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Original Article

Prevalence and Antimicrobial Resistance of *Salmonella* and *Campylobacter* Species Isolated from Laying Duck Flocks in Confinement and Free-grazing Systems

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Abstract

Prevalence and antimicrobial resistance of *Salmonella* and *Campylobacter* species isolated from duck laying flocks in 7 confinement systems and 7 free-grazing systems were determined in central Thailand. In the confinement system, 13.8% (88/639) of *Campylobacter* spp. were isolated from cloacal swabs; 72.7% was identified as *C. jejuni* and 27.3% was identified as *C. coli*. From drinking and surface water samples, 60.0% of *Campylobacter* spp. were isolated; all was identified as *C. jejuni*. In the free-grazing system, 0.3% (2/700) of *Campylobacter* spp. were isolated from cloacal swabs; all was identified as *C. jejuni*. In addition, 52.9% was isolated from drinking and surface water samples; all was identified as *C. jejuni*. Between 67.9% and 81.0% of all *C. jejuni* isolated from both systems was resistant to Streptomycin, Nalidixic acid, Ciprofloxacin, and Levofloxacin. Between 50.0% and 87.5% of all *C. coli* isolated from both systems was resistant to Streptomycin, Nalidixic acid, Ciprofloxacin, and Levofloxacin. In the confinement system, *Salmonella* spp. isolated from cloacal swabs, feed, soil, and water samples were 4.2%, 13.3%, 57.1% and 33.3%, respectively. Among them, 14 serotypes of *Salmonella* spp. were identified. In the free-grazing system, *Salmonella* spp. isolated from cloacal swabs, feed, soil, and water samples were 10.7%, 25.0%, 70.0% and 47.1%, respectively. Among them, 20 serotypes of *Salmonella* spp. were identified. Only one sample (*S.* Typhimurium) from the confinement system was resistant to Cephalothin. Four serotypes (*S.* Agona, *S.* Stanley, *S.* Thompson, and *S.* Corvallis) of five samples from 5 free-grazing flocks were resistant to antimicrobial agents. Consequently, in the confinement system there was a higher prevalence of *Campylobacter* spp. from cloacal swabs than *Salmonella* spp. On the contrary, in the free-grazing system there was a higher prevalence of *Salmonella* spp. from cloacal swabs than *Campylobacter* spp.

Keywords: *Campylobacter*, confinement system, free-grazing system, laying ducks, *Salmonella*

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Introduction

The major cause of diarrhea is the consumption of meats contaminated with foodborne pathogens, especially *Salmonella* and *Campylobacter*. In addition, patients get both bacteria mainly from ingestion of poultry and their eggs (Luber, 2009). In 2011, approximately 220,000 patients in the European Union were infected with Campylobacteriosis, while those infected with Salmonellosis were about 95,000. However, the statistics of salmonellosis in EU have declined continually; 39.7% reduction was found in 2011 with respect to that in 2007. It is because a number of regulations to control the infection were issued, especially in the parent stock level. Besides, several control measures through food chain were launched. As a result, the contamination in the chicken meat declined (EFSA, 2012).

Campylobacter jejuni is the major species causing human Campylobacteriosis. In addition, it is thought that poultry and its products are the main source of infection via direct contact, cooking, and consumption. Besides, the opportunity of human-to-human transmission was found low (Altekruse and Swerdlow, 2002; FSAI, 2002; Silva et al., 2011). Even though the major strains of *Campylobacter* found in poultry do not cause severe diseases in human (Gilbert and Slavik, 2004), a survey from several countries during 1990-2011 indicated that the average prevalence of *Campylobacter* spp. in ducks was 53.0% (0.0-83.3%), in meat and parts was 31.6% (12.5-45.8%), and in the rearing and processing environment was 94.4% (92.0-96.7%) (Adzitey et al., 2012). Moreover, a survey conducted in Taiwan reported that the prevalence of *Campylobacter* spp. in ducks was 43.5% from 92.0% of duck farms; *C. jejuni* (94.8%) and *C. coli* (5.2%) were found in 43.5% of all duck samples (Tsai and Hsiang, 2005). The treatment of Campylobacteriosis is majorly performed by Fluoroquinolone and Erythromycin. In addition, successful treatment is found with Macrolide, Aminoglycoside, Chloramphenicol, and Tetracycline. Treatment with antimicrobial agents provides good results both in humans and animals, especially in poultry farms. As a result, antimicrobial agents have been continuously used since 1990, entailing high incidence of drug resistance both in Europe and the United States of America (Alterkruse and Swerdlow, 2002).

