3.2 Distribution and genetic relatedness of *Campylobacter* isolated from poultry production chain

3.2.1 Description of examined farms

During June to August 2012, six chicken production chains (chains A, B, C, D, E and F) of two poultry companies in Thailand (companies A and B/C) were

chronologically investigated from breeder farm to slaughterhouse (Table 2). However, because of refusal of the company, we could not conduct the investigation in slaughterhouse of chain F. In this study, chicken production units (i.e., breeder farms, hatcheries, broiler farms and slaughterhouses) were located distantly from each other. Investigated broiler flock was supplied by single breeder farm affiliated with the same company. In company 1, fertile eggs from breeder flocks B and C were sent to the same hatchery, while sample collection in hatchery of chain A was taken place in another one. For company 2, fertile egg of three production chains (chains D, E and F) were sent to the same hatchery located in the north eastern province. Broiler farms A, D and E were located in the eastern region of Thailand, while broiler farms B and C were located in the central region. Only broiler farm F was located in the north eastern part of Thailand. Size of broiler farms ranged from 11,200 square meters (farm D) to 384,000 square meters (farm F). Broiler farm A is an antibiotic-free farm with the production capacity of 100,000 chickens per year. Broiler farms B and C are located next to each other. Farm B consisted of 10 houses and produced approximately 1,000,000 chickens per year, while farm C was composed of 7 houses and produced around 700,000 chickens per year. Unlike farms A, B and C, broiler farms D and E had only 1 house with the production capacity of 93,000 and 60,000 chickens per year, respectively. Amongst participating farms, broiler farm F had the largest farm area (384,000 square meters) which could produce 160,000 chickens per year with their four rearing houses. Slaughter age of studied broiler flocks ranged

between 32 to 42 days. Flocks A, B and C were slaughtered in large scale processing plants, whereas flocks D and E were slaughtered in a small scale plant.

Table 2 Information of six broiler production chains participating in this study

Flock	Company	Breeder farm	Hatchery	Broiler farm			Slaughterhouse
				Code name	Farm location	Number of house in farm	
A	A	Br1	H1	A	Prachinburi	2	S1
B	A	Br2-1*	H2	B	Lopburi	10	S2
C	A	Br2-2*	H2	C	Lopburi	7	S2
D	B/C	Br3-1#	H3	D	Prachinburi	1	S3
E	B/C	Br3-2#	H3	E	Prachinburi	1	S3
F	B/C	Br4	H3	F	Nakhon Ratchasima	4	NS

*, # Reared in the same farm but not in the same flock

NS not sampling

3.2.2 Sample collection

In this study, samples were longitudinally collected from breeder farms to slaughterhouses (Table 3). In total, 2,889 samples from breeder flocks, hatcheries, broiler flocks and slaughterhouses were collected from six broiler production chains. *Campylobacter* colonization in breeder flock was determined by cloacal swab samples. Eggs produced from previously sampled breeder flocks were tracked to hatcheries. Egg trays and egg incubators exposed to target egg batches were swabbed on their surface. Egg shell was randomly taken after chicks were hatched. Prior to chick placement, environmental samples of disinfected house were investigated to determine the contamination of *Campylobacter*. Feces-soiled tray liners were collected on the day of chick arrival. Broiler flocks were visited regularly during the rearing period as described in Figure 5. Cloacal swabs from live birds and environmental samples (litter, water from nipple drinkers, water inlet and shoe covers) were taken on each visit. Insects and other pests in farming area were captured as available.

At slaughterhouse, disinfected transport crates were swabbed before being used. Slaughterhouse equipment were sampled at before and after slaughter process of target flock (Figure 5). Three areas on breast comforter surface were randomly swabbed lengthwise. Shackles were sampled at hanging area and evisceration area. Eviscerating equipment and packaging tables were wiped thoroughly. Water samples

were collected from bird washing machine, inside/outside washing machine and chiller tanks. For chicken related samples, cloacal swabs from live birds were collected before they were slaughtered. Carcass rinse was performed after scalding, plucking, evisceration, I/O washer and chilling steps using buffered peptone water. Intact ceca were randomly taken at evisceration area. Meat products from post-chilled chicken i.e., carcass portioning and meat trimming were investigated. All samples were kept on ice during transport to the laboratory and processed within 4 hours after sampling.

Table 3 Sample collection plan of this study

	Estimated number of samples collected per flock	Number of studied flocks	Estimated total number of samples
Breeder flock			
- Cloacal swabs	30	6	180
Hatchery			
- Equipment and environmental samples (e.g., egg tray, incubator and tap water)	30	6	180
Broiler house			
Before rearing period			
- Boot swab samples [a]	5	6	30
- House equipment and environmental samples (e.g., feeder, litter, boots and water)	25	6	150

Table 3 Sample collection plan of this study (Cont.)

	Estimated number of samples collected per flock	Number of studied flocks	Estimated total number of samples
During rearing period (approximately 6 weeks)			
- Boot swab samples [b] (5 samples/week)	30	6	180
- House equipment and environmental samples (e.g., litter, water, pests and feed) (15 samples/week)	90	6	540
- Cloacal swabs (30 samples/week)	180	6	1,080
Slaughterhouse [d]			
- Cloacal swabs	10	5[d]	50
- Equipment and environmental samples (e.g., shackle, chilling water, tap water, etc.)[c]	90	5[d]	450
Total (Approximate)	490		2,840

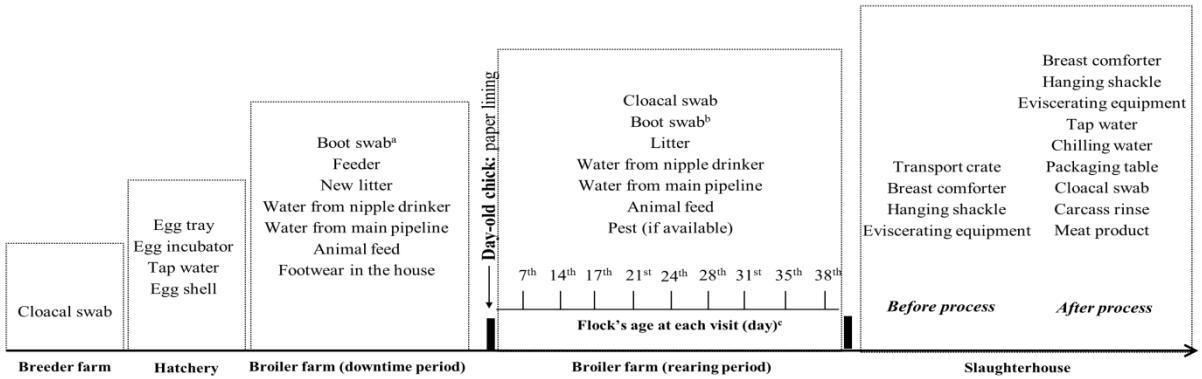
[a] Area of boot swab sampling at downtime period: anteroom of the target house, inside target house and area around the house.

[b] Area of boot swab sampling during rearing period: path-leading to the house, anteroom of the target house, inside the target house, area around the house and inside the adjacent house.

[c] Samples were collected before and during slaughtering process of the selected flock.

[d] Only 5 flocks could be investigated at slaughterhouses.

Figure 5 Types of sample collected throughout the broiler production chain



[a] Area of boot swab sampling at downtime period: anteroom of the target house, inside the target house and area around the house.

[b] Area of boot swab sampling during the rearing period: path-leading to the house, anteroom of the target house, inside the target house, area around the house and inside the adjacent house.

[c] Flocks D and E were visited at 7th, 14th, 21st, 28th, 35th day of the rearing period, while other flocks were visited at 7th, 14th, 17th, 21st, 24th, 28th, 31st, 35th, 38th day of the rearing period.

3.2.3 *Campylobacter* isolation and identification

Samples were examined by direct plating and selective enrichment methods. The direct plating method was used for *Campylobacter* isolation from cloacal swab and cecal samples (Hook et al., 2005). In brief, samples were streaked directly onto *Campylobacter* blood-free selective agar (CM0739; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) supplemented with *Campylobacter* selective supplement (SR0155; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). Samples were incubated at 42 ˚C for 48 hours under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2).

Environmental and meat samples were examined by selective enrichment culturing method. Samples were transferred into Exeter broth consisting of nutrient broth No. 2 (CM0067; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), *Campylobacter* growth supplement (sodium metabisulphite, 250 mg/liter; sodium pyruvate, 250 mg/liter; and ferrous sulfate, 250 mg/liter), *Campylobacter* selective supplement (trimethoprim, 10 mg/liter; rifampicin, 5 mg/liter; polymyxin B, 2,500 IU/liter; cefoperazone, 15 mg/liter; and amphotericin B, 2 mg/liter) and 5% sheep blood. One part of animal feed, egg shell, litter, meat products and chilling water were put into nine parts of Exeter broth. Cotton swabs from any surfaces were immersed into 10 ml of broth. A liter of clean water samples (drinking water and tap water) were filtered through 0.45 µm membrane filters (GN-6 Metricel®, Pall, USA). Then, filtered membrane filters were immersed in the 20 ml of Exeter broth. Insects

(darkling beetles and house flies) were crushed, and then added in 10 ml of broth. Rodents and lizards were tested for *Campylobacter* in their feces and skin surface, respectively. Enrichment broths inoculated with samples were incubated under microaerobic conditions for 48 hours at 37˚C. Thereafter, enriched samples were spread onto mCCDA and incubated in microaerobic conditions for 48 hours at 42˚C. Suspected *Campylobacter* colonies were confirmed by cell morphology and multiplex polymerase chain reaction (Linton et al., 1996; Wang, 2002). *Campylobacter* suspected colonies were suspended in 100 µl of distilled water (Hyclone®, Thermo Scientific, Utah, USA). Cell mixtures were heated at 100 ˚C for 10 minutes and centrifuged to separate cell debris. The supernatant was used as DNA template in PCR reaction (25 µl) containing 2.5 µl of 10x reaction buffer; 200 µM of deoxynucleoside triphosphate; 0.5 µM of *C. jejuni* and *C. coli* primers; 25 ng of DNA template; and 0.625 U of Takara Ex Taq TM (Takara Bio Inc., Japan). PCR were amplified in a thermocycler (Biometra GmbH, Germany) under the conditions as follows: 30 cycles of denaturation at 94 ˚C for 1 minute, amplification at 58 ˚C for 1 minute and extension at 72 ˚C for 1 minute. PCR products were examined on 1.5% agarose gel in 1x Tris-acetate-EDTA (TAE) buffer and visualized by ultraviolet transilluminator. In addition, presumptive *Campylobacter* colonies were stored in skim milk with 30% glycerol at -80˚C for further study.

3.2.4 Genetic characterization of *Campylobacter jejuni*

Randomly selected colonies of *Campylobacter jejuni* isolated from each production unit were primarily subtyped by *flaA* short variable region. Representatives of *flaA* SVR genotypes were further characterized by multilocus sequence typing (MLST). DNA extraction procedure was performed using Wizard® Genomic DNA purification kit (Promega, Madison, USA).

Short variable region of *flaA* gene was amplified with primers FLA242FU (5'-CTA TGG ATG AGC AAT TWA AAA T-3') and FLA625RU (5'-CAA GWC CTG TTC CWA CTG AAG-3') as previously described (Meinersmann et al., 1997a). PCR products were purified by NucleoSpin® Gel and PCR Clean-up kit (MACHEREY-NAGEL, Düren, Germany) and sent for DNA sequencing at First BASE Laboratories (Selangor Darul Ehsan, Malaysia). To determine allelic numbers, nucleotide sequences were submitted into the online database (http://pubmlst.org/*Campylobacter*/flaA/).

MLST was performed according to the previously published protocol (Dingle et al., 2001). Internal fragments of seven housekeeping genes (i.e., *aspA*, aspartase A; *glnA*, glutamine synthetase; *gltA*, citrate synthase; *glyA*, serine hydroxymethyltransferase; *pgm*, phosphoglucomutase; *tkt*, transketolase; and *uncA*, ATP synthase α subunit) were amplified and sequenced (Table 4). Allele numbers, sequence types (STs) and clonal complexes (CCs) were assigned according to the *Campylobacter* MLST database. Phylogenetic reconstruction using neighbour joining

method was performed by importing trimmed sequences into Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0.

Table 4 Set of primers for PCR amplification and sequencing of 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'	

CHAPTER IV
RESULTS

4.1 Prevalence and risk factors associated with *Campylobacter* in broiler flocks

4.1.1 *Campylobacter* colonization in broiler flocks

Of 250 broiler flocks participating in the study, 119 flocks (47.60%; 95% CI 41.41 - 53.79%) were identified as *Campylobacter* positive. Overall, 1,048 *Campylobacter* isolates were recovered from 2,500 cecal samples. The proportion of *Campylobacter* positive cecal samples of each flock was defined as within-flock prevalence which varied from 10 to 100 percent (84.93%, 95% CI 80.77 – 89.09%). In the present study, approximately 80% of broiler flocks had high within-flock prevalence (>75%) (Figure 6). Bacterial enumeration in cecal content ranged from 4.00 $\log_{10}$ to 8.97 $\log_{10}$ CFU per gram (8.06 $\log_{10}$ CFU per gram, SE = 7.49, 95% CI = 7.93 – 8.16). The most of broiler flocks were positive for *Campylobacter jejuni* (84.87%), while only 6.72% of *Campylobacter* positive flocks were colonized with *C. coli*. In addition, the prevalence of mixed infection between *C. jejuni* and *C. coli* was reported as 8.40% (Figure 7).

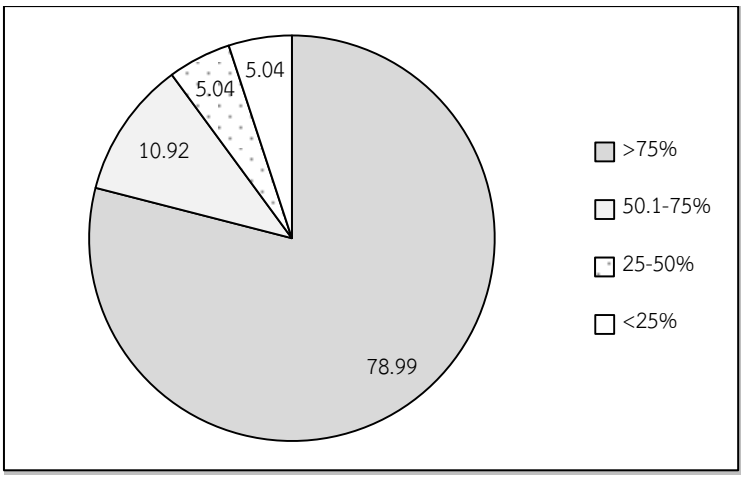


Figure 6 Within-flock prevalence of *Campylobacter* in examined broiler flocks

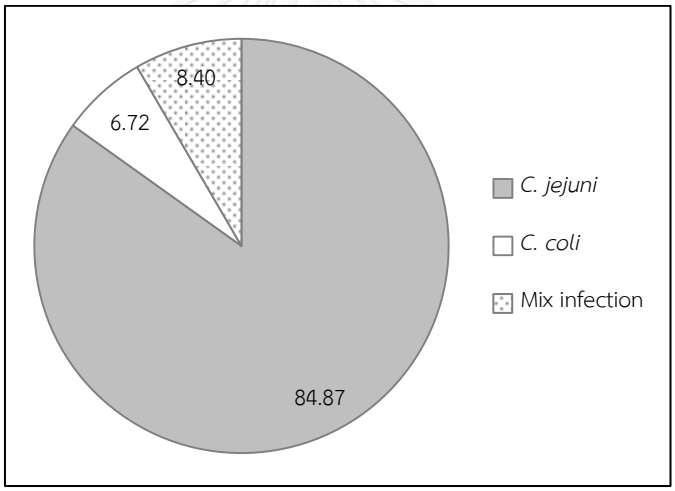


Figure 7 Species identification of *Campylobacter* in examined broiler flocks

To determine monthly prevalence of *Campylobacter* colonization in broiler flocks throughout the year, *Campylobacter* colonization data was categorized by month of sampling (Figure 8). In 2012, *Campylobacter* prevalence was reported ranging from 18.75 to 52.94 percent during January to May, while the sharp increase of *Campylobacter* colonization rate was reported during June to November. Likewise,

in 2013, *Campylobacter* prevalence in July to December was reported from 54.55 to 75.00 percent which was higher than that of the rest in the same year. In 2014, low monthly prevalence was found during January to March and then increase to 100% on April (Figure 8).

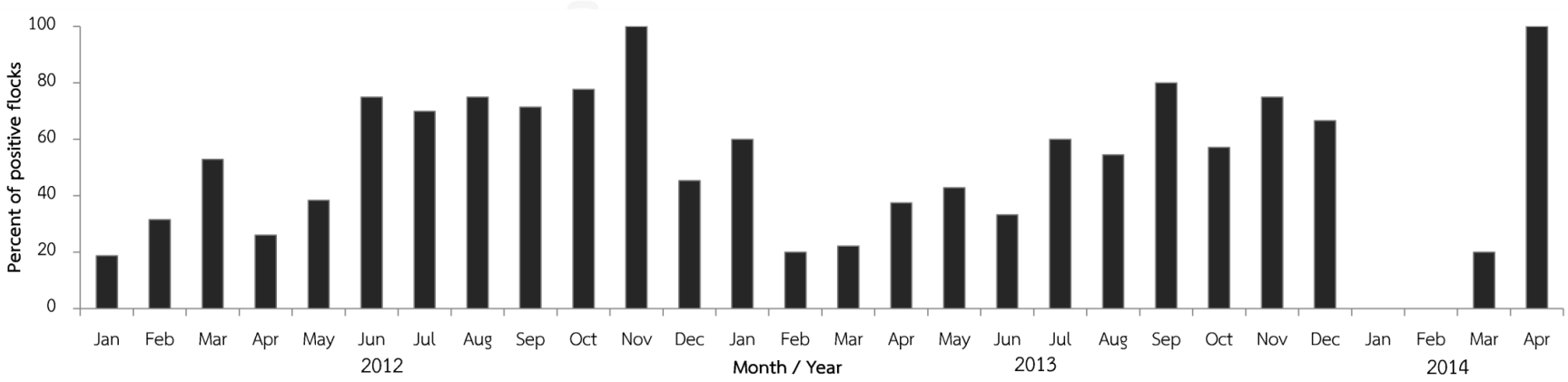


Figure 8 Distribution of *Campylobacter* prevalence in Thai broiler flocks during January 2012 to April 2014

Among 48 preliminary farms, 20 broiler farms were selected for two-year investigation. The actual names of each farm were covered and replaced as A1 – A30 for broiler flock affiliated with company A and B/C1 – B/C19 for broiler flock from company B. Participating broiler farms for two-year investigation were A2, A5, A10, A13, A15, A17, A19, A22, A23, A30, B/C2, B/C3, B/C4, B/C6, B/C7, B/C8, B/C10, B/C11, B/C13 and B/C15. The prevalence of each farm throughout the study was reported between 8.33 and 87.50 percent (Table 5). Farms A5, A17, A22, A23 and B/C6 were highly colonized with *Campylobacter* (from 75.00% to 87.50%), while farms A2, A10 and A30 were colonized once throughout the investigation.

Table 5 Two-year study on *Campylobacter* colonization of 20 Thai broiler farms during 2012 to 2014

Farm	*Campylobacter* colonization status of broiler flocks												Overall prevalence
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle 10	Cycle 11	Cycle 12	(percent)
A2	N	N	N	N	P	N	N	N	N	N	NS	NS	10.00
A5	N	P	P	P	P	P	P	P	P	P	N	N	75.00
A10	N	N	N	N	P	N	N	N	N	N	N	N	8.33
A13	N	N	P	P	P	P	P	N	N	P	N	P	58.33
A15	P	P	P	P	P	P	N	N	N	N	N	N	50.00
A17	N	P	P	P	P	P	P	P	NS	NS	NS	NS	87.50
A19	P	N	N	P	N	P	N	N	N	N	P	N	33.33
A22	P	N	P	P	P	N	P	P	P	P	N	P	75.00
A23	N	P	P	P	P	P	P	P	P	P	P	N	83.33
A30	N	N	N	N	P	N	N	N	N	NS	NS	NS	11.11
B/C2	P	P	P	P	N	P	N	N	NS	NS	NS	NS	62.50
B/C3	N	N	NS	P	NS	N	N	N	P	P	NS	NS	37.50
B/C4	N	P	P	P	P	N	N	N	P	N	NS	NS	50.00
B/C6	P	P	N	P	P	P	NS	NS	NS	NS	NS	NS	83.33
B/C7	N	P	P	P	NS	N	N	N	P	NS	NS	NS	50.00
B/C8	P	P	P	P	P	N	N	N	P	P	P	N	66.67
B/C10	P	N	P	NS	P	P	N	NS	NS	NS	NS	NS	66.67
B/C11	N	N	N	N	P	P	P	P	P	P	NS	NS	60.00
B/C13	P	N	P	P	P	N	N	P	N	NS	NS	NS	55.56
B/C15	P	N	P	P	N	N	N	N	P	P	NS	NS	50.00

P = positive, **N** = negative, **NS** = not sampled

To determine the seasonality of *Campylobacter* in Thai broiler flocks, *Campylobacter* colonization status was repeatedly observed in broiler flocks reared in the same broiler farm for 2 years (during 2012 to 2013). *Campylobacter* colonization data was categorized into 2 groups i.e., wet season (May to October) and dry season (November to April). Out of 250 investigated broiler flocks, 143 flocks were slaughtered in dry season and 107 flocks were slaughtered in wet season. The prevalence of *Campylobacter* was 37.76% (54/143) in dry season and 60.75% (65/107) in wet season (Figure 9). From the raw data of each year (Table 6), most of *Campylobacter* prevalence in dry season was lower than that of wet season. In addition, the statistical analysis show significant difference between prevalence in wet season and prevalence in dry season (chi-square = 12.0590, p = 0.0005).