Salmonella is major zoonotic bacteria. In general, it can be found in feces of animals. In addition, it can contaminate carcass which is the considerable risk to human consumption of animal meats (Humphrey and Jorgensen, 2006). The prevalence of *Salmonella* spp. in ducks from a survey in several global regions between 1997 and 2012 found 19.9% (3.3-56.9%) in ducks, 28.4% (4.4-75.6%) in meat and parts, and 32.5% (10.5-82.6%) in the rearing and processing environments (Adzitey et al., 2012). Some *Salmonella* is resistant to antimicrobial agents, for example, *S.* Typhimurium DT 104 is resistant to Ampicillin, Chloramphenical, Streptomycin, Sulfonamide, and Tetracycline. Besides, it disperses through Europe and North America (Poppe et al., 1998).

Duck meats and eggs are one of the famous protein sources of human. In Thailand, the raising systems for laying ducks include free-grazing and confinement systems. In the free-grazing system, ducks are raised in a rice field together with postharvest rice, shrimps, shells, and fish. When the feed is over, the ducks are transferred to and raised in another rice field. In the confinement system, ducks are accommodated in houses with limited area. They live in the same place and consume commercial concentrated feed throughout their life. Thus, the opportunity to acquire bacterial infection, especially *Salmonella* and *Campylobacter*, of the two raising systems is different. Therefore, the objective of the present study was to survey the prevalence and to perform antimicrobial susceptibility of *Salmonella* and *Campylobacter* in order to establish a database of the prevalence and drug resistance of both bacteria from different laying duck raising systems for further protection and control.

Materials and Methods

Sampling: In total, 1,404 samples were obtained between May 2010 and June 2011 from laying ducks, aged 6-14 months, in seven confinement system flocks and seven free-grazing system flocks. All ducks were accommodated in Nakhon Pathom, Phra Nakhon Si Ayutthaya, and Suphanburi provinces. In each flock, cloacal swab was performed in 77-100 ducks, bringing 1,339 samples in total. In addition, 1-2 environmental samples from water, feed, and soil were conducted from each flock into pooled samples. Finally, 64 environmental samples were obtained.

Species and serovar identifications

Identification of Campylobacter isolates: Isolates were identified by conventional method and multiplex polymerase chain reaction (mPCR), respectively (Denis et al., 1999). In the conventional method, the fecal samples collected by cloacal swab and from environment were applied into Preston broth 9 ml (Nutrient broth No.2, 5.0% (v/v) lysed horse blood, *Campylobacter* growth supplement [Oxoid, USA] and modified Preston *Campylobacter* selective supplement [Oxoid]) and incubated for 48 h at 42°C under microaerophilic condition in anaerobic jars with gas-generating kits (Oxoid). For the environmental samples, 25 ml/g of water, soil, or feed was selected to culture in Preston broth 225 ml at 42°C for 48 h under microaerophilic condition. Following the enrichment in Preston broth, the samples were streaked on Charcoal-Cefoperazone-Deoxycholate Agar (CCDA) (*Campylobacter* blood free selective agar base [Oxoid] with CCDA selective supplement [Oxoid]) at 42°C for 48 h under microaerophilic condition. Afterwards, dark-gray colony was picked up to stain with Victoria blue and gull wing morphology was investigated.

mPCR was conducted by selecting one cultured colony of *Campylobacter* to extract deoxyribonucleic acid (DNA) by commercial DNA extraction kit (Promega, WI, USA). mPCR increased *16S* rRNA to confirm the results at generic level of *Campylobacter*. Besides, *mapA* gene and *ceuE* gene were amplified to confirm the results at specific level of *C. jejuni* and *C. coli*, respectively. PCR procedures were majorly conducted according to the method described by Denis et al. (1999). The differences were that the use of dNTPs concentration was 200 μM, the concentrations of MD16S1 and MD16S2 primer were both 0.5 μM. and the use of *Taq* DNA polymerase (Invitrogen, CA, USA) was 1.2 U. The examination of DNA product used gel electrophoresis by 1% (w/v) agarose gel, 100 volts for 30 min, stained with ethidium bromide, and used UV light to prove DNA size.

Identification of Salmonella isolates: Conventional method and serotyping were used to identify *Salmonella* in the species level according to the principals of Kauffmann-White Schema. Cloacal swab, water, feed, and soil samples were taken to culture on the criterion of ISO 6579:2002 (annex D) (ISO, 2007): cloacal swabs were cultured in 9 ml Buffer Peptone Water (BPW), while 25 ml/g of water, feed and soil samples were cultured in 225 BPW. All samples, afterwards, were incubated at 37°C for 18 h. After incubation, 3 drops from 0.1 ml were transferred to Modified Semi-Solid Rappaport-Vassiliadis (MSRV) agar plate with novobiocine 0.01 g/l at 42°C for 24 h. Growth on MSRV plates suspected to be *Salmonella* spp. was streaked on Brilliant-green Phenol-red Lactose Sucrose (BPLS) agar and Xylose Lysine Deoxycholate (XLD) agar. After incubating at 37°C for 24 h, bacterial identification on BPLS and XLD was biochemically performed on urease agar, triple sugar iron agar, and lysine-decarboxylase broth. If the results showed *Salmonella* spp., those samples were further analyzed for serovar by serological test on the basis of slide agglutination with polyvalent anti-specific O antisera and specific flagellar H antisera (S.A.P. Laboratory, Thailand) to each antigen of the samples. Eventually, the antigen pattern was compared with Kauffmann-White Scheme (antigenic formulas of the *Salmonella* serovars) (Grimont and Weill, 2007).

Antimicrobial susceptibility of *Campylobacter* and *Salmonella*

Campylobacter: Dilution of antimicrobial susceptibility (Broth microdilution) according to the standard method of Clinical and Laboratory Standards Institute (CLSI, 2010) was performed in every sample with *Campylobacter*. The samples were tested with eight antimicrobial agents, including Doxycycline, Levofloxacin, Ciprofloxacin, Erythromycin, Gentamicin, Nalidixic acid, Streptomycin, and Tetracycline at concentration between 0.001 and 512 μg/ml. In addition, *Campylobacter* ATCC 33560 was used as the standard strain in quality control of antimicrobial susceptibility.

Salmonella: Disc diffusion method was applied to every sample with *Salmonella* according to the standard of Clinical and Laboratory Standards Institute (CLSI, 2010). The samples were tested with 16 antimicrobial agents, including Ampicillin (10 μg), Amoxicillin-clavulanic acid (30 μg), Nalidixic acid (30 μg), Kanamycin (30 μg), Ciprofloxacin (5 μg), Norfloxacin (10 μg), Ofloxacin (5 μg), Chloramphenicol (30 μg), Streptomycin (10 μg), Tetracycline (30 μg), Trimethoprim-sulfamethoxazole (25 μg), Gentamicin (μg), Enrofloxacin (5 μg), Ceftiofur (30 μg), Cephalothin (30 μg), and Nitrofurantoin (300 μg). Besides, *E. coli* ATCC 25922 was used as the standard strain in quality control of antimicrobial susceptibility.

Statistical analysis: Chi-squared (χ^2) test was used to analyze the relationship between the prevalence of *Salmonella* and *Campylobacter* isolated from laying ducks in confinement and free-grazing systems. A probability value of less than 5% was considered to be significant.