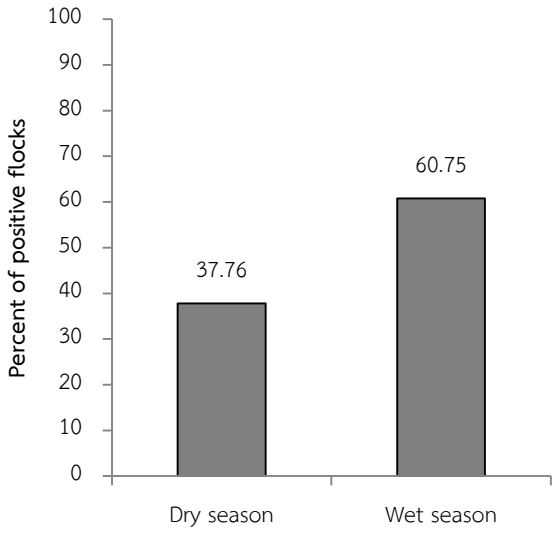


Figure 9 Prevalence of *Campylobacter* in dry and wet seasons

Table 6 *Campylobacter* prevalence in dry and wet seasons

	Season	Prevalence of *Campylobacter* (%)
January 2012 - April 2012	Dry	32.00
May 2012 - October 2012	Wet	66.67
November 2012 - April 2013	Dry	46.94
May 2013 - October 2013	Wet	52.27
November 2013 - April 2014	Dry	36.84

To display the association between climatic factors and *Campylobacter* colonization throughout a year, daily record of 3 climatic factors (i.e., rainfall, ambient temperature and relative humidity) were obtained from Thai meteorological department (TMD). In 2012 and 2013, average daily rainfall during June to November was obviously high comparing to those of the remaining months (Figure 10). Interestingly, prevalence of *Campylobacter* in 2012 was also remarkable during June to November. Similarly, *Campylobacter* colonization rate in 2013 was relatively high during July to December comparing to the remaining months of that year. This finding indicated that *Campylobacter* colonization pattern was consistent to the average daily rainfall. In contrast, the ambient temperature and relative humidity was relatively steady throughout the year (Figures 11 and 12).

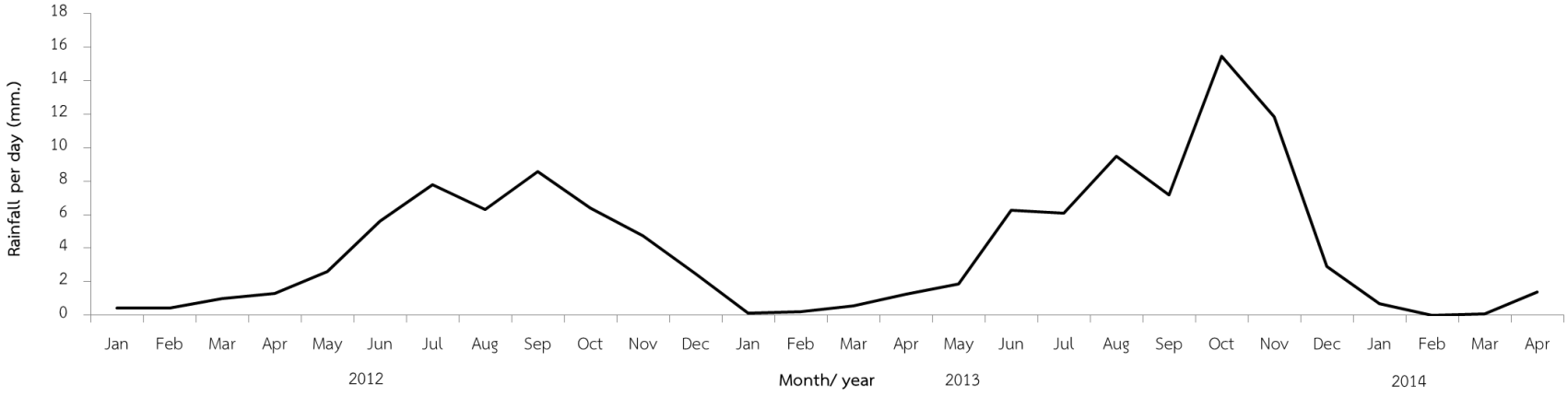


Figure 10 Average daily rainfalls of studied broiler flocks in central region of Thailand during January 2012 to April 2014

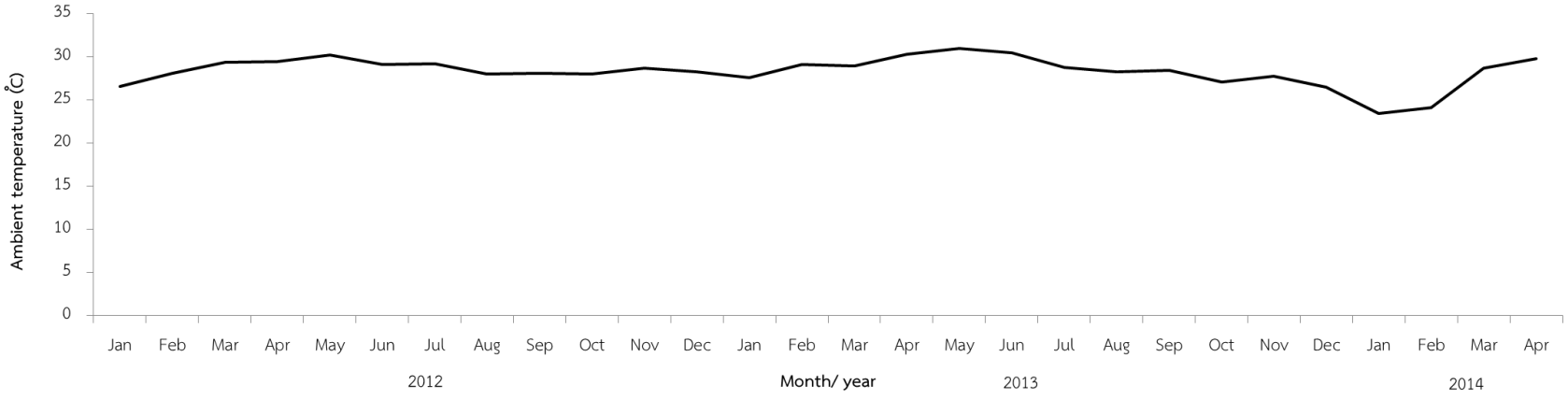


Figure 11 Ambient temperature of studied broiler flocks in central region of Thailand during January 2012 to April 2014

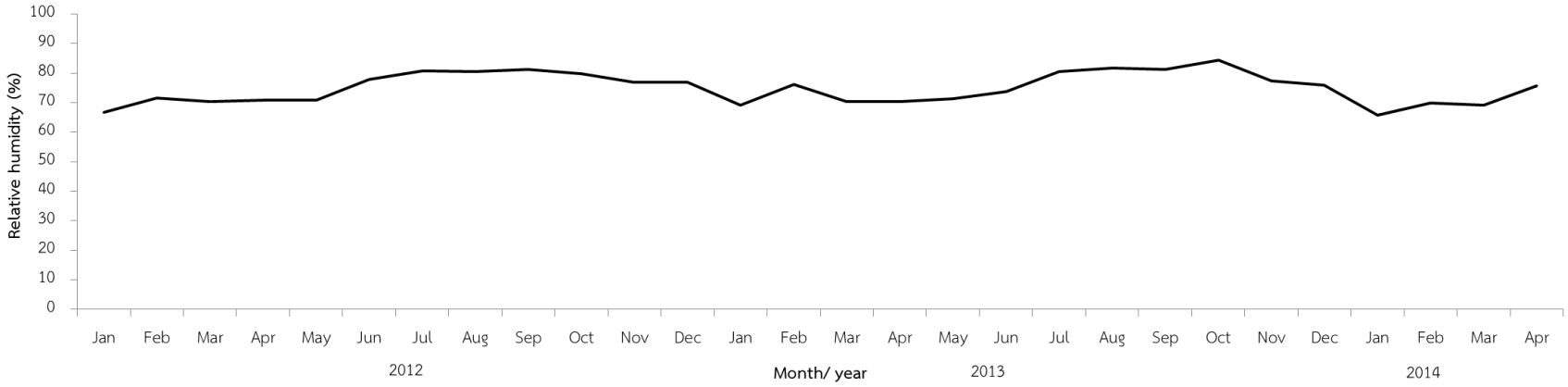


Figure 12 Relative humidity of studied broiler flocks in central region of Thailand during January 2012 to April 2014

4.1.2 Descriptive information of participating broiler farms

To identify risk factors associated with *Campylobacter* colonization in broiler flocks, farm and flock data of each examined flock was collected by structured questionnaires. The obtained data could be categorized into 2 types; 10 continuous variables and 45 categorical variables (Table 7 and 8, respectively).

Participating broiler farms were mainly located in central and eastern parts of Thailand. Wide range of production capacity was described between 36,000 and 2,500,000 chickens per year. Arbor Acre, Ross and Cobb were the major breeds of the examined flocks. All-in all-out system was used in every participating flock without partial depopulation. Moreover, the standard farm management practices such as frequency of footwear disinfectant replacement, dead bird disposal, pest control and downtime period (at least two weeks), was commonly found in this study. The flocks were slaughtered at an average age of 37.87 ± 0.24 days (ranging from 30 to 45 days).

Table 7 Continuous data of participating broiler flocks

Variable	Sample size	Average ± SEM	Minimum - Maximum
Farm size (square meter)	204	70,070.59 ± 7,440.86	8,000 – 384,000
Number of rearing house	243	9.09 ± 1.02	1 – 72
Production capacity (chicken per year)	243	547,270.78 ± 45,779.12	36,000 – 2,500,000
Age of target house (month)	192	110.73 ± 5.56	6 – 360
Average temperature within target rearing house (°C)	158	29.19 ± 0.08	23.00 – 33.11
Humidity within target rearing house (%)	158	69.21 ± 0.60	50.00 – 82.49
Mortality rate (%)	222	2.67 ± 0.11	0.02 – 11.00
Culling rate (%)	211	1.91 ± 0.22	0.03 – 36.38
Slaughter age (days)	227	37.87 ± 0.24	30 - 45
Number of chicken in target rearing house	228	16,023.54 ± 494.11	4,794 – 53,142

Table 8 Categorical data of participating broiler flocks

	Sample size	*Campylobacter* colonization status	
		positive	negative
Feeding system			
Trough feeder	17	10	7
Pan feeder	177	88	89
Both	49	17	32
Drinking water system			
Nipple drinker without cup	57	22	35
Nipple drinker with cup	186	93	93
Bell type	49	17	32
Presence of anteroom			
Yes	197	92	105
No	46	23	23
Presence of damage on target house			
Yes	102	46	56
No	141	69	72
Ground area around target house			
Weed	13	10	3
Dirt	155	77	78
Gravel	24	3	21
Concrete	168	89	79

Table 8 Categorical data of participating broiler flocks (Cont.)

	Sample size	*Campylobacter* colonization status	
		positive	negative
Organic acid supplement use in drinking water			
Yes	213	105	108
No	30	10	20
Antibiotic supplement in food			
Yes	149	80	69
No	90	35	55
Type of disinfectant in water			
No disinfection	22	5	17
Hypochlorite (ClO-)	57	13	44
Chlorine dioxide (ClO_2)	164	97	67
Source of water in farm			
Underground water	229	111	118
Tap water	28	18	10
Surface water	13	2	11
Presence of surface water in surrounding area			
Yes	155	81	74
No	86	33	53
Frequency of dipping water change			
More than once a day	81	42	39
Once a day	160	72	88

Table 8 Categorical data of participating broiler flocks (Cont.)

	Sample size	*Campylobacter* colonization status	
		positive	negative
Cleaning method for drinking water system			
Cleaning with water	79	36	43
Cleaning with acidity solution	153	68	85
Cleaning with disinfectant solution	90	47	43
Pest control management			
Bird	186	101	85
Fly	74	25	49
Darkling beetle	44	25	19
Duration for flock clearance			
Less than 1 day	18	11	7
1 – 2 day (s)	64	26	38
3 – 7 days	161	78	83
Duration for feed depletion			
Less than 6 hours	92	54	38
6 – 8 hours	40	11	29
9 – 12 hours	111	50	61
Presence of domestic animal in farm area			
Yes	55	17	38
No	175	97	78

Table 8 Categorical data of participating broiler flocks (Cont.)

	Sample size	*Campylobacter* colonization status	
		positive	negative
Presence of domestic animal in farm adjacent			
Yes	93	41	52
No	122	63	59
Presence of damaging on watering equipment			
Yes	112	51	61
No	119	58	61
Presence of pest in target house area			
Bird	98	50	48
House lizard	146	71	75
Fly	159	80	79
Darkling beetle	116	60	56
Duration of bird transport to slaughterhouse			
30 minutes – 2 hours	101	34	67
2 – 6 hours	43	26	17
More than 6 hours	86	49	37
Presence of Campylobacter in previous flock			
Yes	96	64	32
No	99	35	64

4.1.3 Risk factors associated with *Campylobacter* colonization in Thai broiler flocks

4.1.3.1 Logistic regression model

Univariate analysis

Variables significantly associated with *Campylobacter* colonization in univariate analysis ($p \leq 0.05$) are displayed in Table 9. From univariate screening, 18 factors were identified as possible risk factors associated with *Campylobacter* colonization in Thai broiler flocks. These factors included the use of trough feeder and pan feeder, age of target house, gravel area around the target house, concrete area around the target house, antibiotic supplement in food, no disinfection in water, use of hypochlorite as a disinfectant in water, use of chlorine dioxide as a disinfectant in water, use of surface water as a main water source in the farm, presence of surface water in the farm area, bird control management, fly control management, feed withdrawal less than 6 hours, feed withdrawal for 6 – 8 hours, presence of domestic animals in farm area, duration of bird transport to slaughterhouse for 30 minutes – 2 hours, duration of bird transport to slaughterhouse more than 6 hours and history of *Campylobacter* in previous flock. Among these variables, four variables (i.e., use of hypochlorite as a disinfectant in water, use of chlorine dioxide as a disinfectant in water, duration of bird transport to slaughterhouse for 30 minutes – 2 hours and history of *Campylobacter* in previous flock) showed the high *p*-value.

Table 9 Results of univariate analysis in logistic regression model

Variable	β	SE	*p*-value
Farm size	0.0052	0.0017	0.0023
Number of house in farm	0.0404	0.0132	0.0021
Production capacity	0.0006	0.0002	0.0031
Use of 2 feeding types (trough feeder and pan feeder)	0.6639	0.3332	0.0463
Age of house	0.00505	0.00221	0.0106
Gravel area around the target house	1.9424	0.6336	0.0022
Concrete area around the target house	-0.7308	0.2879	0.0111
Antibiotic supplement in food	-0.5816	0.2722	0.0326
Type of disinfectant in water: no disinfection	1.2146	0.5266	0.0211
Type of disinfectant in water: hypochlorite	1.3826	0.3496	<0.0001
Type of disinfectant in water: chlorine dioxide	-1.5044	0.3131	<0.0001
Source of water in farm: surface water	1.6782	0.7799	0.0314
Presence of surface water in farm area	-0.5451	0.2748	0.0471
Pest control management: bird	-1.1906	0.3437	0.0005
Pest control management: fly	0.8064	0.2911	0.0056
Feed withdrawal: less than 6 hours	-0.7292	0.2692	0.0068
Feed withdrawal: 6 – 8 hours	1.0288	0.3811	0.0069
Presence of domestic animals in farm area	0.9875	0.3307	0.0028
Duration of bird transport to slaughterhouse: 30 minutes – 2 hours	1.0497	0.2753	0.0001
Duration of bird transport to slaughterhouse: more than 6 hours	-0.7400	0.2748	0.0071
History of *Campylobacter* in previous flock	-1.2808	0.3022	<0.0001

Multivariate analysis

Among 18 candidate variables, 11 variables were neglected by stepwise selection procedure, while including of remaining seven variables in the model resulted in the acceptable significance level (Table 10). To test null hypothesis of the model, the estimation value of parameters (β) in the full model was set equal to zero and evaluated by the likelihood ratio statistic. In this study, we could reject the null hypothesis since the *p*-value of the hypothesis test is less than 0.0001. According to Hosmer and Lemeshow goodness-of-fit test (test for R^2), the *p*-value was 0.4343 indicating that this model has no evidence of lack of fit. Therefore, we can conclude that this model is fit for the data.

Table 10 Results from the multivariate logistic regression model

Parameter	β^*	Odd ratio	95% CI
Intercept	4.1644	64.3263	
Age of target house	-0.0052	0.9950	0.987-1.003
The use of trough feeder and pan feeder	-6.9461	0.0011	<0.001-0.068
Concrete area around the target house	-1.9039	0.1490	0.020-1.085
Feed withdrawal: less than 6 hours	-1.8943	0.1500	0.018-1.263
Presence of domestic animals in farm area	1.6522	5.2180	0.729-37.355
Duration of bird transport to slaughterhouse: 30 minutes – 2 hours	-3.0293	0.0480	0.005-0.474
Presence of *Campylobacter* in previous flock	0.7819	2.1860	0.951-5.024

* Analyzed by maximum likelihood estimates

According to the result of multivariate analysis, seven variables (i.e., age of target house [A], the use of trough feeder and pan feeder [FS], concrete area around the target house [C], feed withdrawal less than 6 hours [FW], presence of domestic animals in farm area [D], duration of bird transport to slaughterhouse for 30 minutes – 2 hours [T] and presence of *Campylobacter* in previous flock [H]) were considered to be the potential variables associated with *Campylobacter* colonization in broiler flocks. Thus, the prediction model could be constructed from these variables as follows:

$$\mathrm{Y} = -0.01(\mathrm{A}) - 6.95(\mathrm{FS}) - 1.90(\mathrm{C}) - 1.89(\mathrm{FW}) + 1.65(\mathrm{D}) - 3.03(\mathrm{T}) + 0.78(\mathrm{H}) + 4.16 \quad \text{Eq. 6}$$

We could calculate the odd ratio from coefficient value and explain the results as follows:

1) Every one-unit increase of the house age will increase the risk of *Campylobacter* colonization in broiler flock for 0.9950 times. Thus, this variable is considered as protective factor.

2) Broiler flock with two types of feeding system i.e., trough feeder and pan feeder will have risk of *Campylobacter* colonization for 0.0011 times comparing to the flock that use only single type of feeding system. Thus, this variable is considered as protective factor.

3) Broiler flock reared in house surrounded by concrete will have risk of *Campylobacter* colonization for 0.1490 times comparing to the flock that does not surrounded by concrete. Thus, this variable is considered as protective factor.

4) The risk of *Campylobacter* colonization in broiler flock that fasted less than 6 hours is 0.15 times of the risk of broiler flock fasted longer than 6 hours. Thus, this variable is considered as protective factor.

5) The risk of *Campylobacter* colonization in broiler flock with the presence of other domestic animals on the farm area is 5.2180 times of the risk of broiler flock that have not evidence of other domestic animals on the farm area. Thus, this variable is considered as risk factor.

6) The broiler flock which is transferred to slaughterhouse during 30 minutes - 2 hours will have the risk of *Campylobacter* colonization in the flock for 0.0408 times comparing to the flock which is transferred more than 2 hours. Thus, this variable is considered as protective factor.

7) The risk of *Campylobacter* colonization in broiler flock with the history of *Campylobacter* positive status is 2.1860 times comparing to the flock that has no history of *Campylobacter*. Thus, this variable is considered as risk factor.

In summary, risk factors identified by logistic regression analysis were the presence of other domestic animals on the farm area and the history of *Campylobacter* positive status, while the remaining factors were defined as protective factor.

4.1.3.2 Generalized estimating equation (GEE)

Univariate analysis

Seventeen variables were significantly associated with *Campylobacter* colonization in broiler flocks (Table 11). These included farm size, number of house in farm, production capacity, gravel area around the target house, no disinfection in water, use of hypochlorite as a disinfectant in water, use of chlorine dioxide as a disinfectant in water, use of tap water as a main water source in the farm, presence of surface water in farm area, bird control management, fly control management, feed withdrawal for less than 6 hours, presence of domestic animals in farm area, duration of bird transport to slaughterhouse for 30 minutes – 2 hours, duration of bird transport to slaughterhouse more than 6 hours, number of chicken in target house and history of *Campylobacter* in previous flock. Twelve out of 17 variables were similar to the results of univariate screening in logistic regression method.