Results and Discussion

Identification of Campylobacter: *Campylobacter* spp. isolated from the cloacal swabs of laying ducks from seven confinement system flocks showed positive result by 13.77% (88 of 639). They were 72.7% (64/88) *C. jejuni* and 27.3% (24/88) *C. coli*. According to the environment from all confinement system flocks, *Campylobacter* spp. were found in the feed, soil, and water by 0.0% (0/15), 0.0% (0/17), and 60.0% (9/15), respectively. All *Campylobacter* spp. detected from the environment were *C. jejuni* from drinking water and ponds in the area (Table 1). It is possible that the drinking water and residing ponds were the reservoir and distributing source of *Campylobacter* in the farms. Therefore, using clean water according to farm standard issued by the Department of Livestock Development, together with changing drinking water regularly, could reduce *Campylobacter* in drinking water. However, reducing *Campylobacter* in a residing pond is relatively difficult since changing water in a pond could be done only after the water is used up which takes approximately 2 years. As a result, the existence and distribution of *Campylobacter* to the ducks remain.

Campylobacter spp. isolated from the cloacal swabs of laying ducks from seven free-grazing system flocks showed positive result by 0.3% (2/700). Both of them were *C. jejuni* and came from the same flock of ducks. In the environment from all free-grazing system flocks, *Campylobacter* spp. were found in the feed, soil, and drinking water by 0.0% (0/16), 0.0% (0/10), and 52.9% (9/17), respectively. All *Campylobacter* spp. detected from the environment were *C. jejuni* from drinking water and ponds in the area (Table 2). According to the cloacal swabs, it was found that the laying ducks raised in the confinement system were more inclined to be contaminated with *Campylobacter* than those accommodated in the free-grazing system. It is because in the confinement system the living area is limited, making it densely crowded with ducks. Moreover, direct defecation to water source for drinking and residing in the confinement system is another significant cause contributing to the dispersal of *Campylobacter* to other ducks.

The examination of environmental samples from all 14 flocks revealed that Campylobacteriosis took place only in the drinking water and residing ponds. This is because water is an appropriate habitat for *Campylobacter*. Besides, all were *C. jejuni*, which has been mostly found in human (Altekruse and Swerdlow, 2002), especially in agricultural areas, such as rice fields. This confirms that natural water sources with living birds such as grazing ducks and water birds are the residence of *Campylobacter*, corresponding with the study of Pitkänen (2013) revealing the relationship among agricultural area, populous water birds, and *Campylobacter* existence. Natural water source, therefore, serves as a reservoir of *Campylobacter* and shedding site for local birds. As a result, people consuming untreated water or birds in such area tend to be infected with *Campylobacter* and might get diarrhea subsequently.

A global survey of the average prevalence of *Campylobacter* spp. in ducks between 1990 and 2011 demonstrated that 53.0% and 94.4% of *Campylobacter* spp. were found in meat-type ducks and environments, respectively. That was higher than what was found in the egg-laying ducks of the present study. This is due to the difference in ages reared and rearing system of both duck breeds. Besides, the proportion of *C. jejuni* was found, in the current study, more than that of *C. coli*, which corresponds with a survey from several regions of the world (Adzitey et al., 2012).

Table 1 *Campylobacter* isolated from laying ducks and environmental samples in confinement system

Flock	Location (district, province)	Type of samples	*C. jejuni*	*C. coli*
A	Muang, Suphan Buri	Cloacal swab	9	2
B	Lat Bua Luang, Phra Nakhon Si Ayutthaya	Cloacal swab	1	
		Drinking water	1	
C	Bang Len, Nakhon Pathom	Cloacal swab	5	
		Drinking water	1	
D	Song Phi Nong, Suphan Buri	Cloacal swab	6	11
		Drinking water	2	
L	Bang Len, Nakhon Pathom	Cloacal swab	28	12
		Drinking water	2	
M	Bang Pa Ma, Suphan Buri	Cloacal swab	1	
		Drinking water	1	
N	Song Phi Nong, Suphan Buri	Cloacal swab	13	
		Drinking water	1	
		Surface water	1	