Table 11 Results of univariate analysis in generalized estimating equation

Variable	β	**SE**	***p*-value**
Farm size	-0.0055	0.0018	0.0017
Number of house in farm	-0.0494	0.0183	0.0068
Production capacity	-0.0008	0.0003	0.0177
Gravel area around the target house	-1.7290	0.5194	0.0009
Type of disinfectant in water: no disinfection	-1.3735	0.5998	0.022
Type of disinfectant in water: hypochlorite	-1.2379	0.5740	0.031
Type of disinfectant in water: chlorine dioxide	1.4489	0.4610	0.0017
Source of water in farm: tap water	1.0316	0.3366	0.0022
Presence of surface water in farm area	1.2340	0.4790	0.0100

Table 11 Results of univariate analysis in generalized estimating equation (Cont.)

Variable	β	SE	*p*-value
Pest control management: bird	1.2482	0.5711	0.0289
Pest control management: fly	-1.0567	0.4977	0.0338
Feed withdrawal: less than 6 hours	1.1097	0.3348	0.0009
Presence of domestic animals in farm area	-1.0724	0.5134	0.0367
Duration of bird transport to slaughterhouse: 30 minutes – 2 hours	-1.3080	0.3436	0.0001
Duration of bird transport to slaughterhouse: more than 6 hours	1.1300	0.3556	0.0015
Number of chicken in target rearing house	-0.0497	0.0214	0.0201
Presence of *Campylobacter* in previous flock	0.9049	0.3738	0.0155

Multivariate analysis

Eight out of 17 candidate variables were included in the model by stepwise backward elimination procedure These variables included number of house on the farm, gravel area around the target house, no disinfection in drinking water, feed withdrawal less than 6 hours, bird control management, the duration of bird transport to slaughterhouse for 30 minutes – 2 hours, number of chicken in the house and presence of *Campylobacter* in previous flock. However, the interaction between two variables i.e., number of chicken in the house and feed withdrawal less than 6 hours, was identified. Moreover, duration of bird transport to slaughterhouse for 30 minutes – 2 hours was identified as confounder of the model. Thus, these variables were removed. The final model contained 5 remaining variables i.e.,

number of house of the farm, gravel area around the target house, no disinfection in water, bird control management and presence of *Campylobacter* in previous flock (Table 12). Exchangeable correlation was specified as the correlation structure of this study. The acceptable correlation between variable (ρ) was displayed by empirical and model-based covariance matrix. Pearson chi-square/DF and mean deviance were 1.0448 and 1.2047, respectively. These values indicated that the model is not over dispersion and good fitted.

Table 12 Results of multivariate analysis by GEE

Parameter	β^*	Odd ratio	95% CI
Intercept	1.3325	3.7900	
Number of house in farm	-0.0504	0.9509	-0.0667-(-0.0340)
Ground area around the target house: gravel	-3.3808	0.0340	-4.2950-(-2.4665)
No disinfection in water	-1.4594	0.2324	-1.9983-(-0.9205)
Bird control management	-1.1580	0.3142	-1.8828-(-0.4332)
Presence of *Campylobacter* in previous flock	0.9200	2.5091	0.1265-1.7135

* Analysis of maximum likelihood estimates

According to the result of multivariate analysis, five variables (i.e., number of house on the farm [N], gravel area around the target house [G], no disinfection in water [D], bird control management [P] and presence of *Campylobacter* in previous flock [H]) were included in the prediction model as follows:

$$Y = -0.05(\mathrm{N}) - 3.38(\mathrm{G}) - 1.46(\mathrm{D}) - 1.16(\mathrm{P}) + 0.92(\mathrm{H}) + 1.33 \qquad \text{Eq. 7}$$

We could calculate the odd ratio from coefficient value and explain the results as follows:

1) One-unit increase of house number in the farm will increase the risk of *Campylobacter* colonization in broiler flocks for 0.9509 times. Thus, this variable is considered as protective factor.

2) The risk of *Campylobacter* colonization in broiler flock with the gravel area around the target house is 0.0340 times comparing to the flock that have no gravel area around the target house. Thus, this variable is considered as protective factor.

3) The risk of *Campylobacter* colonization in broiler flock with no water disinfection is 0.2324 times comparing to the flock that uses the disinfected drinking water. Thus, this variable is considered as protective factor.

4) The risk of *Campylobacter* colonization in broiler flock that uses the bird control management is 0.3142 times comparing to the flock that has no bird controlling programme. Thus, this variable is considered as protective factor.

5) The risk of *Campylobacter* colonization in broiler flock with the history of *Campylobacter* positive status is 2.5091 times comparing to the flock that have no history of *Campylobacter*. Thus, this variable is considered as risk factor.

In summary, the history of *Campylobacter* positive status was identified as the risk factor associated with *Campylobacter* colonization in broiler flocks by generalized estimating equation, while the remaining four variables were defined as protective factor.

4.2 Distribution and genetic relatedness of *Campylobacter* isolated from poultry production chain

4.2.1 Distribution of *Campylobacter* in Thai poultry production chain

To determine the potential sources of *Campylobacter* in broiler flock, six broiler production chains were investigated from breeder to slaughterhouse. Out of 2,889 examined samples, 615 samples were positive for *Campylobacter* species (21.29%). The prevalence in breeders, broiler farms and slaughterhouses were 63.33% (95/150), 13.43% (268/1,995) and 43.52% (252/579), respectively. In breeder flocks, the proportion of flocks colonized with *Campylobacter* ranged from 36.00 to 76.00% (Table 13). Isolates obtained from breeder flocks were mainly identified as *C. coli*. No *Campylobacter* was detected in hatchery-related samples i.e., egg incubators, egg trays, tap water and egg shell. Likewise, *Campylobacter* were absent in feces-soiled lining papers and environmental samples from the broiler house before chick placement.

During the rearing period, 0.00 to 48.75% of cloacal swab samples obtained from six broiler flocks were positive for *Campylobacter* (Table 14). In contrast to breeder isolates, all isolates recovered from broiler flocks were identified as *C. jejuni*. At the first visit (7^{th} day), no *Campylobacter* was detected in any examined samples, while *Campylobacter* colonization the first identified on the 14^{th} day in flocks D and E. For large farms (flocks A, B and C), *Campylobacter* could be isolated from chickens after 4 weeks of age. No *Campylobacter* isolate was recovered in broilers of flock F,

although boot swab from path-leading to the target house was tested positive for this organism. Within-flock prevalence varied among positive broiler flocks from 3.33 to 93.33% (Table 14). Unlike the cloacal swab samples, less than 7 percent of samples from farm environment, such as boot swabs inside and outside the house, darkling beetles, flies and drinking water, were contaminated with *Campylobacter*. Generally, environmental samples were commonly tested positive after flock colonization.

In slaughterhouse, the high prevalence of *Campylobacter* was found in chicken related samples (caecum, cloacal swab, meat product and carcass rinse) ranging from 37.88 to 90.00% (Table 13). Several types of slaughterhouse equipment and environmental samples (e.g., breast comforter, shackle, eviscerating equipment, chilling water and packaging table) were contaminated with *Campylobacter* at the prevalence between 6.45 and 38.03%. *Campylobacter* were mostly recovered from environment and equipment in slaughterhouses after slaughter process was conducted, while a few of the disinfected equipment (i.e., transport crate, eviscerating equipment and hanging shackle) were occasionally positive for *Campylobacter*. In addition, no *Campylobacter* was found in tap water collected from the slaughterhouses. Similar to broiler flocks, *C. jejuni* was the predominant species found in slaughterhouses.

Table 13 Distribution of *Campylobacter* in 6 chicken meat production chains in Thailand

Production chain	Production unit	Chicken-related sample[a]			Environmental sample[b]		
		No. of positive samples /Total (%)	Species identification (%)		No. of positive samples /Total (%)	Species identification (%)	
			C. jejuni	*C. coli*		*C. jejuni*	*C. coli*
A	Breeder farm	11/30 (36.67)	5/11 (45.45)	6/11 (54.55)	NS[c]	NS	NS
	Hatchery	0/10 (0.00)	n/a[d]	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	58/220 (26.36)	58/58 (100.00)	0/58 (0.00)	7/113 (6.19)	7/7 (100.00)	0/7 (0.00)
	Slaughterhouse	45/56 (80.36)	45/45 (100.00)	0/45 (0.00)	13/70 (18.57)	13/13 (100.00)	0/13 (0.00)
B	Breeder farm	23/30 (76.67)	6/23 (26.09)	17/23 (73.91)	NS	NS	NS
	Hatchery	0/10 (0.00)	n/a	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	80/220 (36.36)	80/80 (100.00)	0/80 (0.00)	0/113 (0.00)	n/a	n/a
	Slaughterhouse	34/66 (51.52)	34/34 (100.00)	0/34 (0.00)	27/71 (38.03)	27/27 (100.00)	0/27 (0.00)
C	Breeder farm	21/30 (70.00)	8/21 (38.10)	13/21 (61.90)	NS	NS	NS
	Hatchery	0/10 (0.00)	n/a	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	2/250 (0.80)	2/2 (100.00)	0/2 (0.00)	1/123 (0.81)	1/1 (100.00)	0/1 (0.00)
	Slaughterhouse	25/66 (37.88)	25/25 (100.00)	0/25 (0.00)	25/71 (35.21)	25/25 (100.00)	0/25 (0.00)
D	Breeder farm	17/24 (70.83)	2/17 (11.76)	15/17 (88.24)	NS	NS	NS
	Hatchery	0/10 (0.00)	n/a	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	32/160 (20.00)	32/32 (100.00)	0/32 (0.00)	4/135 (2.96)	4/4 (100.00)	0/4 (0.00)
	Slaughterhouse	36/40 (90.00)	36/36 (100.00)	0/36 (0.00)	11/37 (29.73)	11/11 (100.00)	0/11 (0.00)

Table 13 Distribution of *Campylobacter* in 6 chicken meat production chains in Thailand (cont.)

Production chain	Production unit	Chicken-related sample[a]			Environmental sample[b]		
		No. of positive samples /Total (%)	Species identification (%)		No. of positive samples /Total (%)	Species identification (%)	
			C. jejuni	*C. coli*		*C. jejuni*	*C. coli*
E	Breeder farm	17/24 (70.83)	8/17 (47.06)	9/17 (52.94)	NS	NS	NS
	Hatchery	0/6 (0.00)	n/a	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	78/160 (48.75)	78/78 (100.00)	0/78 (0.00)	5/133 (3.76)	5/5 (100.00)	0/5 (0.00)
	Slaughterhouse	32/40 (80.00)	32/32 (100.00)	0/32 (0.00)	4/62 (6.45)	4/4 (100.00)	0/4 (0.00)
F	Breeder farm	6/12 (50.00)	3/6 (50.00)	3/6 (50.00)	NS	NS	NS
	Hatchery	0/10 (0.00)	n/a	n/a	0/17 (0.00)	n/a	n/a
	Broiler farm	0/220 (0.00)	n/a	n/a	1/148 (0.68)	1/1 (100)	0/1 (0.00)
	Slaughterhouse	NS	NS	NS	NS	NS	NS

[a] Chicken-related samples include cloacal swab, carcass rinse, caecum, meat product.

[b] Environmental samples include samples from hatchery (i.e., egg tray, egg incubator, tap water and egg shell), samples from broiler farm (i.e., boot swab, feeder, litter, water from nipple drinker, water from main pipeline, animal feed, footwear in the house and pest) and samples from slaughterhouse (i.e., transport crate, breast comforter, hanging shackle, eviscerating equipment, chilling water and packaging table).

[c] NS, not sample.

[d] n/a, not applicable.

4.2.2 Genetic characterization of *Campylobacter* isolated from poultry production chain

Since *C. jejuni* was the predominant *Campylobacter* species in this study, representative *C. jejuni* isolated from 5 positive broiler production chains (i.e., chains A, B, C, D and E) were selected for genetic characterization. *Campylobacter* isolated from chain F was not included in the genetic characterization due to the absence of sample collection in slaughterhouse and negative *Campylobacter* status in the broilers. Amongst 311 *C. jejuni* isolates characterized by *flaA* SVR sequencing and 108 isolates further genotyped by multilocus sequence typing (MLST), 29 *flaA* SVR alleles and 17 sequence types were identified. Fifteen sequence types were clustered into 10 clonal complexes, while 2 sequence types (ST-2131 and ST-2409) could not be grouped in any available clonal complex (Figure 13). Novel allelic sequences (asp 358, tkt 546 and tkt 553) and new sequence types (ST-6876, ST-6995 and ST-6996) were assigned. The most common clonal complex found in this study was CC-353 (e.g., ST-1075, ST-1232, ST-5213 and ST-5247), followed by CC-45 (e.g., ST-45 and ST-583). These clonal complexes were found to be distributed in every examined production chain, except for chain B.

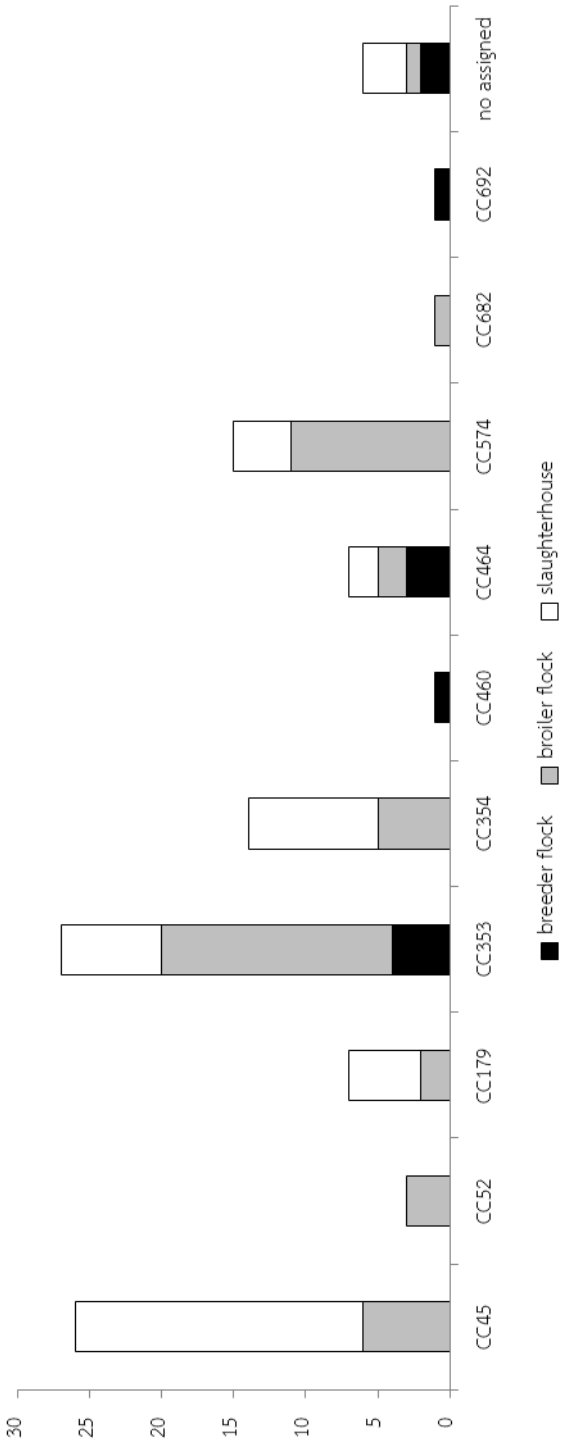


Figure 13 Distribution of clonal complexes identified in five broiler production chains

In chains A, C, D and E, most of sequence types and *flaA* SVR genotypes of *C. jejuni* isolated from breeders and their respective progenies were distantly related (Figure 14). In contrast, genetic similarity between *C. jejuni* isolated from breeders and broilers was observed in chain B. A single dominant genotype (ST-464 or *flaA* SVR allele 54) was identified throughout the chicken meat production of chain B, even though a few strains i.e., ST-354 (or *flaA* SVR allele 18) and *flaA* SVR allele 783 were occasionally present. However, for the other production chains, multiple genotypes of *C. jejuni* were identified (Table 14). Substitution of the initial predominant genotype in broiler flock A was demonstrated, where the predominant strain changed from ST-574 (or *flaA* SVR allele 57) to ST-45 (or *flaA* SVR allele 22) during the rearing period (Table 15). This ST-45 strain was also reported as the predominant sequence type in the slaughterhouse. For the late colonized flock (flock C), a single strain (ST-2209 or *flaA* SVR allele 629) was identified. Although this sequence type was predominantly found in the chicken intestinal tract until slaughter, it was dominated by another sequence type (ST-354 or *flaA* SVR allele 18) on chicken carcasses (Table 15). Genetic diversity of *C. jejuni* was more frequently noticed at the end of the rearing period. This finding was obvious in flock E where multiple *flaA* SVR genotypes (i.e., 18, 45, 253, 255, 287, 854 and 1527) were detected, particularly at the day before the birds were sent to slaughterhouse (Table 14). In general, most *C. jejuni* contaminating on the slaughterhouse environment, equipment, carcass rinses and meat products were genetically similar to those found

in broiler flocks and caeca. In addition, meat products could be sometimes found the sequence types which were not reported in broiler flock in the same production line.

To reveal the source of *Campylobacter* in broiler farms, genetic comparison between *C. jejuni* isolated from broiler house surrounding before chick placement and *C. jejuni* isolated from broiler flocks was conducted (Table 15). In this study, no *Campylobacter* was recovered from farm environment before chick placement, while only one isolate was obtained from environmental samples collected before *Campylobacter* detection in broilers. However, this isolate which was recovered from water from nipple drinker of flock C (ST-45) was genetically different from those colonized in broiler flock (ST-2209). Generally, *Campylobacter* were recovered from the house environment after flocks became positive and predominant sequence types identified in both house environment and broiler flocks were quite similar. For instance, predominant strains of the birds in flocks A, D and E (i.e., ST-574, ST-1232 and ST-5247, respectively) were found to be the main sequence types in environmental samples such as boot swab, water from nipple drinker, flies and darkling beetles. The above findings indicated that the majority of *C. jejuni* present in farm environment mainly originated from broilers. The definite source of *Campylobacter* during rearing period is still unclear.

Table 14 Within-flock prevalence and predominant genotypes of *Campylobacter* during the rearing period

Flock		Flock age (days)					
		14	21	28	31	35	38
A	Prevalence (percent)	0	0	0	70.00	36.67	86.67[a]
	Predominant sequence type (*flaA* SVR type)	n/a[b]	n/a	n/a	ST-574 (57)	ST-574 (57)	ST-45 (22)
B	Prevalence (percent)	0	0	0	90.00	86.67	90.00[a]
	Predominant sequence type (*flaA* SVR type)	n/a	n/a	n/a	ST-464 (54)	ST-464 (54)	ST-464 (54)
C	Prevalence (percent)	0	0	0	0	0	6.67[a]
	Predominant sequence type (*flaA* SVR type)	n/a	n/a	n/a	n/a	n/a	ST-2209 (629)
D	Prevalence (percent)	26.67	46.67	3.33	30.00[a]	n/a	n/a
	Predominant sequence type (*flaA* SVR type)	ST-1232 (783)	ST-1232 (783)	ST-1232 (783)	ST-1232 (783)	n/a	n/a
E	Prevalence (percent)	30.00	n/a	93.33	90.00[a]	n/a	n/a
	Predominant sequence type (*flaA* SVR type)	ST-5247 (287)	ST-5247 (287)	ST-5247 (287)	ST-1919 (253)	n/a	n/a

[a] The last visit before the flock was sent to slaughterhouse.

[b] n/a, not applicable.

Table 15 *Campylobacter* genotypes detected in chicken meat production units

Flock	Production unit	Sample	Day	Number of isolate examined	Genotype	
					flaA SVR	MLST[c]
A	Breeding farm	Cloacal swab	-	5	353, 506, 783, 1211, 1485	**1232**[d], 6876
	Broiler farm	Cloacal swab day 31	31	11	18, 22, **57**, 312	354, **574**
		Boot swab inside the target house [a]	31	1	22	45
		Cloacal swab day 35	35	6	57, 312	574
		Boot swab from path-leading to target house [b]	35	1	57	574
		Boot swab inside the target house [a]	35	1	57	574
		Boot swab from area around the house [b]	35	1	57	574
		Boot swab from adjacent house [b]	35	1	22	45
		Water from nipple drinker [a]	35	1	18	354
		Darkling beetle [a]	35	1	57	574
		Cloacal swab day 38	38	19	18, 22, **57**	45, 354, **574**
	Slaughterhouse	Cloacal swab and cecum	-	10	18, **22**, 57, 312	**45**, 354, 574
		Transport crate	-	3	**45**	**2409**
		Slaughterhouse equipment (before used)	-	2	18, 22	45, 354
		Chilling water	-	3	**22**	**45**
		Meat and carcass rinse	-	15	18, **22**, 57, 177	**45**, 354, 574, 583
B	Breeding farm	Cloacal swab	-	2	**54**	**464**
	Broiler farm	Cloacal swab day 31	31	15	**54**	**464**
		Cloacal swab day 35	35	13	**54**, 18	**464**, 354
		Cloacal swab day 38	38	6	**54**	**n/a**
	Slaughterhouse	Cloacal swab and cecum	-	4	**54**	**464**
		Slaughterhouse equipment (after used)	-	8	**54**, 783	**n/a**
		Chilling water	-	3	**54**	**n/a**
		Meat and carcass rinse	-	10	**54**	**n/a**

Table **15** *Campylobacter* genotypes detected in chicken meat production units (Cont.)