Table 2 *Campylobacter* isolated from laying ducks and environmental samples in free-grazing system

Flock	Location (district, province)	Type of samples	*C. jejuni*	*C. coli*
E	Song Phi Nong, Suphan Buri	Not detected in all samples	-	-
F	Kamphaeng Saen, Nakhon Pathom	Drinking water	1	
		Surface water	1	
G	Kamphaeng Saen, Nakhon Pathom	Not detected of all samples	-	-
H	Song Phi Nong, Suphan Buri	Cloacal swab	2	
		Drinking water	1	
		Surface water	1	
I	Song Phi Nong, Suphan Buri	Surface water	1	
J	Song Phi Nong, Suphan Buri	Surface water	2	
K	Bang Sai, Phra Nakhon Si Ayutthaya	Surface water	2	

Antimicrobial susceptibility of Campylobacter: Eight antimicrobial agents were used to test the antimicrobial susceptibility of *Campylobacter* spp. in ducks from all 14 flocks. The antimicrobial resistance of *C. jejuni* was highest to Streptomycin (81.0%). In addition, it was resistant to Fluoroquinolone: Nalidixic acid (76.2%), Ciprofloxacin (70.2%), Levofloxacin (67.9%); Tetracycline: Tetracycline (32.1%) and Doxycycline (28.6%); and Gentamicin (25.0%). The lowest resistance was found to Erythromycin (4.8%).

According to the antimicrobial susceptibility testing from eight antimicrobial agents of *C. coli* in ducks from all 14 flocks, the resistance test corresponded with that of *C. coli* and *C. jejuni* in every order. It had the highest resistance to Streptomycin (87.5%). Moreover, it was resistant to Fluoroquinolone: Nalidixic acid (58.3%), Ciprofloxacin (54.2%), and Levofloxacin (50.0%); and Tetracycline: Tetracycline (12.5%) and Doxycycline (0.0%). However, the resistance to Gentamicin and Erythromycin was not detected.

Due to researches on antimicrobial susceptibility of *Campylobacter* spp. to Doxycycline, Ciprofloxacin, Erythromycin, Gentamicin, Nalidixic acid, Streptomycin, and Tetracycline in ducks from European, African, and Asean countries during 2000-2011, the highest resistance of *C. jejuni* was found to Tetracycline (72.6%). Furthermore, *C. jejuni* was resistant to Nalidixic acid (50.1%), Ciprofloxacin (28.9%), Streptomycin (17.4%), Erythromycin (10.8%), and Gentamicin (7.8%). Nevertheless, the resistance to Doxycycline was not observed. In case of *C. coli*, Tetracycline was the most resistant antimicrobial agents (68.2%). In addition, antimicrobial resistance was found in Nalidixic acid (62.6%), Ciprofloxacin (38.0%), Streptomycin (2.5%), and Erythromycin 12.1%. However, it was not resistant to Gentamicin (Adzitey et al., 2012a). These imply that both *C. jejuni* and *C. coli* are resistant more than 50.0% to only

Tetracycline and Nalidixic acid. Besides, the present study found the resistance more than 50.0% to four antimicrobial agents: Streptomycin, Nalidixic acid, Ciprofloxacin, and Levofloxacin.