Flock	Production unit	Sample	Day	Number of isolate examined	Genotype	
					flaA SVR	MLST[c]
C	Breeding farm	Cloacal swab	-	6	30, **34**, 54, 312	460, 574, **6996**
	Broiler farm	Water from nipple drinker	14	1	22	45
		Cloacal swab	38	2	**629**	**2209**
	Slaughterhouse	Cloacal swab and cecum	-	4	**68, 629, 1340**	**2209**
		Transport crate	-	2	783	5213
		Slaughterhouse equipment (after used)	-	1	783	5213
		Chilling water	-	1	1340	n/a
		Meat and carcass rinse	-	17	**18**, 68, 783, 1340	**354**, 2209
D	Breeding farm	Cloacal swab	-	1	677	2131
	Broiler farm	Cloacal swab	15	5	**783**	**1232**
		Water from nipple drinker	15	1	**783**	**1232**
		Boot swab inside the target house	15	1	**783**	**1232**
		Cloacal swab	21	13	**783**	**1232**
		Cloacal swab	28	1	**783**	**1232**
		Cloacal swab	32	9	48, **783**	**1232**, 2131, 5213
		Boot swab inside the target house	32	1	**783**	**1232**
	Slaughterhouse	Cloacal swab and cecum	-	13	**783**	**1232, 5213**
		Slaughterhouse equipment (after used)	-	5	22 ,**783**	1075
		Meat and carcass rinse	-	9	**783**	1232

Table **15** *Campylobacter* genotypes detected in chicken meat production units (Cont.)

Flock	Production unit	Sample	Day	Number of isolate examined	Genotype	
					flaA SVR	MLST[c]
E	Breeding farm	Cloacal swab		5	21, 54, 45, 402, 48	2131
	Broiler farm	Cloacal swab	14	5	57, **287**	**5247**
		Cloacal swab	21	14	253, **287**	1919, **5247**
		Boot swab from path-leading to target house	21	1	255	
		Boot swab inside the target house	21	1	1239	
		Boot swab from area around the house	21	1	1397	6995
		Flies	21	1	**287**	**5247**
		Cloacal swab	28	14	253, **255, 287**	1919, **5247**
		Boot swab from area around the house	28	1	287	n/a
		Cloacal swab	32	19		
	Slaughterhouse	Cloacal swab and cecum	-	4	253, **783**,1527	n/a[e]
		Slaughterhouse equipment (after used)	-	3	45, 253, 652	n/a
		Meat and carcass rinse	-	6	45, **287**, 312, 652	**5247**

[a] Environment inside the target house

[b] Environment outside the target house

[c] Approximately 30% of *flaA* SVR sequencing tested samples were further genotyped by MLST.

[d] Bold letter stands for predominant strain.

[e] n/a, not applicable.

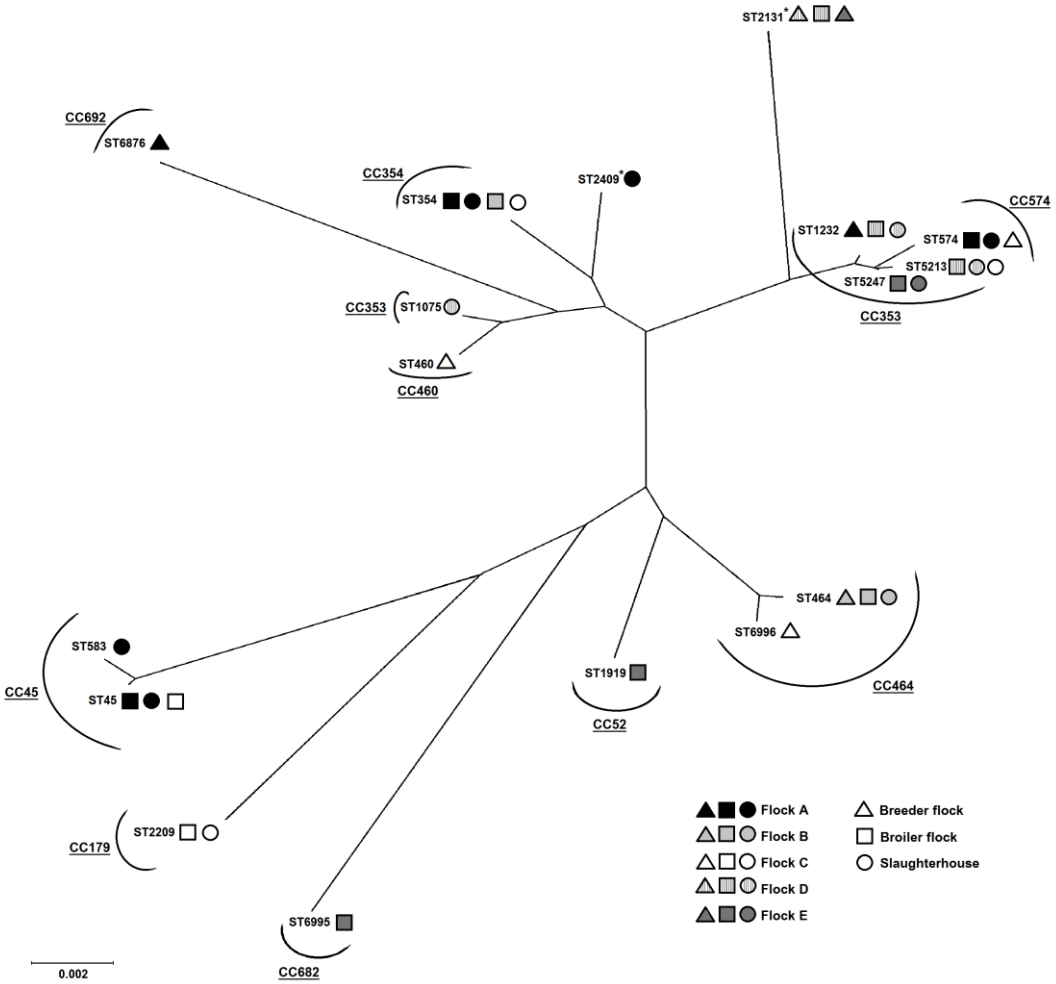


Figure 14 Phylogenetic relationship of *Campylobacter jejuni* from various sources of broiler production processes. Distribution of sequence types in each production chain (i.e., A, B, C, D and E) and production unit (breeder farm, broiler farm and slaughterhouse) was represented by different shading pattern and geometric shape, respectively. Asterisk (*) defined as unassigned clonal complexes.

Mainly, *flaA* SVR sequencing provided the concordant results with MLST. However, these two methods sometimes produce the self-contradictory result. For example, *flaA* SVR 22 isolated from chains A and C were commonly identified as ST-45 but this *flaA* SVR type could be identified as the different sequence type i.e., ST-1075 (Table 16).

Table 16 Correlation between MLST and *flaA* SVR sequencing

Clonal complex	Sequence type	*flaA* SVR types	Number of matched isolates
45	45	22	25
	583	177	1
52	1919	253	3
179	2209	68	2
		629	3
		1340	2
353	1075	22	1
	1232	353	1
		783	14
		1211	1
		1485	1
	5213	783	5
	5247	287	4
354	354	18	14
460	460	34	1
464	464	54	4
	6996*	34	1
		54	1
574	574	57	15
		312	1
682	6995*	1397	1
692	6876*	506	1
No assigned	2131	48	2
		677	1
	2409	45	3

*Novel sequence type

CHAPTER V
DISCUSSIONS

5.1 Prevalence and risk factors associated with *Campylobacter* colonization in broiler flocks

In this part, the prevalence, seasonality, species identification and bacterial enumeration along with risk factors associated with *Campylobacter* colonization in Thai broiler flocks were revealed. Generally, *Campylobacter* prevalence in wet season was mostly higher than that of dry season. Wide range of bacterial number in cecal content was reported with *Campylobacter jejuni* as a predominant species. Although risk factors identified by two statistical methods were different from each other, except for the history of *Campylobacter* colonization in previous flock.

5.1.1 Prevalence of *Campylobacter* in broiler flock

Wide range of *Campylobacter* prevalence has been reported in various climatic and geographical areas. For example, the prevalence of *Campylobacter* in broiler flocks from European member countries was reported ranging from 2 to 100 percent (EFSA, 2011a). In Asian countries, 31.90 and 83.30% of broiler flocks were *Campylobacter* positive (Chen et al., 2010; Rejab et al., 2012; Carrique-Mas et al., 2014). In this study, 47.60% of broiler flocks reared in central region of Thailand was colonized with *Campylobacter*. This finding was lower than the study of Padungtod and Kaneene (2005) which reported that 64% of broiler ceca in northern region of Thailand were positive for *Campylobacter*, while the study of Chokboonmongkol et

al. (2013) reported the relatively low prevalence (11.2%) in cecal samples collected from chickens in the northern region of Thailand. The mean of within-flock prevalence in the present study (84.93%) was higher than those previously reported in UK (81.60%) and Spain (60.50%) (Evans and Sayers, 2000; Torralbo et al., 2014). Although previous study in Thailand reported *C. coli* as the predominant species (Padungtod and Kaneene, 2005), our study and previous publications (Cardinale et al., 2004; McDowell et al., 2008; Sasaki et al., 2011) found that *C. jejuni* was the major species identified in broiler flocks. The difference of *Campylobacter* species between previous report in Thailand and our study could be explained by the variation of climate, farm management or surrounding area.

5.1.2 Risk factors associated with *Campylobacter* colonization in Thai broiler flocks

Although logistic regression model (LRM) is commonly performed in survey research, this method was sometimes limited by their own assumptions, including independence of the data. Generalized estimating equations (GEE) were invented to handle the study which has the correlated response variable within the same cluster. However, ignorance of repeated data was frequently found in many publications since the similarity between GEE and LRM results could be found in some cases. In this study, the data was obtained from subsequent broiler flocks reared under the same farm management which originated from the different production cycles. The dependency of response data was still doubtful and could be defined into two distinguishable ways: dependent and independent assumptions. Thus, GEE and LRM

were used in this study to see whether the results of these two methods different from each other. With GEE approach, number of house in farm, ground area around the target house: gravel, type of disinfectant in water: no disinfection, pest control management: bird and presence of *Campylobacter* in previous flock were identified as the risk factors associated with *Campylobacter* colonization in broiler flocks, while 7 risk factors (i.e., age of the house, use of trough feeder and pan feeder, concrete area around the target house, feed withdrawal less than 6 hours, the presence of other domestic animals in farm area, duration of bird transport to slaughterhouse: 30 minutes – 2 hours and presence of *Campylobacter* in previous flock) were identified by LRM. From these findings, presence of *Campylobacter* in previous flock was the only common risk factor identified by both methods with the quite close estimating correlation value and standard error. The reason for the difference between these two methods is the way that outcome is modelled. For GEE, 49 independent clusters (farm) were included, while logistic regression have approximately 200 independent outcome measures.

Our finding indicated that the presence of *Campylobacter* in previous flock was strongly associated with *Campylobacter* colonization in Thai broiler flocks since this factor was identified as the risk factor by both LRM and GEE approaches. Although these organisms were not often recovered from the house environment after disinfection, *Campylobacter* could be introduced into broiler flocks by several ways. Farm environment such as puddle, organic matter or fecal material are the

good support for survival of *Campylobacter* outside the bird. Alternatively, pest or wild animal surrounding the farm could be the potential reservoirs which carry *Campylobacter* to the next flocks (Newell et al., 2011).

5.1.3 Seasonal effect of *Campylobacter* prevalence in Thai broiler flocks

To identify the seasonality and climatic factors associated with *Campylobacter* colonization in Thai broiler flocks, broiler flocks from the same house were continually investigated for 2 years in order to minimize the effect from other factors such as farm management.

Seasonal variation of *Campylobacter* in broiler flocks was previously described, particularly in northern hemispheres. Several investigations in Norway, Denmark and Japan described the low prevalence of *Campylobacter* in broiler flocks during winter time, while the highest peak of *Campylobacter* colonization rate was found in summer (Boysen et al., 2011; Jonsson et al., 2012). In contrast, no evidence of seasonality of *Campylobacter* prevalence was reported in the UK study (Evans and Sayers, 2000). Several climatic factors, such as temperature, sunlight, humidity and rainfall, were suggested as the ecological factors influencing the survival of *Campylobacter* in environment (Sandberg et al., 2006; Hartnack et al., 2009). In temperate zone, climatic conditions in summer were associated with increasing of house flies which could support *Campylobacter* invasion into chicken flocks (Hald et al., 2007). Unlike the temperate zone, seasons of Thailand is mostly under the

influence of monsoon winds which correspond to the changes of rainfall, relative humidity and ambient temperature. In the present study, rainfall and relative humidity in wet season was higher than those of dry season, while the ambient temperature between seasons was not much different (Figures 10, 11 and 12). Unsurprisingly, seasonal pattern of *Campylobacter* prevalence in this study was distinct from previous reports.

5.2 Distribution and genetic relatedness of *Campylobacter* isolated from poultry production chain

Over the last decade, *Campylobacter* in the poultry production chain have been widely investigated in many countries. Although strategies for reducing the organism in poultry and poultry products were continuously progressed, the prevalence of *Campylobacter* was still found to be high (EFSA, 2011a). To improve the efficiency of *Campylobacter* interventions, the epidemiology and population biology of these bacteria in poultry need to be elucidated. This part of the study demonstrated the distribution and population structure of *Campylobacter* in Thai poultry production processes.

5.2.1 Correlation between MLST and *flaA* SVR sequencing

Combination between genotyping methods for *Campylobacter* was conducted in order to increase the discriminatory power for source tracking and epidemiological study (Behringer et al., 2011). Using of MLST along with *flaA* SVR sequencing usually produced the satisfied discriminatory results (Price et al., 2006). Although *flaA* SVR is limited to identify genotype in the long-term study, it was useful for screening before applying by more powerful method instead (Pittenger et al., 2009). Thus, combination between these two methods provided both short-term and long-term information of genetic diversity in *Campylobacter* population (Price et al., 2006). In present study, MLST and *flaA* SVR genotyping provided the concordant genotyping results even if some cases showed the different result (Table 16).

5.2.2 Distribution and genetic relatedness of *Campylobacter* isolated from poultry production process

In this study, all breeder flocks were colonized with *Campylobacter*, while none of the organism was recovered from hatchery samples or tray liners of day-old-chicks. Differences in *Campylobacter* genotypes identified in breeders and their following production units indicate that vertical transmission might not be the major route of *Campylobacter* transmission in Thai broiler production chain.

The presence of multiple strains of *Campylobacter* was identified in each broiler flock, particularly at the end of the rearing period. Additional strains were intermittently recovered from the flocks along the rearing period. These indicate

breaches of biosecurity on the farms allowing ingress of *Campylobacter i*nto the broiler house. Interestingly, most of those new strains were distantly related to the pre-existing strains (Figure 14). In the past, several sources e.g., domestic and wild animals, contaminated water, farm staff and house equipment were identified as risk factors associated with *Campylobacter* colonization in broilers (Hermans et al., 2012). However, the evidence of potential source of *Campylobacter* was still unclear in this study. Improvement in personnel hygiene practices and biosecurity on the poultry farm should be the primary strategy to prevent *Campylobacter* introduction into broiler flocks at this moment.

Implementation of strict biosecurity practice was considered as the effective method to prevent or postpone *Campylobacter* colonization time in broiler flocks during the rearing period (Hermans et al., 2011). From the studies in Norway and Denmark, improvement of biosecurity was mentioned as the significant protective factor for *Campylobacter* colonization in poultry farms (Hofshagen and Kruse, 2005). In broiler farms B and C, which were located adjacent to each other, the predominant sequence types present in these farms (ST-464 and ST-2209, respectively) were unrelated (Figure 14). This finding indicated that proper farm management and farm biosecurity might be the effective way for *Campylobacter* prevention and control in broiler flocks.

From previous investigation, broiler flocks reared on larger farms were more likely to be colonized with *Campylobacter* than those reared on small farms

(Arsenault et al., 2007). In contrast, early colonization (14th day) observed in the present study was found in small-scale farms (i.e., farms D and E). Meanwhile, *Campylobacter* were firstly detected in the late rearing period (31st to 38th day) of larger farms (i.e., farms A, B and C). According to farm data, large-scale farms in this study were operated with strict biosecurity and good management practices, while lower level of farm biosecurity was described in small-scale farms. Differences in farm management and biosecurity practices might be one of the explanations for this finding.

Meat products from *Campylobacter*-positive broiler flocks were more likely to be contaminated with this organism than the products from *Campylobacter*-free flocks (Reich et al., 2008). Increasing numbers of *Campylobacter* on carcasses was commonly reported after plucking and eviscerating procedure (Sasaki et al., 2013). In the present study, genetic relatedness between *Campylobacter* isolated from intestinal tract of broilers and samples collected from slaughterhouses e.g., eviscerating equipment, shackles, carcass rinses and meat products, was revealed. The existence of *Campylobacter* after disinfection is of concern. To minimize the spreading of *Campylobacter* on poultry carcasses, the prevention of intestinal content leakage as well as effective cleaning and disinfection of slaughterhouse environment during slaughtering process should be emphasized. Management interventions e.g., logistic slaughter were also suggested as the supporting preventive methods (Sasaki et al., 2013).

The main clonal complexes identified in this study were ST-45, ST-353, ST-354 and ST-574 complex. ST-45 complex is known as one of the most common clonal complexes identified in human cases, various types of animal hosts and environmental samples (Habib et al., 2009). There is evidence indicating that members of the ST-45 complex were environmentally adapted strains, which can survive under unfavorable conditions better than other strains (Sheppard et al., 2007). Similar to the ST-45 complex, the ST-353 one was also mentioned as one of the common clonal complexes recovered from human cases and poultry (Ragimbeau et al., 2008). In the present study, at least one isolate from each production chain, except for chain B, was belonging to the ST-353 complex. Although the ST-354 and ST-574 complexes are not common at the global level, they were commonly found in this study. According to the MLST database, ST-354 and ST-574 were reported as the predominant strains found in human and poultry samples of Thailand. Interestingly, our study could not detect any ST-21 complex which was extensively known as the most common clonal complex identified in wide-ranging sources and associated with human infection worldwide (Sheppard et al., 2009). However, this clonal complex was not predominantly detected in Thailand. In addition, according to the MLST database (http://pubmlst.org/*Campylobacter*/), most of the clonal complexes identified in the present study were similar to clonal complexes previously reported in human cases in Thailand. This finding emphasizes

the importance of poultry as one of the significant sources of *Campylobacter* infection in humans.

Our findings reveal that *Campylobacter* were distributed throughout the Thai broiler production process. Flock colonization and carcass contamination with various genotypes of *Campylobacter* reflect the presence of several sources of *Campylobacter* during the poultry production process. To minimize *Campylobacter* contamination in chicken, interventions should be conducted both at the broiler farm and in the slaughterhouse. This study suggests that standard hygienic practices and biosecurity seem to be the most practical strategies for prevention and control of *Campylobacter* during broiler production process.

CONCLUSION AND SUGGESTION

Over the last decade, the presence of *Campylobacter* in poultry and poultry products were frequently reported. To generate the effective control and prevention strategies for *Campylobacter*, the epidemiology of these bacteria in broiler production process should be thoroughly investigated. In this study, *Campylobacter* colonization rate and within-flock prevalence in broiler flocks was 47.60% (95% CI 41.41 - 53.79%) and 84.93% (95% CI 80.77 – 89.09%), respectively. The prevalence of *Campylobacter* was high during May to October which is considered as the wet season in Thailand, while previous investigations, which were mostly conducted in temperate zones, reported the high prevalence of *Campylobacter* during summer. History of *Campylobacter* positive in previous flocks was strongly associated with *Campylobacter* colonization in broiler flocks in this study.

Genetic characterization of *C. jejuni* revealed that these organisms were distributed throughout the production process. Flock colonization and carcass contamination with various genotypes of *Campylobacter* reflect the presence of various sources of *Campylobacter* during poultry production process. Vertical transmission was unlikely considered as the major route of *Campylobacter* transmission in broiler production chain in our study since the difference of *Campylobacter* genotypes identified in breeders and their progenies was found.

Instead, horizontal transmission could be the potential transmission route in this study.

For poultry producers and farmers, strict biosecurity and good management practices on broiler farms are the primary strategies for controlling *Campylobacter* at the pre-harvest level. At the post-harvest level, proper disinfection process seems like the most suitable method to reduce *Campylobacter* since *Campylobacter* contamination is usually inevitable in the slaughterhouse environment after the introduction of *Campylobacter* positive chickens into the slaughtering process. To minimize *Campylobacter* contamination in chicken meat, interventions should be focused at both farm and slaughterhouse levels.