Table 3 Counts of *C. jejuni* with different resistance profiles from laying ducks and environmental samples in confinement and free-grazing systems

Profile	No. of isolates	Occurrence (%)[a]
No resistance demonstrated	1	1.2
Total	1	1.2
Resistance to one agent		
NAL[b]	1	1.2
GEN	1	1.2
STR	11	13.1
Total	13	15.5
Resistance to two agents		
LEV, CIP	2	2.4
NAL, STR	4	4.8
GEN, STR	1	1.2
Total	7	8.3
Resistance to three agents		
LEV, CIP, NAL	4	4.8
LEV, CIP, STR	1	1.2
LEV, NAL, STR	1	1.2
CIP, NAL, STR	1	1.2
CIP, GEN, STR	1	1.2
NAL, DOX, TET	1	1.2
STR, DOX, TET	1	1.2
Total	10	11.9
Resistance to four agents		
LEV, CIP, NAL, GEN	1	1.2
LEV, CIP, NAL, STR	10	11.9
LEV, CIP, NAL, DOX	1	1.2
LEV, NAL, GEN, STR	1	1.2
CIP, NAL, GEN, STR	1	1.2
CIP, NAL, STR, TET	1	1.2
NAL, STR, DOX, ERY	1	1.2
Total	16	19.0
Resistance to five agents		
LEV, CIP, NAL, GEN, STR	10	11.9
LEV, CIP, NAL, STR, DOX	1	1.2
LEV, CIP, NAL, STR, TET	4	4.7
LEV, CIP, NAL, DOX, TET	3	3.6
LEV, CIP, NAL, DOX, ERY	1	1.2
LEV, CIP, GEN, STR, TET	1	1.2
LEV, NAL, STR, DOX, TET	1	1.2
CIP, NAL, STR, DOX, TET	1	1.2
Total	22	26.2
Resistance to six agents		
LEV, CIP, NAL, GEN, STR, TET	1	1.2
LEV, CIP, NAL, GEN, STR, ERY	1	1.2
LEV, CIP, NAL, STR, DOX, TET	10	11.9
Total	12	14.3
Resistance to seven agents		
LEV, CIP, NAL, GEN, STR, DOX, TET	2	2.4
LEV, CIP, NAL, STR, DOX, TET, ERY	1	1.2
Total	3	3.6

[a]The number of isolates tested was 84.
[b]CIP = Ciprofloxacin, DOX = Doxycycline, ERY = Erythromycin, GEN = Gentamicin, LEV = Levofloxacin, NAL = Nalidixic acid, STR = Streptomycin, TET = Tetracycline

Comparing the antimicrobial resistance of *C. jejuni* and *C. coli*, it was found that *C. jejuni* was more resistant to the antimicrobial agents than *C. coli*. This did not correspond with former studies that *C. coli* was more resistant to antimicrobial agents than *C. jejuni* (Miflin et al., 2007). In some countries such as Australia, Canada, Norway, and Brazil, Fluoroquinolone usage is prohibited. Thus, no drug resistance was observed in these countries. Meanwhile, Fluoroquinolone resistance of *Campylobacter* spp. was found in the USA by 19.0% and EU countries by 14.9% for *C. jejuni* and 39.6% for *C. coli* (Miflin et al., 2007). In the current study, *C. jejuni* was highly resistant to Fluoroquinolone: Nalidixic acid (76.2%), Ciprofloxacin (70.2%), and Levofloxacin (67.9%), whereas *C. coli* was resistant to Nalidixic acid (58.3%), Ciprofloxacin (52.4%), and Levofloxacin (50.0%). This demonstrates the excessive use of antimicrobial agents in laying ducks raised in central Thailand, resulting in poor response to Fluoroquinolone in patients infected with resistant *Campylobacter* which contributes to prolonged treatment, severe symptoms, and increased cost of treatment according to drug alteration. Besides, weak or immunodeficient patients might die from this situation.

Minimum inhibitory concentration (MIC) of *Campylobacter* spp. from 97 samples of laying ducks in all confinement system flocks showed 24.7% of *C. coli* (24/97) and 75.3% of *C. jejuni* (73/97). Only 3 *Campylobacter* samples were not resistant to the 8 antimicrobial agents. Besides, multidrug resistance of *Campylobacter* was 16.5% (16/97): 15 samples of *C. jejuni* and 1 sample of *C. coli*. In addition, there were 5 samples of *C. coli* from the same farm which were resistant to the same 4 antimicrobial agents, i.e. Levofloxacin, Ciprofloxacin, Nalidixic acid, and Streptomycin. This indicates the dispersal of resistant *Campylobacter* to other ducks in the same flock. Subsequently, it might also contaminate the eggs.

Table 4 Counts of *C. coli* with different resistance profiles from laying ducks and environmental samples in confinement and free-grazing systems

Profile	No. of isolates	Occurrence (%)[a]
No resistance demonstrated	2	8.3
Total	2	8.3
Resistance to one agent		
CIP[b]	2	8.3
STR	4	16.7
Total	6	25
Resistance to two agents		
LEV, STR	1	4.2
CIP, STR	1	4.2
STR, DOX	1	4.2
Total	3	12.5
Resistance to three agents		
LEV, CIP, NAL	5	20.8
LEV, NAL, DOX	1	4.2
CIP, NAL , STR	1	4.2
NAL, STR, DOX	1	4.2
Total	8	33.3
Resistance to four agents		
LEV, CIP, NAL, STR	5	20.8
Total	5	20.8

[a]The number of isolates tested was 24
[b]CIP = Ciprofloxacin, DOX = Doxycycline, LEV = Levofloxacin, NAL = Nalidixic acid, STR = Streptomycin

Table 5 *Salmonella* isolated from laying ducks and environmental samples in confinement system

Flock	Type of samples	*Salmonella* serotype (No. of isolates)
A	Cloacal swab	*S.* Amsterdam (4)
	Cloacal swab	*S.* Mbandaka (1)
	Feed	*S.* Hvittingfoss (1), *S.* Mbandaka (1)
	Drinking water	*S.* Hvittingfoss (1), *S.* Mbandaka (1)
	Soil	*S.* I 4,5,12:i:- (1)
B	Cloacal swab	*S.* Agona (2), *S.* Hvittingfoss (1), *S.* Kentucky (2), *S.* Tennessee (1)
	Feed	*S.* Mbandaka (1)
	Soil	*S.* Cubana (1)
	Drinking water	*S.* Tennessee (1)
C	Cloacal swab	*S.* Weltevreden (1)
D	Cloacal swab	*S.* Cerro (1), *S.* Mbandaka (2), *S.* Stanley (4)
	Soil	*S.* Mbandaka (1)
	Drinking water	*S.* Chester (1)
L	Cloacal swab	*S.* Chicago (1), *S.* I 4,5,12:i:- (4), *S.* Typhimurium (3)
	Drinking water	*S.* I 4,5,12:i:- (1)
M	Soil	*S.* Amsterdam (1)
N	Drinking water	*S.* Weltevreden (1)

According to MIC from 11 samples of *C. jejuni* in laying ducks from all free-grazing system flocks, all samples were resistant to at least one antimicrobial agent. Besides, 63.6% (7/11) of the samples from four sites showed multidrug resistance. Most of them (6/11) came from nearby areas, i.e. a border between Song Phi Nong district of Suphan Buri province and Kamphaeng Saen district of Nakhon Pathom province. Furthermore, multidrug resistance took place with the same six antimicrobial agents: Tetracycline, Doxycycline, Nalidixic acid, Ciprofloxacin, Levofloxacin, and Streptomycin. It was thought that these grazing ducks received the drug-resistant *C. jejuni* from the field environment, which was the habitat of those ducks. The grazing ducks carrying drug-resistant *C. jejuni* then defecated to the field environment or irrigating water, spreading the same drug-resistant *C. jejuni* to both adjacent districts.