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APPENDIX

Appendix A

Culture media for *Campylobacter* isolation

1. *Campylobacter* enrichment broth (Exeter)

Nutrient broth No. 2

5% Lysed horse blood

Campylobacter selective supplement

Campylobacter growth supplement

2. *Campylobacter* selective supplement (Exeter)

Antimicrobial agent	Concentration in medium (mg/litre)
Amphotericin B	2
Cefoperazone	15
Polymyxin B	2,500 IU
Rifampicin	5
Trimethoprim	10

3. *Campylobacter* growth supplement

Typical formula	Concentration in medium (mg/litre)
Sodium pyruvate	250
Sodium metabisulphite	250
Ferrous sulphate	250

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

Typical formula	(gm/litre)
Nutrient broth No.2	25.00
Bacteriological charcoal	4.00
Casein hydrolysate	3.00
Sodium desoxycholate	1.00
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.00

pH 7.4 ± 0.2 @ 25°C

5. CCDA selective supplement

Antimicrobial agent	Concentration in medium (mg/litre)
Cefoperazone	32
Amphotericin B	10

Appendix B

Prevalence and risk factors associated with *Campylobacter* in Thai broiler flocks

Questionnaire (farm section)

Farm description

- Size of farm..... Rai/square meter
- Number of houses in farm.....
- Production capacity .... chickens per year
- Breed of chicken

☐ Ross ☐ Cobb ☐ Arber acres

House structure

- Type of the selected house

☐ Opened system ☐ Closed system

- Age of the target house.....month
- Type of feeder

☐ Trough feeder ☐ Pan feeder ☐ Both

- Type of drinker

☐ Nipple drinker ☐ Bell drinker ☐ Both type

- Presence of anteroom

☐ Yes ☐ No

- Anteroom sharing between houses

☐ Yes ☐ No

House condition

- Roof and wall condition

☐ Normal ☐ Having some cracks or damage

- Environment surrounding the house

☐ Weed ☐ Soil ☐ Gravel ☐ Concrete

Medicine & chemical

- Organic acid in drinking water

☐ Yes ☐ No

- Antibiotic mixing in animal feed

☐ Yes ☐ No

- Antibiotic usage in chicken

☐ Yes ☐ No

- Feed additive provided

☐ Yes ☐ No

- Vaccination program

☐ New castle disease ☐ Infectious bronchitis ☐ Infectious bursal disease

Watering system

- Sources of water

☐ underground water ☐ tap water

☐ surface water ☐ other sources.....

- Disinfectants for water treatment

☐ Hypochlorite ☐ Chlorine dioxide

☐ Oxidizing disinfectant ☐ Organic acid

☐ Others..... ☐ No water disinfection

- Surface water in farm area

☐ Yes ☐ No

Sanitary practice

- Duration of down time

☐ Less than 1 week ☐ 1 – 2 weeks ☐ More than 2 weeks

- Foot dip at the house entrance

☐ Yes ☐ No

- Frequency of foot dip changing

☐ More than 1 time/day ☐ 1 times/day ☐ every 2- 3 day

- Chemical for nipple drinker cleaning

☐ Water ☐ Acid ☐ Disinfectant

- Waste management (i.e. used- litter, feces)

☐ Discarded (outside the farm) ☐ Buried ☐

Incinerated

- Management of dead chickens

☐ Incinerated ☐ Buried ☐ Sale

- Type of pest that controlling program was available

☐ Rodents ☐ Birds

☐ Flies ☐ Other.....

Animal management

- Chicken intensity .....chicken per m^2

- The shortest lighting time during rearing period.....hours/day

- Depopulation time (in the farm)

☐ <1 day ☐ 1-2 day ☐ 3-7 day ☐ > 1 week

- Feed withdrawal time

☐ <6 hours ☐6-8 hours ☐ 9-12 hours ☐>12 hours

Pets in the farm

- Presence of pets in farm

☐ Dog ☐ Cat ☐ Cattle ☐ Swine

☐ Small ruminant ☐ Bird ☐ Duck/goose ☐ other.....

- Presence of pets in adjacent area

☐ Dog ☐ Cat ☐ Cattle ☐ Swine

☐ Small ruminant ☐ Bird ☐ Duck/goose ☐ other.....

- In case of cats are present in farm, the cats have access to

☐ Poultry houses ☐ Feed stores ☐ Outside farming area

Questionnaire (flock section)

Farm record

- Average temperature .....

- Average humidity.....

- Mortality rate.....

- Culling rate.....

- Condition of drinking waterer

☐ Normal ☐ Damage ☐ etc.

- Carcasses disposal

☐ 1 time/day ☐ 2 times/day ☐ more than 3 times/day

- Litter replacement during rearing period

☐ Yes.....times during rearing period

☐ No

- Chicken transferring between flocks

☐ Yes

☐ No

- Health problem of the flock

☐ Pododermatitis ☐ Hock burn

☐ Avian pathogenic E.coli ☐ Other health problem.....

- Extensive death during rearing period

☐ Yes (please specify the suspected cause).....

☐ No

- Antibiotic use during rearing period

☐ Yes (please specify the objective of use).....

☐ No

- Presence of the pest

☐ Bird ☐ Rodent

☐ House lizard ☐ Cockroach

☐ Fly ☐ Darkling beetle

☐ No pest in the house

- Duration of bird catching for slaughter

☐ Less than 30 minutes

☐ Between 30 minutes – 2 hours

☐ Between 2 – 6 hours

☐ More than 6 hours

- Slaughter age.....days

Table B-1 Definition of independent variable in the questionnaires

Independent variable	Definition
Farm size (square meter)	The area of target farm in square meter (SI unit)
Number of rearing house	The number of broiler house located in the target farm
Production capacity (chicken per year)	The number of chicken produced from the target farm per year
Age of target rearing house (month)	Approximate age of target house since from the first employed
Average temperature within target rearing house (°C)	Average temperature recorded in the house during rearing period
Humidity within target rearing house (%)	Average humidity recorded in the house during rearing period
Mortality rate (%)	Summary of mortality rate at the end of rearing period
Culling rate (%)	Summary of culling rate at the end of rearing period
Slaughter age (days)	Age of birds at the day of flock clearance
Number of chicken in target rearing house	The number of chicken in the flock at the end of rearing period
Feeding system	Type of feeding equipment providing in the house i.e., trough feeder, pan feeder and both types
Drinking water system	Type of waterer system providing in the house i.e., nipple drinker without cup, nipple drinker with cup and bell waterer
Presence of anteroom	Presence of the store room in the front of target house
Damage on target house structure	Presence of damage on the structure of target house

Independent variable	Definition
Ground area around the target house	Type of ground area around the target house i.e., weed, gravel, dirt and concrete
Organic acid supplement use in drinking water	Using of organic acid in drinking water
Antibiotic supplement in animal feed	Presence of antibiotic addition in animal feed
Type of disinfectant in water	Type of disinfectant for water disinfection or no disinfection
Source of water in farm	Source of water supply for farm; underground water, tap water and surface water
Presence of surface water in surrounding area	Presence of surface water on surrounding area of the farm
Frequency of foot dip disinfectant change	Frequency of foot dip disinfectant change; once a day and more than once a day
Cleaning method for drinking water equipment	Cleaning method for waterer equipment; cleaning with water, cleaning with acidity solution and cleaning with disinfectant solution
Pest control management	Presence of pest control program or facility in the target house
Duration for flock clearance (within the farm)	Duration of flock clearance in the farm; start from the first house until the last house in the farm
Duration for bird catching	Duration of bird catching for slaughter in the target house
Duration for feed withdrawal	Duration of feed withdrawal before slaughter

Independent variable	Definition
Presence of domestic animal in farm area/adjacent	Presence of domestic animal, such as dog, cat, cattle, swine or bird, within farm area or adjacent to farm area
Presence of any damage on waterer equipment	Presence of crack, leaking or break on waterer equipment
Presence of pest in target house area	Presence of pest found in target house area; bird, house lizard, fly and darkling beetle
Seasonal time at slaughter	The season that target flock was slaughtered; dry and wet season
History of *Campylobacter* in previous flock	Presence of *Campylobacter* positive status on previous broiler flock

Table B-2 Raw data obtained from questionnaires

No.	Farm	Company	Farm size (m^2)	House number	Production capacity (chicken/year)	Chicken breed
1	A1	A	352,000	72	1620000	n/a
2	A2	A	352,000	72	1620000	n/a
3	A3	A	80,000	12	700000	n/a
4	A4	A	25,600	3	222500	n/a
5	A5	A	144,000	17	2000000	n/a
6	A6	A	8,000	5	340000	n/a
7	A7	A	40,000	12	1200000	n/a
8	A8	A	128,000	10	1435000	Arber acre & Ross
9	A9	A	n/a	22	2019500	Arber acre & Ross
10	A10	A	8,000	3	230400	n/a
11	A11	A	n/a	10	1512000	Arber acre & Ross
12	A12	A	128,000	10	1537500	Arber acre & Ross
13	A13	A	32,000	10	1000000	Arber acre
14	A14	A	n/a	27	2484000	Arber acre& Ross
15	A15	A	24,000	7	700000	Arber acre
16	A16	A	64,000	12	1035000	Arber acre & Ross
17	A17	A	16,000	2	105000	Arber acre & Ross
18	A18	A	n/a	1	80000	n/a
19	A19	A	32,000	2	112000	Arber acre & Ross
20	A20	A	48,000	10	735000	Arber acre & Ross
21	A21	A	32,000	5	400000	Arber acre & Ross
22	A22	A	n/a	9	985000	Arber acre & Ross
23	A23	A	n/a	2	100000	n/a
24	A24	A	n/a	7	705500	Arber acre & Ross
25	A25	A	n/a	14	1298500	Arber acre & Ross
26	A26	A	n/a	3	280000	n/a
27	A27	A	n/a	7	685000	Arber acre & Ross
28	A28	A	n/a	3	192000	Arber acre & Ross
29	A29	A	80,000	14	2250000	Arber acre & Ross
30	A30	A	272,000	30	2500000	Arber acre

No.	Farm	Company	Farm size (m^2)	House number	Production capacity (chicken/year)	Chicken breed
31	B/C2	B/C	11,200	1	93,000	Cobb, Arber acre
32	B/C3	B/C	22,400	1	36,000	Cobb, Arber acre
33	B/C4	B/C	17,600	1	60,000	Cobb, Arber acre
34	B/C6	B/C	11,200	1	96,000	Cobb, Arber acre
35	B/C7	B/C	8,000	1	60,000	Cobb, Arber acre
36	B/C8	B/C	19,200	1	90,000	Cobb, Arber acre
37	B/C9	B/C	19,200	1	90,000	Cobb, Arber acre
38	B/C10	B/C	11,200	1	96,000	Cobb, Arber acre
39	B/C11	B/C	19,200	1	72,000	Cobb, Arber acre
40	B/C13	B/C	19,200	1	72,000	Cobb, Arber acre
41	B/C14	B/C	16,000	1	72,000	Cobb, Arber acre
42	B/C15	B/C	19,200	1	45,000	Cobb, Arber acre
43	B/C12-1	B/C	384,000	4	160,000	Cobb
44	B/C12-2	B/C	384,000	4	160,000	Cobb
45	B/C1	B/C	16,000	1	72,000	Cobb, Arber acre

No.	Farm	Company	Type of house	Feeding system	Drinking water system	Presence of anteroom
1	A1	A	Close system	Pan feeder	Nipple without cup	Yes
2	A2	A	Close system	Pan feeder	Nipple without cup	Yes
3	A3	A	Close system	Pan feeder	Nipple with cup	No
4	A4	A	Close system	Both type	Nipple with cup	Yes
5	A5	A	Close system	Trough feeder	Nipple with cup	Yes
6	A6	A	Close system	Pan feeder	Nipple with cup	No
7	A7	A	Close system	Pan feeder	Nipple with cup	Yes
8	A8	A	Close system	Pan feeder	Nipple without cup	No
9	A9	A	Close system	Pan feeder	Nipple and bell drinker	No
10	A10	A	Close system	Both type	Nipple with cup	Yes
11	A11	A	Close system	Pan feeder	Nipple and bell drinker	No
12	A12	A	Close system	Pan feeder	Nipple without cup	No
13	A13	A	Close system	Pan feeder	Nipple without cup	Yes
14	A14	A	Close system	Pan feeder	Nipple and bell drinker	No
15	A15	A	Close system	Pan feeder	Nipple without cup	Yes
16	A16	A	Close system	Pan feeder	Nipple with cup	Yes
17	A17	A	Close system	Pan feeder	Nipple without cup	Yes
18	A18	A	Close system	Pan feeder	Nipple with cup	No
19	A19	A	Close system	Both type	Nipple with cup and bell drinker	Yes
20	A20	A	Close system	Pan feeder	Nipple with cup	Yes
21	A21	A	Close system	Pan feeder	Nipple with cup	Yes
22	A22	A	Close system	Both type	Nipple and bell drinker	No
23	A23	A	Close system	Pan feeder	Nipple and bell drinker	No
24	A24	A	Close system	Both type	Nipple and bell drinker	Yes
25	A25	A	Close system	Pan feeder	Nipple and bell drinker	No
26	A26	A	Close system	Pan feeder	Nipple with cup	No
27	A27	A	Close system	Pan feeder	Nipple and bell drinker	No
28	A28	A	Close system	Both type	Nipple with cup	No
29	A29	A	Close system	Pan feeder	Nipple with cup	Yes
30	A30	A	Close system	Both type	Nipple without cup	Yes

No.	Farm	Company	Type of house	Feeding system	Drinking water system	Presence of anteroom
31	B/C2	B/C	Close system	Pan feeder	Nipple with cup	Yes
32	B/C3	B/C	Close system	Pan feeder	Nipple with cup	Yes
33	B/C4	B/C	Close system	Pan feeder	Nipple with cup	Yes
34	B/C6	B/C	Close system	Pan feeder	Nipple with cup	Yes
35	B/C7	B/C	Close system	Pan feeder	Nipple with cup	Yes
36	B/C8	B/C	Close system	Pan feeder	Nipple with cup	Yes
37	B/C9	B/C	Close system	Pan feeder	Nipple with cup	Yes
38	B/C10	B/C	Close system	Pan feeder	Nipple with cup	Yes
39	B/C11	B/C	Close system	Pan feeder	Nipple with cup	Yes
40	B/C13	B/C	Close system	Pan feeder	Nipple with cup	Yes
41	B/C14	B/C	Close system	Pan feeder	Nipple with cup	Yes
42	B/C15	B/C	Close system	Pan feeder	Nipple with cup	Yes
43	B/C12-1	B/C	Close system	Trough feeder	Nipple with cup	Yes
44	B/C12-2	B/C	Close system	Trough feeder	Nipple with cup	Yes
45	B/C1	B/C	Close system	Pan feeder	Nipple with cup	Yes

No.	Farm	Company	Age of house (month)	Damage of target house	Ground area around the target house	Organic acid supplement in drinking water
1	A1	A	300	Yes	Concrete	Yes
2	A2	A	300	Yes	Concrete	Yes
3	A3	A	24	Yes	Concrete	Yes
4	A4	A	81	Yes	Soil gravel	No
5	A5	A	120	Yes	Weed concrete	Yes
6	A6	A	240	Yes	Concrete	Yes
7	A7	A	120	No	Concrete	Yes
8	A8	A	6	No	Gravel	Yes
9	A9	A	n/a	No	Concrete	Yes
10	A10	A	132	Yes	Soil gravel	No
11	A11	A	n/a	No	Concrete	Yes
12	A12	A	6	No	Gravel	Yes
13	A13	A	96	No	Concrete	Yes
14	A14	A	n/a	No	Concrete	Yes
15	A15	A	96	No	Concrete	Yes
16	A16	A	72	Yes	Soil	No
17	A17	A	72	Yes	Soil	No
18	A18	A	n/a	Yes	Concrete	Yes
19	A19	A	n/a	Yes	Soil	Yes
20	A20	A	72	Yes	Soil	No
21	A21	A	60	Yes	Soil	Yes
22	A22	A	n/a	Yes	Soil	Yes
23	A23	A	n/a	Yes	Concrete	Yes
24	A24	A	n/a	Yes	Weed	No
25	A25	A	n/a	No	Gravel	Yes
26	A26	A	n/a	Yes	Concrete	Yes
27	A27	A	n/a	No	Concrete	Yes
28	A28	A	n/a	Yes	Soil	Yes
29	A29	A	24	Yes	Soil	Yes
30	A30	A	36	No	Soil	Yes

No.	Farm	Company	Age of house (month)	Damage of target house	Ground area around the target house	Organic acid supplement in drinking water
31	B/C2	B/C	12	No	Soil, Concrete	Yes
32	B/C3	B/C	132	No	Soil, Concrete	Yes
33	B/C4	B/C	120	No	Soil, Concrete	Yes
34	B/C6	B/C	24	No	Soil, Concrete	Yes
35	B/C7	B/C	108	No	Soil, Concrete	Yes
36	B/C8	B/C	120	No	Soil, Concrete	Yes
37	B/C9	B/C	48	No	Soil, Concrete	Yes
38	B/C10	B/C	12	No	Soil, Concrete	Yes
39	B/C11	B/C	84	No	Soil, Concrete	Yes
40	B/C13	B/C	120	No	Soil, Concrete	Yes
41	B/C14	B/C	120	No	Soil, Concrete	Yes
42	B/C15	B/C	120	No	Soil, Concrete	Yes
43	B/C12-1	B/C	360	Yes	Gravel	No
44	B/C12-2	B/C	360	Yes	Gravel	No
45	B/C1	B/C	120	No	Soil, Concrete	Yes

No.	Farm	Company	Antibiotic supplement in animal feed	Type of disinfectant in water	Source of water in farm	Presence of surface water in surrounding
1	A1	A	No	Hypochlorite	Underground	No
2	A2	A	No	Hypochlorite	Underground	No
3	A3	A	Yes	Chlorine dioxide	Surface	Yes
4	A4	A	Yes	Hypochlorite	Surface	Yes
5	A5	A	No	Chlorine dioxide	Underground	Yes
6	A6	A	Yes	Chlorine dioxide	Underground & Surface	No
7	A7	A	Yes	Chlorine dioxide	Underground	Yes
8	A8	A	N/A	Chlorine dioxide	Surface	No
9	A9	A	No	No disinfection	Underground	No
10	A10	A	Yes	Hypochlorite	Underground	Yes
11	A11	A	No	No disinfection	Underground	No
12	A12	A	N/A	Chlorine dioxide	Surface	No
13	A13	A	Yes	Chlorine dioxide	Underground	No
14	A14	A	No	No disinfection	Underground	No
15	A15	A	Yes	Chlorine dioxide	Underground	No
16	A16	A	No	Hypochlorite	Underground	No
17	A17	A	No	Hypochlorite	Underground	No
18	A18	A	Yes	Chlorine dioxide	Underground	No
19	A19	A	No	No disinfection	Underground	Yes
20	A20	A	No	Hypochlorite	Underground	No
21	A21	A	No	Hypochlorite	Surface	No
22	A22	A	No	Chlorine dioxide	Underground	Yes
23	A23	A	Yes	Chlorine dioxide	Underground	No
24	A24	A	No	Hypochlorite	n/a	Yes
25	A25	A	No	Hypochlorite	n/a	No
26	A26	A	Yes	Chlorine dioxide	Underground	No
27	A27	A	No	No disinfection	Underground	No
28	A28	A	No	No disinfection	Underground	n/a
29	A29	A	No	Hypochlorite	Surface	No
30	A30	A	No	Hypochlorite	Underground	Yes

No.	Farm	Company	Antibiotic supplement in animal feed	Type of disinfectant in water	Source of water in farm	Presence of surface water in surrounding
31	B/C2	B/C	Yes	Chlorine dioxide	Underground, tap water	Yes
32	B/C3	B/C	Yes	Chlorine dioxide	Underground	Yes
33	B/C4	B/C	Yes	Chlorine dioxide	Underground	Yes
34	B/C6	B/C	Yes	Chlorine dioxide	Underground, tap water	Yes
35	B/C7	B/C	Yes	Chlorine dioxide	Underground, tap water	Yes
36	B/C8	B/C	Yes	Chlorine dioxide	Underground	Yes
37	B/C9	B/C	Yes	Chlorine dioxide	Underground	Yes
38	B/C10	B/C	Yes	Chlorine dioxide	Underground, tap water	Yes
39	B/C11	B/C	Yes	Chlorine dioxide	Underground	Yes
40	B/C13	B/C	Yes	Chlorine dioxide	Underground	Yes
41	B/C14	B/C	Yes	Chlorine dioxide	Underground	Yes
42	B/C15	B/C	Yes	Chlorine dioxide	Underground	Yes
43	B/C12-1	B/C	No	Hypochlorite	Underground	No
44	B/C12-2	B/C	No	Hypochlorite	Underground	No
45	B/C1	B/C	Yes	Chlorine dioxide	Underground	Yes

No.	Farm	Company	Frequency of foot dip disinfectant change	Reagent for drinking equipment cleaning	Pest control management
1	A1	A	once a day	Acid	Rodent
2	A2	A	once a day	Acid	Rodent
3	A3	A	> once a day	Water and disinfectant	Rodent, bird, darkling beetle
4	A4	A	once a day	Water and acid	Rodent, fly
5	A5	A	> once a day	Water and acid	Rodent, fly
6	A6	A	> once a day	Water and disinfectant	Rodent, bird, darkling beetle
7	A7	A	> once a day	Water and disinfectant	Rodent, bird, fly, darkling beetle
8	A8	A	once a day	Disinfectant	Rodent, fly
9	A9	A	> once a day	Water and acid	Rodent, fly
10	A10	A	once a day	Water and acid	Rodent, fly
11	A11	A	> once a day	Water and acid	Rodent, fly
12	A12	A	once a day	Disinfectant	Rodent, fly
13	A13	A	> once a day	Water and disinfectant	Rodent, bird, fly, darkling beetle
14	A14	A	> once a day	Water and acid	Rodent, fly
15	A15	A	> once a day	Water and disinfectant	Rodent, bird, fly, darkling beetle
16	A16	A	> once a day	Disinfectant	Rodent, bird
17	A17	A	> once a day	Disinfectant	Rodent, bird
18	A18	A	> once a day	Water and disinfectant	Rodent, bird, darkling beetle
19	A19	A	once a day	Disinfectant	Rodent, bird
20	A20	A	> once a day	Disinfectant	Rodent, bird
21	A21	A	> once a day	Disinfectant	Rodent, bird
22	A22	A	once a day	Disinfectant	Rodent, bird
23	A23	A	> once a day	Water and disinfectant	Rodent, bird, darkling beetle
24	A24	A	once a day	Disinfectant	Rodent
25	A25	A	> once a day	Water and acid	Rodent, fly
26	A26	A	> once a day	Water and disinfectant	Rodent, bird, darkling beetle
27	A27	A	> once a day	Water and acid	Rodent, fly
28	A28	A	Every 2-3 days	Disinfectant	Rodent, bird
29	A29	A	> once a day	Disinfectant	Rodent, bird
30	A30	A	once a day	Acid	Rodent, bird, fly

No.	Farm	Company	Frequency of foot dip disinfectant change	Reagent for drinking equipment cleaning	Pest control management
31	B/C2	B/C	once a day	Acid	Rodent, bird
32	B/C3	B/C	once a day	Acid	Rodent, bird
33	B/C4	B/C	once a day	Acid	Rodent, bird
34	B/C6	B/C	once a day	Acid	Rodent, bird
35	B/C7	B/C	once a day	Acid	Rodent, bird
36	B/C8	B/C	once a day	Acid	Rodent, bird
37	B/C9	B/C	once a day	Acid	Rodent, bird
38	B/C10	B/C	once a day	Acid	Rodent, bird
39	B/C11	B/C	once a day	Acid	Rodent, bird
40	B/C13	B/C	once a day	Acid	Rodent, bird
41	B/C14	B/C	once a day	Acid	Rodent, bird
42	B/C15	B/C	once a day	Acid	Rodent, bird
43	B/C12-1	B/C	once a day	Acid	Rodent
44	B/C12-2	B/C	once a day	Acid	Rodent
45	B/C1	B/C	once a day	Acid	Rodent, bird

No.	Farm	Company	Duration for flock clearance in broiler farm	Duration for feed withdrawal	Presence of domestic animal in farm area/adjacent
1	A1	A	3-7 days	6-8 hours	Yes
2	A2	A	3-7 days	6-8 hours	Yes
3	A3	A	3-7 days	9-12 hours	Yes
4	A4	A	1-2 day(s)	9-12 hours	Yes
5	A5	A	3-7 days	6-8 hours	Yes
6	A6	A	1-2 day(s)	9-12 hours	Yes
7	A7	A	3-7 days	9-12 hours	Yes
8	A8	A	3-7 days	6-8 hours	n/a
9	A9	A	3-7 days	9-12 hours	Yes
10	A10	A	1-2 day(s)	6-8 hours	Yes
11	A11	A	3-7 days	9-12 hours	Yes
12	A12	A	3-7 days	6-8 hours	n/a
13	A13	A	1-2 day(s)	9-12 hours	Yes
14	A14	A	3-7 days	9-12 hours	Yes
15	A15	A	1-2 day(s)	9-12 hours	Yes
16	A16	A	3-7 days	9-12 hours	Yes
17	A17	A	1-2 day(s)	9-12 hours	Yes
18	A18	A	Less than 1 day	9-12 hours	Yes
19	A19	A	1-2 day(s)	9-12 hours	Yes
20	A20	A	3-7 days	9-12 hours	Yes
21	A21	A	1-2 day(s)	9-12 hours	Yes
22	A22	A	3-7 days	9-12 hours	Yes
23	A23	A	Less than 1 day	9-12 hours	No
24	A24	A	3-7 days	9-12 hours	Yes
25	A25	A	3-7 days	9-12 hours	Yes
26	A26	A	1-2 day(s)	9-12 hours	Yes
27	A27	A	3-7 days	9-12 hours	Yes
28	A28	A	1-2 day(s)	9-12 hours	Yes
29	A29	A	3-7 days	9-12 hours	Yes
30	A30	A	3-7 days	9-12 hours	n/a

No.	Farm	Company	Duration for flock clearance in broiler farm	Duration for feed withdrawal	Presence of domestic animal in farm area/adjacent
31	B/C2	B/C	3-7 days	< 6 hours	No
32	B/C3	B/C	3-7 days	< 6 hours	No
33	B/C4	B/C	3-7 days	< 6 hours	No
34	B/C6	B/C	3-7 days	< 6 hours	No
35	B/C7	B/C	3-7 days	< 6 hours	No
36	B/C8	B/C	3-7 days	< 6 hours	No
37	B/C9	B/C	3-7 days	< 6 hours	No
38	B/C10	B/C	3-7 days	< 6 hours	No
39	B/C11	B/C	3-7 days	< 6 hours	No
40	B/C13	B/C	3-7 days	< 6 hours	No
41	B/C14	B/C	3-7 days	< 6 hours	No
42	B/C15	B/C	3-7 days	< 6 hours	No
43	B/C12-1	B/C	Less than 1 day	9-12 hours	Yes
44	B/C12-2	B/C	Less than 1 day	9-12 hours	Yes
45	B/C1	B/C	3-7 days	< 6 hours	No

No.	Farm	Company	Average temperature within the house (°C)	Humidity within the house (%)	Mortality rate (%)
1	A1	A	27.3 – 29.0	73.0 – 75.0	4.13 – 4.34
2	A2	A	26.7 – 29.0	64.5 – 82.5	1.21 – 2.89
3	A3	A	29.0 – 29.7	60.0 – 74.9	3.33 – 11.00
4	A4	A	30	60.0	3.70
5	A5	A	26.0 – 30.0	62.0 – 72.0	1.23 – 5.17
6	A6	A	28.5 – 30.0	57.5 – 76.0	2.00 – 2.37
7	A7	A	29.0	60.0 – 65.0	0.78 – 5.82
8	A8	A	28.0	60.0 – 65.0	1.99 – 2.10
9	A9	A	30.0	60.0	n/a
10	A10	A	23.0 – 31.0	50.0 – 80.0	1.00 - 2.56
11	A11	A	30.0	60.0	n/a
12	A12	A	28.0	60.0	8.92
13	A13	A	28.0 – 31.0	60.0 – 77.5	0.65 – 2.02
14	A14	A	30.0	60.0	n/a
15	A15	A	27.0 – 32.0	60.0 – 77.5	0.94 - 3.1
16	A16	A	30.0	70.0	2.50
17	A17	A	27.0 – 34.0	59.0 – 75.0	0.50 – 1.51
18	A18	A	29.0	50.0	5.68
19	A19	A	27.7 – 31.5	65.7 - 75.0	1.06 – 3.68
20	A20	A	28.0	70.0 – 75.0	1.50
21	A21	A	30.0 – 32.0	70.0 – 75.0	1.50 – 2.50
22	A22	A	26.5 – 30.2	64.0 - 75.1	1.66 – 9.86
23	A23	A	27.2 - 33.1	55.6 – 78.0	0.98 - 3.34
24	A24	A	30.0	70.0	2.48
25	A25	A	30.0	60.0	1.70 -2.60
26	A26	A	29.0	60.0	2.88
27	A27	A	30.0	60.0	n/a
28	A28	A	28.5 – 30.0	60.0 – 75.0	1.63 – 1.86
29	A29	A	30.0	70.0 – 75.0	1.50 – 2.50
30	A30	A	27.3 – 30.9	73.0 - 80.0	1.54 - 5.08

No.	Farm	Company	Average temperature within target house (°C)	Humidity within target rearing house (%)	Mortality rate (%)
31	B/C2	B/C	28 - 29	76	1.97 – 7.61
32	B/C3	B/C	28 - 29	76	2.07 – 4.92
33	B/C4	B/C	28 - 29	76	1.79 – 3.62
34	B/C6	B/C	28 - 29	76	1.57 – 3.73
35	B/C7	B/C	28 - 29	76	1.96 – 4.47
36	B/C8	B/C	28 - 29	76	2.66 – 4.82
37	B/C9	B/C	28 - 29	76	3.11 – 3.59
38	B/C10	B/C	28 - 29	76	2.75 – 8.51
39	B/C11	B/C	28 - 29	76	0.77 – 4.14
40	B/C13	B/C	28 - 29	76	0.63 – 6.25
41	B/C14	B/C	28 - 29	76	3.40 – 3.63
42	B/C15	B/C	28 - 29	76	1.92 – 7.26
43	B/C12-1	B/C	n/a	n/a	n/a
44	B/C12-2	B/C	n/a	n/a	n/a
45	B/C1	B/C	28 - 29	76	2.06

No.	Farm	Company	Culling rate (%)	Slaughter age (days)	Number of chicken in target house	Presence of damage on waterer
1	A1	A	2.31 – 2.32	39	14,280	Yes
2	A2	A	0.63 – 4.57	39 – 41	12,852 – 16,830	Yes
3	A3	A	4.08 – 8.56	39	12,036 – 13,260	Yes
4	A4	A	0.80	41	15,000	Yes
5	A5	A	0.36 – 1.8	39 – 42	20,910 – 27,336	Yes
6	A6	A	1.37 – 2.19	41	8,058 – 9,996	No
7	A7	A	1.21 – 4.36	40 – 42	21,930 – 22,032	Yes
8	A8	A	2.00	42	31,008 – 31,212	No
9	A9	A	n/a	39 -41	15,810 – 17,034	No
10	A10	A	1.91 – 7.03	39 – 42	14,658 – 53,142	Yes
11	A11	A	n/a	39	24,888 – 29,478	No
12	A12	A	n/a	40	27,030	No
13	A13	A	0.49 – 2.85	37 -42	19,380 – 24,480	Yes
14	A14	A	n/a	37 -42	15,606 – 16,524	No
15	A15	A	0.62 - 3.12	38 – 41	23,256 – 29,070	Yes
16	A16	A	5.00	41	17,850	No
17	A17	A	1.11– 3.50	37 – 40	9,486 – 12,500	Yes
18	A18	A	6.71	40	20,400	No
19	A19	A	0.32 – 1.53	38 – 42	9,200 – 11,730	Yes
20	A20	A	3.50 – 5.00	39	16,500	No
21	A21	A	2.50 – 3.50	41	9,200	No
22	A22	A	0.32 - 36.38	38 – 43	18,054 – 21,930	Yes
23	A23	A	0.95 – 3.06	39 – 41	9,486 – 11,730	No
24	A24	A	n/a	42	19,894	Yes
25	A25	A	5.6 - 6.8	39 – 40	11,832 – 12,138	Yes
26	A26	A	2.02	41	16,320	Yes
27	A27	A	n/a	37 – 40	17,136	Yes
28	A28	A	0.32	42	10,210 – 10,710	No
29	A29	A	3.00 – 5.00	40 – 41	17,850 – 24,200	No
30	A30	A	1.01 - 22.28	30 - 43	13,260 – 16,524	Yes

No.	Farm	Company	Culling rate (%)	Slaughter age (days)	Number of chicken in target house	Presence of damage on waterer
31	B/C2	B/C	0.24 – 1.25	31 – 38	13,260 – 16,320	Yes
32	B/C3	B/C	0.08 – 1.42	31 – 37	4,794 – 6,630	Yes
33	B/C4	B/C	0.08 – 1.18	31 – 42	9,690 – 11,220	Yes
34	B/C6	B/C	0.12 – 1.03	31 – 40	15,300 – 16,320	Yes
35	B/C7	B/C	0.26 – 2.15	31 – 40	9,690 – 11,730	Yes
36	B/C8	B/C	0.29 – 1.75	31 - 39	14,790 – 16,320	Yes
37	B/C9	B/C	1.04 – 1.74	31 – 33	15,000 – 16,014	Yes
38	B/C10	B/C	0.26 – 1.53	31 – 36	15,045 – 16,320	Yes
39	B/C11	B/C	0.24 – 2.24	31 – 41	10,200 – 12,750	Yes
40	B/C13	B/C	0.29 – 3.54	30 – 38	11,730 – 12,750	Yes
41	B/C14	B/C	0.40 – 0.90	31 – 38	12,000 – 12,240	Yes
42	B/C15	B/C	0.03 – 2.63	31 – 41	7,500 – 8,466	Yes
43	B/C12-1	B/C	n/a	n/a	n/a	n/a
44	B/C12-2	B/C	n/a	n/a	n/a	n/a
45	B/C1	B/C	0.77	32	12,000	No

No.	Farm	Company	Presence of pest in target house area	Duration for bird catching
1	A1	A	House lizard, fly, darkling beetle	30 minutes – 2 hours
2	A2	A	Bird, House lizard, fly, darkling beetle	30 minutes – 2 hours
3	A3	A	Fly, darkling beetle	30 minutes – 2 hours
4	A4	A	House lizard	< 30 minutes
5	A5	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
6	A6	A	Fly, darkling beetle	30 minutes – 2 hours
7	A7	A	House lizard, fly	30 minutes – 2 hours
8	A8	A	Fly, darkling beetle	2 – 6 hours
9	A9	A	None	30 minutes – 2 hours
10	A10	A	Fly, darkling beetle	2 – 6 hours
11	A11	A	None	30 minutes – 2 hours
12	A12	A	Fly, darkling beetle	2 – 6 hours
13	A13	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
14	A14	A	None	30 minutes – 2 hours
15	A15	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
16	A16	A	House lizard	30 minutes – 2 hours
17	A17	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
18	A18	A	Fly, darkling beetle	30 minutes – 2 hours
19	A19	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
20	A20	A	House lizard	30 minutes – 2 hours
21	A21	A	House lizard	30 minutes – 2 hours
22	A22	A	Bird, House lizard, fly, rodent, darkling beetle	2 – 6 hours
23	A23	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
24	A24	A	Bird, House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
25	A25	A	None	30 minutes – 2 hours
26	A26	A	Fly, darkling beetle	30 minutes – 2 hours
27	A27	A	None	30 minutes – 2 hours
28	A28	A	House lizard, fly, rodent, darkling beetle	30 minutes – 2 hours
29	A29	A	House lizard	30 minutes – 2 hours
30	A30	A	Bird, House lizard, fly, darkling beetle	30 minutes – 2 hours

No.	Farm	Company	Presence of pest in target house area	Duration for bird catching
31	B/C2	B/C	Bird, House lizard, fly, rodent, cockroach, darkling beetle	> 6 hours
32	B/C3	B/C	Bird, House lizard, fly, rodent, cockroach, darkling beetle	> 6 hours
33	B/C4	B/C	Bird, House lizard, fly, rodent, cockroach, darkling beetle	> 6 hours
34	B/C6	B/C	Bird, House lizard, fly, rodent, cockroach, darkling beetle	> 6 hours
35	B/C7	B/C	Bird, House lizard, fly, rodent, cockroach, darkling beetle	> 6 hours
36	B/C8	B/C	Bird, House lizard, fly, darkling beetle	> 6 hours
37	B/C9	B/C	House lizard, fly, darkling beetle	> 6 hours
38	B/C10	B/C	Bird, House lizard, fly, cockroach, darkling beetle	> 6 hours
39	B/C11	B/C	Bird, House lizard, fly, darkling beetle	> 6 hours
40	B/C13	B/C	Bird, House lizard, fly, darkling beetle	> 6 hours
41	B/C14	B/C	House lizard, fly, darkling beetle	> 6 hours
42	B/C15	B/C	Bird, House lizard, fly, cockroach, darkling beetle	> 6 hours
43	B/C12-1	B/C	n/a	n/a
44	B/C12-2	B/C	n/a	n/a
45	B/C1	B/C	House lizard, darkling beetle	> 6 hours

Table B-3 *Campylobacter* isolations from broiler flocks in Thailand

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
A1	16/1/2012	negative					
	22/3/2012	negative					
A2	16/1/2012	negative					
	26/3/2012	negative					
	1/6/2012	negative					
	13/8/2012	negative					
	27/10/2012	positive	9/10 (90)	1.12E+08	100	0	0
	7/1/2013	negative					
	18/3/2013	negative					
	3/6/2013	negative					
	7/8/2013	negative					
	21/10/2013	negative					
A3	17/1/2012	positive	9/10 (90)	5.60E+06	100	0	0
	27/3/2012	positive	8/10 (80)	8.76E+07	100	0	0
A4	17/1/2012	negative					
A5	26/1/2012	negative	10/10 (100)	1.00E+07	100	0	0
	4/4/2012	positive	10/10 (100)	4.47E+07	100	0	0
	11/6/2012	positive	10/10 (100)	5.99E+07	100	0	0
	20/8/2012	positive	10/10 (100)	3.25E+08	100	0	0
	27/10/2012	positive	9/10 (90)	3.68E+07	100	0	0
	11/1/2013	positive	10/10 (100)	1.49E+08	100	0	0
	2/4/2013	positive					
A5	12/6/2013	positive	10/10 (100)	1.25E+08	100	0	0
	20/8/2013	positive	6/10 (60)	1.02E+08	100	0	0
	1/11/2013	positive	10/10 (100)	N/A	100	0	0
	10/1/2014	negative					
	15/3/2014	negative					
A6	26/1/2012	negative					
	2/4/2012	negative					
A7	26/1/2012	negative					
	7/4/2012	negative					
A8	31/1/2012	negative					
	27/4/2012	negative					
A9	31/1/2012	negative					
	9/4/2012	negative					

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
A10	1/2/2012	negative					
	7/4/2012	negative					
	14/6/2012	negative					
	25/8/2012	negative					
	29/10/2012	positive	10/10 (100)	5.35E+05	100	0	0
	3/1/2013	negative					
	18/3/2013	negative					
	4/6/2013	negative					
	15/8/2013	negative					
A10	26/10/2013	negative					
	9/1/2014	negative					
	24/3/2014	negative					
A11	1/2/2012	negative					
	10/4/2012	negative					
A12	1/2/2012	negative					
	2/5/2012	negative					
A13	2/2/2012	negative					
	11/4/2012	negative					
	20/6/2012	positive	5/10 (50)	3.20E+05	100	0	0
	31/8/2012	positive	10/10 (100)	8.63E+07	100	0	0
	6/11/2012	positive	10/10 (100)	N/A	100	0	0
	17/1/2013	positive	10/10 (100)	N/A	100	0	0
	6/4/2013	positive	10/10 (100)	1.49E+08	100	0	0
	20/6/2013	negative					
	28/8/2013	negative					
	7/11/2013	positive	10/10 (100)	N/A	100	0	0
	18/1/2014	negative					
	27/3/2014	positive	8/10 (80)	2.64E+07	100	0	0
A14	2/2/2012	negative					
	11/4/2012	negative					
A15	4/2/2012	positive	10/10 (100)	2.80E+06	100	0	0
	10/3/2012	positive	10/10 (100)	4.53E+08	0	100	0
	14/5/2012	positive	8/10 (80)	<104	100	0	0
	23/7/2012	positive	2/10 (20)	<104	100	0	0
	29/9/2012	positive	10/10 (100)	N/A	100	0	0
	13/12/2012	positive	9/10 (90)	N/A	100	0	0
	1/3/2013	negative					

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
A15	15/5/2013	negative					
	22/7/2013	negative					
	2/10/2013	negative					
	13/12/2013	negative					
	19/2/2014	negative					
A16	16/1/2012	negative					
A17	17/1/2012	negative					
	27/3/2012	positive	10/10 (100)	1.41E+08	100	0	0
	9/8/2012	positive	10/10 (100)	2.59E+07	20	80	0
	17/12/2012	positive	8/10 (80)	5.54E+07	100	0	0
	23/2/2013	positive	10/10 (100)	3.68E+07	100	0	0
	2/5/2013	positive	10/10 (100)	1.11E+08	100	0	0
	6/7/2013	positive	8/10 (80)	1.57E+07	100	0	0
	14/9/2013	positive	8/10 (80)	1.02E+08	100	0	0
A18	18/1/2012	negative					
A19	26/1/2012	positive	10/10 (100)	2.94E+08	100	0	0
	22/3/2012	negative					
	23/5/2012	negative					
	30/7/2012	positive	2/10 (20)	3.50E+05	100	0	0
	9/10/2012	negative					
	11/12/2012	positive	10/10 (100)	1.85E+08	100	0	0
	18/2/2013	negative					
	16/4/2013	negative					
	4/7/2013	negative					
	13/9/2013	negative					
	14/11/2013	positive	7/10 (70)	3.90E+07	100	0	0
	17/1/2014	negative					
A20	26/1/2012	negative					
	7/4/2012	negative					
A21	31/1/2012	negative					
	19/4/2012	negative					
A22	31/1/2012	positive	10/10 (100)	1.87E+08	0	100	0
	10/4/2012	negative					
	22/6/2012	positive	8/10 (80)	3.73E+07	100	0	0
	1/9/2012	positive	8/10 (80)	7.50E+06	100	0	0
	8/11/2012	positive	9/10 (90)	4.57E+07	88.89	11.11	0
	17/1/2013	negative					

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
	2/4/2013	positive	10/10 (100)	2.71E+07	100	0	0
A22	28/6/2013	positive	10/10 (100)	1.66E+06	100	0	0
	18/9/2013	positive	10/10 (100)	2.74E+08	100	0	0
	3/12/2013	positive	6/10 (60)	1.00E+04	100	0	0
	6/2/2014	negative					
	25/4/2014	positive	10/10 (100)	5.46E+08	0	100	0
A23	1/2/2012	negative					
	26/3/2012	positive	10/10 (100)	3.23E+07	100	0	0
	31/5/2012	positive	7/10 (70)	9.60E+07	100	0	0
	6/8/2012	positive	10/10 (100)	7.47E+07	100	0	0
	16/10/2012	positive	2/10 (20)	<1.00E+04	100	0	0
	26/12/2012	positive	10/10 (100)	2.94E+08	100	0	0
	11/3/2013	positive	10/10 (100)	3.15E+08	100	0	0
	30/5/2013	positive	10/10 (100)	3.22E+07	100	0	0
	6/8/2013	positive	10/10 (100)	1.79E+07	100	0	0
	18/10/2013	positive	10/10 (100)	9.42E+07	100	0	0
	23/12/2013	positive	10/10 (100)	6.35E+07	100	0	0
	17/3/2014	negative					
A24	1/2/2012	positive	1/10 (10)	<1.00E+04	100	0	0
A25	2/2/2012	positive	10/10 (100)	8.90E+07	100	0	0
	10/4/2012	negative					
A26	3/2/2012	negative					
A27	4/2/2012	negative					
	12/4/2012	negative					
A28	6/2/2012	negative					
	9/4/2012	positive	10/10 (100)	2.56E+08	100	0	0
A29	6/2/2012	negative					
	18/4/2012	negative					
A30	24/2/2012	negative					
	5/5/2012	negative					
	13/7/2012	negative					
	25/9/2012	negative					
	19/12/2012	positive	3/10 (30)	3.26E+06	100	0	0
	7/3/2013	negative					
	19/5/2013	negative					
	28/7/2013	negative					
	12/12/2013	negative					

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
B/C1	15/2/2012	positive	6/10 (60)	1.40E+05	100	0	0
B/C2	23/2/2012	positive	10/10 (100)	1.74E+07	100	0	0
	23/4/2012	positive	3/10 (30)	1.31E+08	100	0	0
	23/6/2012	positive	9/10 (90)	9.24E+08	0	100	0
	18/8/2012	positive	10/10 (100)	1.29E+06	100	0	0
	19/10/2012	negative					
	1/12/12	positive	30/30 (100)	2.87E+08	100	0	0
B/C2	15/2/2013	negative					
	7/5/2013	negative					
B/C3	23/2/2012	negative					
	20/4/2012	negative					
	14/8/2012	positive	3/10 (30)	<1.00E+04	100	0	0
	26/12/2012	negative					
	2/3/2013	negative					
	24/6/2013	negative					
	27/8/2013	positive	9/10 (90)	1.19E+07	100	0	0
	16/10/2013	positive	6/10 (60)	N/A	100	0	0
B/C4	27/2/2012	negative					
	19/4/2012	positive	10/10 (100)	N/A	100	0	0
	15/6/2012	positive	8/10 (80)	5.21E+08	100	0	0
	11/8/2012	positive	10/10 (100)	4.32E+06	100	0	0
	15/10/2012	positive	26/30 (86.67)	9.02E+05	100	0	0
	1/12/12	negative					
	18/2/2013	negative					
	19/4/2013	negative					
	19/6/2013	positive	7/10 (70)	7.95E+07	100	0	0
	15/8/2013	negative					
B/C5	27/2/2012	Positive	9/10 (90)	3.50E+07	100	0	0
B/C6	13/3/2012	positive	8/10 (80)	6.50E+06	100	0	0
	17/5/2012	positive	5/10 (50)	2.25E+08	100	0	0
	6/7/2012	negative					
	5/9/2012	positive	7/10 (70)	2.17E+08	100	0	0
	1/11/2012	positive	10/10 (100)	7.65E+05	100	0	0
	3/1/2013	positive	10/10 (100)	1.51E+08	100	0	0
B/C7	14/3/2012	negative					
	17/5/2012	positive	10/10 (100)	1.71E+08	100	0	0
	14/7/2012	positive	10/10 (100)	4.55E+06	100	0	0

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
B/C7	8/9/2012	positive	6/10 (60)	2.95E+08	0	100	0
	9/1/2013	negative					
	13/3/2013	negative					
	6/5/2013	negative					
	14/8/2013	positive	10/10 (100)	1.60E+05	100	0	0
B/C8	14/3/2012	positive	8/10 (80)	1.76E+08	100	0	0
	11/5/2012	positive	1/10 (10)	<1.00E+04	100	0	0
	5/7/2012	positive	10/10 (100)	2.89E+08	100	0	0
	28/8/2012	positive	10/10 (100)	1.06E+08	100	0	0
	26/10/2012	positive	10/10 (100)	3.18E+07	90	10	0
	29/12/2012	negative					
	4/3/2013	negative					
	3/5/2013	negative					
B/C8	1/7/2013	positive	7/10 (70)	N/A	71.43	28.57	0
	18/8/2013	positive	6/10 (60)	9.27E+07	100	0	0
	15/10/2013	positive	10/10 (100)	5.04E+06	100	0	0
B/C9	16/3/2012	positive	9/10 (90)	5.53E+06	100	0	0
	3/5/2012	negative					
	28/6/2012	positive	10/10 (100)	3.48E+07	60	40	0
B/C10	16/3/2012	positive	10/10 (100)	1.32E+07	100	0	0
	11/5/2012	negative					
	9/7/2012	positive	9/10 (90)	3.36E+08	0	88.89	11.11
	5/11/2012	positive	9/10 (90)	3.00E+05	100	0	0
	4/1/2013	positive	5/10 (50)	1.51E+08	100	0	0
	8/3/2013	negative					
B/C11	19/3/2012	negative					
	14/5/2012	negative					
	13/7/2012	negative					
	13/9/2012	negative					
	12/11/2012	positive	10/10 (100)	2.69E+08	100	0	0
	11/1/2013	positive	10/10 (100)	8.78E+07	100	0	0
	13/3/2013	positive	10/10 (100)	9.70E+06	100	0	0
	9/5/2013	positive	10/10 (100)	1.30E+07	100	0	0
	11/9/2013	positive	9/10 (90)	6.03E+06	66.67	33.33	0
	14/10/2013	positive	10/10 (100)	1.29E+08	0	30	70
B/C12-1	23/3/2012	negative					

Farm	Slaughter date	*Campylobacter* colonization	Positive sample/ Examined sample (%)	Bacterial number (CFU/g)	Species identification (%)		
					C. jejuni	*C. coli*	*C. jejuni* and *C. coli*
B/C12-2	23/3/2012	negative					
	16/6/2012	positive	1/10 (10)	<1.00E+04	0	100	0
	30/8/2012	negative					
	2/12/2012	negative					
B/C13	23/3/2012	positive	9/10 (90)	1.46E+06	100	0	0
	8/5/2012	negative					
	4/7/2012	positive	10/10 (100)	6.48E+07	100	0	0
	30/8/2012	positive	10/10 (100)	2.58E+08	50		50
	22/10/2012	positive	9/10 (90)	6.49E+08	0	100	0
	27/12/2012	negative					
	30/4/2013	negative					
	23/8/2013	positive	8/10 (80)	4.65E+07	87.5	12.5	
	18/10/2013	negative	N/A				
B/C14	30/3/2012	negative					
	24/5/2012	positive	8/10 (80)	<1.00E+04	100	0	0
B/C15	30/3/2012	positive	10/10 (100)	1.35E+07	100	0	0
	22/5/2012	negative					
	15/7/2012	positive	8/10 (80)	2.71E+08	0	100	0
	19/9/2012	positive	10/10 (100)	2.65E+06	100	0	0
	11/12/2012	negative					
	20/2/2013	negative					
B/C15	17/4/2013	negative					
	10/6/2013	negative					
	31/7/2013	positive	10/10 (100)	2.13E+07	100	0	0
	25/9/2013	positive	6/10 (60)	4.95E+07	100	0	0
B/C16	20/4/2012	negative					
	15/6/2012	positive	9/10 (90)	6.18E+07	100	0	0
B/C17	17/4/2012	positive	6/10 (60)	<104	100	0	0
	11/6/2012	negative					
B/C18	30/4/2012	negative					

Appendix C Distribution of *Campylobacter* in broiler production chains

Table C-1 *Campylobacter* isolation from broiler production chain of Chain A23

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Broiler flock					
	Cloacal swab	30	11/30 (36.67)	5/11 (45.45)	6/11 (54.55)
Hatchery					
Day 1	Egg tray	10	0/10 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/1 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
Day 7	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 7	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
Day 14	Cloacal swab	30	0/29 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Rodent	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 17	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	N/A		
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 21	Flies	1	0/1 (0)	n/a	n/a
Day 24	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 28	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 31	Cloacal swab	30	21/30 (70)	21/21 (100)	0/21 (0)
	Boot swab inside the target house	1	1/1 (100)	1/1 (100)	0/1 (0)
Day 35	Cloacal swab	30	11/30 (36.67)	11/11 (100)	0/11 (0)
	Boot swab from path-leading to the house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab inside the target house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from area around the house	2	1/2 (50)	1/1 (100)	0/1 (0)
	Boot swab inside the adjacent house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	1/6 (16.67)	1/1 (100)	0/1 (0)
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	1/1 (100)	1/1 (100)	0/1 (0)

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 38	Cloacal swab	30	26/30 (86.67)	26/26 (100)	0/26 (0)
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Slaughterhouse					
Before process	Transport crate	5	3/5 (60)	3/3 (100)	0/3 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	0/10 (0)	n/a	n/a
	Eviscerating equipment	15	2/15 (13.33)	2/2 (100)	0/2 (0)
After process	Cloacal swab	5	5/5 (100)	5/5 (100)	0/5 (0)
	Carcass rinse	25	20/25 (80)	20/20 (100)	0/20 (100)
	Meat product	16	10/16 (62.50)	10/10 (100)	0/10 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	3/10 (30)	3/3 (100)	0/3 (0)
	Eviscerating equipment	15	0/15 (0)	n/a	n/a
	Tap water (I/O washer and bird washer)	2	0/2 (0)	n/a	n/a
	Chilling water	6	4/6 (66.67)	4/4 (100)	0/4 (0)
	Packaging table	1	1/1 (100)	1/1 (100)	0/1 (0)

Table C-2 *Campylobacter* isolation from broiler production chain of Chain A13

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Breeder flock					
	Cloacal swab	30	23/30 (76.67)	6/23 (26.09)	17/23 (73.91)
Hatchery					
Day 1	Egg tray	10	0/10 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
Day 7	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 7	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 14	Cloacal swab	30	n/a	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 17	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 21	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 24	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 28	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 31	Cloacal swab	30	27/30 (90)	27/27 (100)	0/27 (0)
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 35	Cloacal swab	30	26/30 (86.67)	26/26 (100)	0/26 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 35	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 38	Cloacal swab	30	27/30 (90)	27/27 (100)	0/27 (0)
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Slaughterhouse					
Before process	Transport crate	5	0/5 (0)	n/a	n/a
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	0/10 (0)	n/a	n/a
	Eviscerating equipment	15	1/15 (6.67)	1/1 (100)	0/1 (0)
After process	Cloacal swab	15	0/15 (0)	n/a	n/a
	Carcass rinse	25	17/25 (68)	17/17 (100)	0/17 (0)
	Meat product	16	7/16 (43.75)	7/7 (100)	0/7 (0)
	Breast comforter	3	1/3 (33.33)	1/1 (100)	0/1 (0)
	Hanging shackle	10	10/10 (100)	10/10 (100)	0/100 (0)
	Eviscerating equipment	15	11/15 (73.33)	11/11 (100)	0/11 (0)
	Tap water (I/O washer and bird washer)	2	0/2 (0)	n/a	n/a
	Chilling water	4	3/4 (75)	3/3 (100)	0/3 (0)
	Packaging table	4	1/4 (25)	1/1 (100)	0/1 (0)

Table C-3 *Campylobacter* isolation from broiler production chain of Chain A15

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Breeder flock					
	Cloacal swab	30	21/30 (70)	8/21 (38.1)	13/21 (61.9)
Hatchery					
Day 1	Egg tray	10	0/10 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/1 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
Day 7	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 7	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 14	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	1/6 (16.67)	1/1 (100)	0/1 (0)
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 17	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 21	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a	n/a	n/a	n/a
Day 24	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 28	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Pest	n/a			
Day 31	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Day 35	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 35	Water from main pipeline	1	0/1 (0)	n/a	n/a
	pest	n/a	n/a	n/a	n/a
Day 38	Cloacal swab	30	2/30 (6.67)	2/2 (100)	0/2 (0)
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
Slaughterhouse					
Before process	Transport crate	5	2/5 (40)	2/2 (100)	0/2 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	0/10 (0)	n/a	n/a
	Eviscerating equipment	15	1/15 (6.67)	1/1 (100)	0/1 (0)
After process	Cloacal swab	15	6/15 (40)	6/6 (100)	0/6 (0)
	Carcass rinse	25	10/25 (40)	10/10 (100)	0/10 (0)
	Meat product	16	9/16 (56.25)	9/9 (100)	0/9 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	9/10 (90)	9/9 (100)	0/9 (0)
	Eviscerating equipment	15	10/15 (66.67)	10/10 (100)	0/10 (0)
	Tap water (I/O washer and bird washer)	2	0/2 (0)	n/a	n/a
	Chilling water	4	3/4 (75)	3/3 (100)	0/3 (0)
	Packaging table	4	0/4 (0)	n/a	n/a

Table C-4 *Campylobacter* isolation from broiler production chain of Chain B/C2

Date	Samples	Sample number	Species identification		
			Positive /total (%)	*C. jejuni* (%)	*C. coli* (%)
Breeder farm					
	Cloacal swab	24	17/24 (70.83)	2/17 (11.76)	15/17 (88.24)
Hatchery					
Day 1	Egg tray	10	0/10 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/1 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 0	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
Day 7	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 14	Cloacal swab	30	8/30 (26.67)	8/8 (100)	0/8 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	1/6 (16.67)	1/1 (100)	0.1 (0)
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 14	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	14/30 (46.67)	14/14 (100)	0/14 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	1/6 (16.67)	1/1 (100)	0/1 (0)
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 28	Cloacal swab	30	1/30 (3.33)	1/1 (100)	0/1 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 32	Cloacal swab	30	9/30 (30)	9/9 (100)	0/9 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Slaughterhouse					
Before process	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	1/10 (10)	1/1 (100)	0/1 (0)
	Eviscerating equipment	6	0/6 (0)	n/a	n/a
After process	Cloacal swab	5	5/5 (100)	5/5 (100)	0/5 (0)
	Carcass rinse	25	21/25 (84)	21/21 (100)	0/21 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	5	4/5 (80)	4/4 (100)	0/4 (0)
	Eviscerating equipment	6	5/6 (83.33)	5/5 (100)	0/5 (0)
	Tap water (I/O washer)	1	0/1 (0)	n/a	n/a
	Chilling water	2	0/2 (0)	n/a	n/a
	Packaging table	1	1/1 (100)	1/1 (100)	0/1 (0)

Table C-5 *Campylobacter* isolation from broiler production chain of Chain B/C 4

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Breeder flock					
	Cloacal swab	24	17/24 (70.83)	8/17 (47.06)	9/17 (52.94)
Hatchery					
Day 1	Egg tray	6	0/6 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/1 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 0	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
Day 7	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
Day 14	Cloacal swab	30	9/30 (30)	9/9 (100)	0/9 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 14	Flies	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	14/30 (46.67)	14/14 (100)	0/14 (0)
	Boot swab from path-leading to the house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from area around the house	2	1/2 (50)	1/1 (100)	0/1 (0)
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	1/6 (16.67)	1/1 (100)	0/1 (0)
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	1/1 (100)	1/1 (100)	0/1 (0)
Day 28	Cloacal swab	30	28/30 (93.33)	28/28 (100)	0/28 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	1/2 (50)	1/1 (100)	0/1 (0)
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 32	Cloacal swab	30	27/30 (90)	27/27 (100)	0/27 (0)
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 32	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Darkling beetle	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Slaughterhouse					
Before process	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	0/10 (0)	n/a	n/a
	Eviscerating equipment	6	0/6 (0)	n/a	n/a
After process	Cloacal swab	5	5/5 (100)	5/5 (100)	0/5 (0)
	Carcass rinse	25	17/25 (68)	17/17 (100)	0/17 (0)
	Breast comforter	3	0/3 (0)	n/a	n/a
	Hanging shackle	10	0/10 (0)	n/a	n/a
	Eviscerating equipment	5	2/5 (40)	2/2 (100)	0/2 (0)
	Tap water (I/O washer)	1	0/1 (0)	n/a	n/a
	Chilling water	2	0/2 (0)	n/a	n/a
	Packaging table	1	1/1 (100)	1/1 (100)	0/1 (0)

Table C-6 *Campylobacter* isolation from broiler production chain of Chain B/C12-2

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Breeder flock					
	Cloacal swab	12	6/12 (50)	3/6 (50)	3/6 (50)
Hatchery					
Day 1	Egg tray	10	0/10 (0)	n/a	n/a
	Tap water	1	0/1 (0)	n/a	n/a
Day 18	Egg incubator	6	0/6 (0)	n/a	n/a
Day 21	Egg shell	10	0/10 (0)	n/a	n/a
Downtime period					
Downtime period (before chicken placement)	Feeder for small chicken	6	0/6 (0)	n/a	n/a
	Feeder for the old chicken	6	0/6 (0)	n/a	n/a
	New litter	3	0/3 (0)	n/a	n/a
	Footwear in the house	2	0/2 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
Rearing period					
Day 0	Paper lining	10	0/10 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 0	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
Day 7	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
Day 14	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	1/1 (100)	1/1 (100)	0/1 (0)
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 14	Dust	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a
Day 21	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Rodent	1	0/1 (0)	n/a	n/a
Day 28	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
	Flies	1	0/1 (0)	n/a	n/a

Date	Samples	Sample number	Species identification		
			Positive/total (%)	*C. jejuni* (%)	*C. coli* (%)
Day 35	Cloacal swab	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Litter	3	0/3 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a
	Animal feed	1	0/1 (0)	n/a	n/a
	Dust	1	0/1 (0)	n/a	n/a
Day 43	Cloacal swab day 42	30	0/30 (0)	n/a	n/a
	Cloacal swab day 43	30	0/30 (0)	n/a	n/a
	Boot swab from path-leading to the house	1	0/1 (0)	n/a	n/a
	Boot swab from anteroom of target house	1	0/1 (0)	n/a	n/a
	Boot swab inside the target house	1	0/1 (0)	n/a	n/a
	Boot swab from area around the house	2	0/2 (0)	n/a	n/a
	Boot swab inside the adjacent house	1	0/1 (0)	n/a	n/a
	Water from nipple drinker	6	0/6 (0)	n/a	n/a
	Water from main pipeline	1	0/1 (0)	n/a	n/a

Appendix D Genetic characterization of *Campylobacter*

Table D-1 Genetic characterization of *Campylobacter* isolated from Chain A23

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Breeder	Cloacal swab1	783	1232	ST-353 complex
Breeder	Cloacal swab2	1485	1232	ST-353 complex
Breeder	Cloacal swab3	1211	1232	ST-353 complex
Breeder	Cloacal swab4	506	6876	ST-692 complex
Breeder	Cloacal swab5	353	1232	ST-353 complex
Broiler day 31	Cloacal swab1	57	574	ST-574 complex
Broiler day 31	Cloacal swab2	57	574	ST-574 complex
Broiler day 31	Cloacal swab3	57	574	ST-574 complex
Broiler day 31	Cloacal swab4	18	354	ST-354 complex
Broiler day 31	Cloacal swab5	18	354	ST-354 complex
Broiler day 31	Cloacal swab6	312	NT*	
Broiler day 31	Cloacal swab7	57	NT	
Broiler day 31	Cloacal swab8	22	NT	
Broiler day 31	Cloacal swab9	57	NT	
Broiler day 31	Cloacal swab10	57	NT	
Broiler day 31	Cloacal swab11	57	NT	
Broiler day 31	Boot swab inside the target house	22	45	ST-45 complex
Broiler day 35	Cloacal swab1	57	574	ST-574 complex
Broiler day 35	Cloacal swab2	57	574	ST-574 complex
Broiler day 35	Cloacal swab3	57	574	ST-574 complex
Broiler day 35	Cloacal swab4	57	NT	
Broiler day 35	Cloacal swab5	312	NT	
Broiler day 35	Cloacal swab6	57	NT	
Broiler day 35	Boot swab from path-leading to the house	57	574	ST-574 complex
Broiler day 35	Boot swab inside the target house	57	574	ST-574 complex
Broiler day 35	Boot swab from area around the house	57	574	ST-574 complex
Broiler day 35	Water from nipple drinker	18	354	ST-354 complex
Broiler day 35	Darkling beetle	57	574	ST-574 complex

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Broiler day 35	Boot swab inside the adjacent house	22	45	ST-45 complex
Broiler day 38	Cloacal swab1	57	574	ST-574 complex
Broiler day 38	Cloacal swab2	22	45	ST-45 complex
Broiler day 38	Cloacal swab3	22	45	ST-45 complex
Broiler day 38	Cloacal swab4	18	354	ST-354 complex
Broiler day 38	Cloacal swab5	18	NT	
Broiler day 38	Cloacal swab6	22	45	ST-45 complex
Broiler day 38	Cloacal swab7	22	NT	
Broiler day 38	Cloacal swab8	22	NT	
Broiler day 38	Cloacal swab9	18	NT	
Broiler day 38	Cloacal swab10	22	NT	
Broiler day 38	Cloacal swab11	22	NT	
Broiler day 38	Cloacal swab12	22	NT	
Broiler day 38	Cloacal swab13	22	NT	
Broiler day 38	Cloacal swab14	22	NT	
Broiler day 38	Cloacal swab15	18	NT	
Broiler day 38	Cloacal swab16	22	NT	
Broiler day 38	Cloacal swab17	57	NT	
Broiler day 38	Cloacal swab18	57	NT	
Broiler day 38	Cloacal swab19	18	NT	
Slaughterhouse	Transport crate1	45	2409	NT
Slaughterhouse	Transport crate2	45	2409	NT
Slaughterhouse	Transport crate3	45	2409	NT
Slaughterhouse	Eviscerating equipment (before used)	22	45	ST-45 complex
Slaughterhouse	Knife (before used)	18	354	ST-354 complex
Slaughterhouse	Chilling water1	22	45	ST-45 complex
Slaughterhouse	Chilling water2	22	45	ST-45 complex
Slaughterhouse	Chilling water3	22	45	ST-45 complex
Slaughterhouse	Carcass rinse (after scalding process) 1	57	574	ST-574 complex
Slaughterhouse	Carcass rinse (after scalding process) 2	22		NT
Slaughterhouse	Carcass rinse (after scalding process) 3	22		NT

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Slaughterhouse	Carcass rinse (after defeathering process) 1	22	45	ST-45 complex
Slaughterhouse	Carcass rinse (after defeathering process) 2	312	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 3	18	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 4	22	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 1	22	45	ST-45 complex
Slaughterhouse	Carcass rinse (after eviscerating process) 2	22	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 3	312	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 4	18	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 1	22	45	ST-45 complex
Slaughterhouse	Carcass rinse (after inside-outside washing process) 2	22	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 3	1582	NT	
Slaughterhouse	Carcass rinse (after chilling process)	22	45	ST-45 complex
Slaughterhouse	Cloacal swab1	18	354	ST-354 complex
Slaughterhouse	Cloacal swab2	22	45	ST-45 complex
Slaughterhouse	Cloacal swab3	312	574	ST-574 complex
Slaughterhouse	Cloacal swab4	22	45	ST-45 complex
Slaughterhouse	Cloacal swab5	18	354	ST-354 complex
Slaughterhouse	Cecum1	22	45	ST-45 complex
Slaughterhouse	Cecum2	22	45	ST-45 complex
Slaughterhouse	Cecum3	22	45	ST-45 complex
Slaughterhouse	Cecum4	22	45	ST-45 complex
Slaughterhouse	Cecum5	57	574	ST-574 complex
Slaughterhouse	Fillet (untrimmed)	22	45	ST-45 complex
Slaughterhouse	Breast (untrimmed)1	57	574	ST-574 complex
Slaughterhouse	Breast (untrimmed)2	22	45	ST-45 complex
Slaughterhouse	Wing (untrimmed)	22	45	ST-45 complex
Slaughterhouse	Thigh (untrimmed)1	177	583	ST-45 complex
Slaughterhouse	Thigh (untrimmed)2	22	45	ST-45 complex
Slaughterhouse	Thigh (untrimmed)3	22	45	ST-45 complex
Slaughterhouse	Fillet (trimmed)	18	354	ST-354 complex
Slaughterhouse	Thigh (trimmed)	18	354	ST-354 complex
Slaughterhouse	Wing (trimmed)	18	354	ST-354 complex

* NT =not test

Table D-2 Genetic characterization of *Campylobacter* isolated from Chain A13

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Breeder	Cloacal swab1	54	464	ST-464
Breeder	Cloacal swab2	54	NT	
Broiler day 31	Cloacal swab1	54	NT	
Broiler day 31	Cloacal swab2	54	NT	
Broiler day 31	Cloacal swab3	54	NT	
Broiler day 31	Cloacal swab4	54	NT	
Broiler day 31	Cloacal swab5	54	NT	
Broiler day 31	Cloacal swab6	54	NT	
Broiler day 31	Cloacal swab7	54	NT	
Broiler day 31	Cloacal swab8	54	464	ST-464
Broiler day 31	Cloacal swab9	54	NT	
Broiler day 31	Cloacal swab10	54	NT	
Broiler day 31	Cloacal swab11	54	NT	
Broiler day 31	Cloacal swab12	54	NT	
Broiler day 31	Cloacal swab13	54	NT	
Broiler day 31	Cloacal swab14	54	NT	
Broiler day 31	Cloacal swab15	54	NT	
Broiler day 35	Cloacal swab1	54	NT	
Broiler day 35	Cloacal swab2	54	NT	
Broiler day 35	Cloacal swab3	54	NT	
Broiler day 35	Cloacal swab4	54	NT	
Broiler day 35	Cloacal swab5	54	NT	
Broiler day 35	Cloacal swab6	54	NT	
Broiler day 35	Cloacal swab7	54	NT	
Broiler day 35	Cloacal swab8	54	464	ST-464
Broiler day 35	Cloacal swab9	54	NT	
Broiler day 35	Cloacal swab10	54	NT	
Broiler day 35	Cloacal swab11	18	NT	
Broiler day 35	Cloacal swab12	18	354	ST-354

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Broiler day 35	Cloacal swab13	18	NT	
Broiler day 38	Cloacal swab1	54	NT	
Broiler day 38	Cloacal swab2	54	NT	
Broiler day 38	Cloacal swab3	54	NT	
Broiler day 38	Cloacal swab4	54	NT	
Broiler day 38	Cloacal swab5	54	NT	
Broiler day 38	Cloacal swab6	54	NT	
Slaughterhouse	Breast comforter	54	NT	
Slaughterhouse	Carcass trimming table	54	NT	
Slaughterhouse	Eviscerating equipment1	783	NT	
Slaughterhouse	Eviscerating equipment2	783	NT	
Slaughterhouse	Knife1	54	NT	
Slaughterhouse	Knife 2	783	NT	
Slaughterhouse	Vent gun1	783	NT	
Slaughterhouse	Vent gun2	783	NT	
Slaughterhouse	Breast (untrimmed) 1	54	NT	
Slaughterhouse	Wing (untrimmed) 2	54	NT	
Slaughterhouse	Fillet (untrimmed) 3	54	NT	
Slaughterhouse	Fillet (untrimmed) 4	54	NT	
Slaughterhouse	Breast (trimmed) 1	54	NT	
Slaughterhouse	Wing (trimmed) 2	54	NT	
Slaughterhouse	Carcass rinse (after scalding process)1	54	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process)1	54	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process)2	54	NT	
Slaughterhouse	Cecum1	54	NT	
Slaughterhouse	Cecum2	54	464	ST-464
Slaughterhouse	Cecum3	54	NT	
Slaughterhouse	Cecum4	54	NT	
Slaughterhouse	Chilling water1	54	NT	
Slaughterhouse	Chilling water2	54	NT	
Slaughterhouse	Chilling water3	54	NT	

Table D-3 Genetic characterization of *Campylobacter* isolated from Chain A15

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Breeder	Cloacal swab1	312	574	ST-574
Breeder	Cloacal swab2	54	6996	ST-464
Breeder	Cloacal swab3	30	NT	
Breeder	Cloacal swab4	34	NT	
Breeder	Cloacal swab5	34	460	ST-460
Breeder	Cloacal swab6	34	6996	ST-464
Broiler day 14	Water from nipple drinker	22	45	ST-45
Broiler day 38	Cloacal swab1	629	2209	ST-179
Broiler day 38	Cloacal swab2	629	2209	ST-179
Slaughterhouse	Cloacal swab1	629	2209	ST-179
Slaughterhouse	Cloacal swab2	629	NT	
Slaughterhouse	Cloacal swab3	1340	2209	ST-179
Slaughterhouse	Knife	783	5213	ST-353
Slaughterhouse	Transport crate1	783	5213	ST-353
Slaughterhouse	Transport crate2	783	NT	
Slaughterhouse	Carcass rinse (after defeathering process)1	18	354	ST-354
Slaughterhouse	Carcass rinse (after defeathering process)2	18	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 1	68	2209	ST-179
Slaughterhouse	Carcass rinse (after inside-outside washing process) 1	1340	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 2	1340	2209	ST-179
Slaughterhouse	Carcass rinse (after inside-outside washing process) 3	18	NT	
Slaughterhouse	Carcass rinse (after chilling process) 1	18	NT	
Slaughterhouse	Carcass rinse (after chilling process) 2	1340	NT	
Slaughterhouse	Carcass rinse (after chilling process) 3	18	NT	
Slaughterhouse	Cecum 1	68	2209	ST-179
Slaughterhouse	Chilling water	1340	NT	
Slaughterhouse	Breast (untrimmed) 1	18	NT	
Slaughterhouse	Breast (untrimmed) 2	18	354	ST-354
Slaughterhouse	Wing (untrimmed) 3	18	NT	
Slaughterhouse	Wing (untrimmed) 4	783	NT	
Slaughterhouse	Breast (trimmed)1	18	NT	
Slaughterhouse	Wing (trimmed) 2	18	354	ST-354
Slaughterhouse	Thigh (untrimmed) 3	18	NT	

Table D-4 Genetic characterization of *Campylobacter* isolated from Chain B/C2

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Breeder	Cloacal swab	677	2131	n/a
Broiler day 15	Cloacal swab1	783	1232	ST-353
Broiler day 15	Cloacal swab2	783	1232	ST-353
Broiler day 15	Cloacal swab3	783	NT	
Broiler day 15	Cloacal swab4	783	NT	
Broiler day 15	Cloacal swab5	783	NT	
Broiler day 15	Water from nipple drinker	783	1232	ST-353
Broiler day 15	Boot swab inside the target house	783	1232	ST-353
Broiler day 21	Cloacal swab1	783	1232	ST-353
Broiler day 21	Cloacal swab2	783	1232	ST-353
Broiler day 21	Cloacal swab3	783	NT	
Broiler day 21	Cloacal swab4	783	NT	
Broiler day 21	Cloacal swab5	783	NT	
Broiler day 21	Cloacal swab6	783	NT	
Broiler day 21	Cloacal swab7	783	NT	
Broiler day 21	Cloacal swab8	783	NT	
Broiler day 21	Cloacal swab9	783	NT	
Broiler day 21	Cloacal swab10	783	NT	
Broiler day 21	Cloacal swab11	783	NT	
Broiler day 21	Cloacal swab12	783	NT	
Broiler day 21	Cloacal swab13	783	NT	
Broiler day 21	Water from nipple drinker	783	NT	
Broiler day 28	Cloacal swab	783	1232	ST-353
Broiler day 32	Cloacal swab1	783	1232	ST-353
Broiler day 32	Cloacal swab2	783	5213	ST-353
Broiler day 32	Cloacal swab3	783	1232	ST-353
Broiler day 32	Cloacal swab4	783	2131	n/a
Broiler day 32	Cloacal swab5	783	NT	
Broiler day 32	Cloacal swab6	783	NT	
Broiler day 32	Cloacal swab7	783	NT	
Broiler day 32	Cloacal swab8	783	NT	
Broiler day 32	Cloacal swab9	783	NT	
Broiler day 32	Boot swab inside the target house	48	1232	ST-353
Slaughterhouse	Cloacal swab1	783	NT	
Slaughterhouse	Cloacal swab2	783	1232	ST-353

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Slaughterhouse	Cloacal swab3	783	5213	ST-353
Slaughterhouse	Cecum 1	783	5213	ST-353
Slaughterhouse	Cecum 2	783	NT	
Slaughterhouse	Cecum 3	783	1232	ST-353
Slaughterhouse	Cecum 4	783	NT	
Slaughterhouse	Cecum 5	783	NT	
Slaughterhouse	Cecum 6	783	NT	
Slaughterhouse	Cecum 7	783	NT	
Slaughterhouse	Cecum 8	783	NT	
Slaughterhouse	Cecum 9	783	NT	
Slaughterhouse	Cecum 10	783	NT	
Slaughterhouse	Knife	783	NT	
Slaughterhouse	Eviscerating equipment	783	NT	
Slaughterhouse	Hanging shackle 1	783	NT	
Slaughterhouse	Hanging shackle 2	783	NT	
Slaughterhouse	Carcass trimming table	22	1075	ST-353
Slaughterhouse	Carcass rinse (after scalding process)1	783	NT	
Slaughterhouse	Carcass rinse (after scalding process) 2	783	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 1	783	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 2	783	NT	
Slaughterhouse	Carcass rinse (after eviscerating process)	783	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 1	783	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 2	783	NT	
Slaughterhouse	Carcass rinse (after chilling process) 1	783	NT	
Slaughterhouse	Carcass rinse (after chilling process) 2	783	NT	

Table D-5 Genetic characterization of *Campylobacter* isolated from Chain B/C4

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Breeder	Cloacal swab1	45	NT	
Breeder	Cloacal swab2	402	NT	
Breeder	Cloacal swab3	48	2131	n/a
Breeder	Cloacal swab4	21	NT	
Breeder	Cloacal swab5	54	NT	
Broiler day 14	Cloacal swab1	287	NT	
Broiler day 14	Cloacal swab2	287	NT	
Broiler day 14	Cloacal swab3	287	NT	
Broiler day 14	Cloacal swab4	287	5247	ST-353
Broiler day 14	Cloacal swab5	57	NT	
Broiler day 21	Cloacal swab1	287	NT	
Broiler day 21	Cloacal swab2	287	NT	
Broiler day 21	Cloacal swab3	287	5247	ST-353
Broiler day 21	Cloacal swab4	253	1919	ST-52
Broiler day 21	Cloacal swab5	287	NT	
Broiler day 21	Cloacal swab6	253	1919	ST-52
Broiler day 21	Cloacal swab7	287	NT	
Broiler day 21	Cloacal swab8	287	NT	
Broiler day 21	Cloacal swab9	287	NT	
Broiler day 21	Cloacal swab10	287	NT	
Broiler day 21	Cloacal swab11	287	NT	
Broiler day 21	Cloacal swab12	287	NT	
Broiler day 21	Cloacal swab13	253	NT	
Broiler day 21	Cloacal swab14	287	NT	
Broiler day 21	Boot swab from path-leading to the house	255	NT	
Broiler day 21	Boot swab inside the target house	1239	NT	
Broiler day 21	Boot swab from area around the house		NT	
Broiler day 21	Flies	287	5247	ST-353

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Broiler day 28	Cloacal swab1	287	NT	
Broiler day 28	Cloacal swab2	287	NT	
Broiler day 28	Cloacal swab3	287	NT	
Broiler day 28	Cloacal swab4	253	1919	ST-52
Broiler day 28	Cloacal swab5	287	5247	ST-353
Broiler day 28	Cloacal swab6	287	NT	
Broiler day 28	Cloacal swab7	287	NT	
Broiler day 28	Cloacal swab8	287	NT	
Broiler day 28	Cloacal swab9	287	NT	
Broiler day 28	Cloacal swab10	255	NT	
Broiler day 28	Cloacal swab11	287	NT	
Broiler day 28	Cloacal swab12	287	NT	
Broiler day 28	Cloacal swab13	287	NT	
Broiler day 28	Cloacal swab14	287	NT	
Broiler day 28	Boot swab from area around the house	287	NT	
Broiler day 32	Cloacal swab1	253	NT	
Broiler day 32	Cloacal swab2	253	NT	
Broiler day 32	Cloacal swab3	255	NT	
Broiler day 32	Cloacal swab4	253	NT	
Broiler day 32	Cloacal swab5	253	NT	
Broiler day 32	Cloacal swab6	253	NT	
Broiler day 32	Cloacal swab7	255	NT	
Broiler day 32	Cloacal swab8	854	NT	
Broiler day 32	Cloacal swab9	287	NT	
Broiler day 32	Cloacal swab10	45	NT	
Broiler day 32	Cloacal swab11	253	NT	
Broiler day 32	Cloacal swab12	45	NT	
Broiler day 32	Cloacal swab13	18	NT	
Broiler day 32	Cloacal swab14	253	NT	
Broiler day 32	Cloacal swab15	1527	NT	
Broiler day 32	Cloacal swab16	253	NT	

Sample		*flaA*-SVR	Sequence type	Clonal complex
Production unit	Type of sample			
Broiler day 32	Cloacal swab17	45	NT	
Broiler day 32	Cloacal swab18	1527	NT	
Broiler day 32	Cloacal swab19	253	NT	
Slaughterhouse	Carcass rinse (after scalding process)1	287	NT	
Slaughterhouse	Carcass rinse (after scalding process) 2	57	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 1	287	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 2	312	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 3	287	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 4	45	NT	
Slaughterhouse	Carcass rinse (after defeathering process) 5	253	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 1	652	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 2	287	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 3	45	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 4	45	NT	
Slaughterhouse	Carcass rinse (after eviscerating process) 5	287	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 1	287	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 2	287	NT	
Slaughterhouse	Carcass rinse (after inside-outside washing process) 3	287	NT	
Slaughterhouse	Carcass rinse (after chilling process) 1	287	NT	
Slaughterhouse	Carcass rinse (after chilling process) 2	287	NT	
Slaughterhouse	Carcass rinse (after chilling process) 3	22	NT	
Slaughterhouse	Carcass rinse (after chilling process) 4	253	NT	
Slaughterhouse	Carcass rinse (after chilling process) 5	57	NT	
Slaughterhouse	Cecum 1	1527	NT	
Slaughterhouse	Cecum 2	253	NT	
Slaughterhouse	Cecum 3	783	NT	
Slaughterhouse	Cecum 4	783	NT	
Slaughterhouse	Knife	253	NT	
Slaughterhouse	Eviscerating equipment	45	NT	
Slaughterhouse	Carcass trimming table	652	NT	

Appendix E New sequence type identified in this study

asp 358

ATGATAGGTGAAGATATACAAAGAGTATTAGAAGCTAGAAAATTGATTTTAGAGATCAATTTGGGTGGAACTGC
TATTGGAACAGGAATTAATTCTCATCCTGATTATCCGAAGGTTGTAGAAAGAAAAATAAGAGAAGTGACAGGTT
TTGAATATACTGTGGCTGAGGATTTGATCGAGGCGACTCAAGATACGGGAGCTTATGTACAAATTTCAGGTGT
TTTAAAACGTGTTGCAACAAAACTTTCTAAAGTATGTAATGACTTAAGACTTTTAAGTAGTGGTCCAAAATGTG
GTCTTAATGAGATTAATCTTCCAAAAATGCAACCAGGTAGTTCTATCATGCCAGGTAAAGTAAATCCTGTTATT
CCTGAAGTAGTTAATCAAGTTTGTTATTTTGTTATTGGAGCAGATGTAACTGTAACTTTTGCTTGTGAGGGTGG
ACAATTACAACTTAATGTTTTTGAACCAGTTGTA

tkt 546

TTACATTTGAGCGGCTATGACTTAAGCTTAGAAGATCTTAAAAATTTCCGCCAACTTCATTCTAAAACCCCTGG
ACACCCTGAAATTTCAACTCTTGGAGTAGAAATCGCTACAGGCCCTTTAGGACAAGGCGTTGCCAATGCTGTA
GGCTTTGCTATGGCAGCAAAAAAAGCACAAAATTTGCTAGGCAGTGATTTAATCGATCATAAAATTTATTGTCT
TTGCGGAGATGGGGATTTACAAGAAGGCATTTCTTATGAAGCTTGTTCTTTAGCAGGACTTCACAAACTTGATA
ACTTCATACTCATTTATGATAGCAACAATATCTCCATAGAAGGCGATGTAGGTTTAGCCTTTAACGAAAATGTA
AAAATGCGTTTTGAAGCACAAGGATTTGAAGTTTTAAGTATAAATGGACACGATTATGAAGAAATCAATAAAGC
CTTAGAACAAGCTAAA

tkt 553

TTACATTTAAGTGGCTATGATTTAAGCTTAGAAGATCTTAAAAATTTCCGCCAACTTCATTCTAAAACCCCAGG
ACACCCTGAAATTTCAACTCTTGGAGTAGAAATCGCTACAGGTCCTTTAGGACAAGGCGTTGCCAATGCTGTA
GGCTTTGCTATGGCGGCAAAAAAAAGCACAAAATTTACTAGGTAGCAATTTAATCGATCATAAAATTTATTGTCT
TTGCGGAGATGGAGATTTACAAGAAGGCATTTCTTATGAAGCTTGTTCTTTAGCAGGACTTCACAAACTTGATA
ACTTCATACTCATTTATGATAGCAACAATATCTCCATAGAAGGCGATGTAGGTTTAGCCTTTAATGAAAATGTA
AAAATGCGTTTTGAAACACAAGGATTTGAAGTTTTAAGTATAAATGGACATGATTATGAAGAAATTAATAAAGC
CTTAGAACAAGCTAAA

VITA

Miss Sakaoporn Prachantasena was born on September 19, 1985 in Bangkok, Thailand. She completed with the Degree of Veterinary Sciences (D.V.M.) from the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand in 2010. After that, she enrolled in Doctor of Philosophy Program at the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University since academic year 2010.