According to Table 3, all *C. jejuni* were from the samples of ducks in both confinement and free-grazing systems; 1.2% (1/84) of them did not show drug resistance to the eight antimicrobial agents. Besides, 34 patterns of antimicrobial resistance were found from *C. jejuni*: 31.0% (26/84) was multidrug resistance. Fluoroquinolone (Levofloxacin, Ciprofloxacin, and Nalidixic acid) resistance was found to be 58.8%. Besides, the resistance to Tetracycline (Tetracycline and Doxycycline) and Aminoglycosides (Streptomycin and Gentamicin) were 23.5% and 21.2%, respectively. Multidrug resistance of *C. jejuni* from 14 flocks was found in the same pattern such as LEV, CIP, NAL, STR, DOX, TET, indicating that such bacteria were dominant and epidemic. As for *C. coli*, 8.3% of the samples were not resistant to the eight antimicrobial agents. Ten patterns of drug resistance were found; 4.2% (1/24) multidrug resistance was found in one sample to Nalidixic acid, Streptomycin and Doxycycline (NAL, STR, DOX). In addition, the resistance to Fluoroquinolone (Levofloxacin, Ciprofloxacin, and Nalidixic acid) was 41.7% as shown in Table 4. Furthermore, the multidrug resistance of *C. coli* from 14 flocks was lower than that of *C. jejuni*. However, several groups had the same drug resistance pattern such as LEV, CIP, NAL, and STR.

Table 6 *Salmonella* isolated from laying ducks and environmental samples in free-grazing system

Flock	Type of samples	*Salmonella* serotype (No. of isolates)
E	Cloacal swab	*S.* Bangkok (1), *S.* Hvittingfoss (2), *S.* Mbandaka (2), *S.* Newport (1), *S.* Typhimurium (1)
	Feed	*S.* Hvittingfoss (1)
	Soil	*S.* Mbandaka (1), *S.* Weltevreden (1)
	Drinking water	*S.* Mbandaka (1)
	Surface water	*S.* Agona (1), *S.* Weltevreden (1)
F	Cloacal swab	*S.* Eastbourne (1), *S.* Hvittingfoss (2), *S.* I 4,5,12:i:- (6), *S.* Javiana (2), *S.* Mbandaka (3), *S.* Typhimurium (1)
	Feed	*S.* Javiana (1)
	Soil	*S.* Mbandaka (1), *S.* Stanley (1)
	Surface water	*S.* Hvittingfoss (1), *S.* Newport (1)
G	Cloacal swab	*S.* Agona (1), *S.* Thompson (1), *S.* Weltevreden (1)
	Soil	*S.* Thompson (1)
H	Cloacal swab	*S.* Virchow (1)
	Feed	*S.* Stanley (1)
I	Surface water	*S.* Mbandaka (1)
J	Cloacal swab	*S.* Mbandaka (2), *S.* Paratyphi B var.Java (1), *S.* Thompson (1)
	Soil	*S.* Javiana (1), *S.* Paratyphi B var. Java (1)
	Surface water	*S.* Paratyphi B var. Java (1)
K	Cloacal swab	*S.* Bareilly (4), *S.* Corvallis (4), *S.* Dublin (6), *S.* Hvittingfoss (20), *S.* Javiana (3), *S.* Newport (2), *S.* Ramatgan (1), *S.* Saintpaul (1), *S.* Schwarzengrund (1), *S.* Stanley (9), *S.* Virchow (1)
	Soil	*S.* Hvittingfoss (1)
	Surface water	*S.* Hvittingfoss (2)

Table 7 Relationship between prevalence of *Salmonella* spp. and *Campylobacter* spp. isolated from laying ducks in confinement and free-grazing systems

Bacterial isolated	Laying duck samples			
	Confinement system		Free-grazing system	
	No. of isolates	%	No. of isolates	%
Salmonella spp.	27	4.2	75	10.7
Campylobacter spp.	88	13.8	2	0.3

χ^2= 101.2, df=1, p-value < 0.05

Identification of Salmonella: The cloacal swab of laying ducks from seven confinement system flocks showed positive results of *Salmonella* spp. by 4.2% (27/639). In the environment, *Salmonella* spp. were found in the feed, soil, and water by 13.3% (2/15), 57.1% (4/7), and 33.3% (5/15), respectively. Besides, 14 different serotypes were found: *S.* Agona, *S.* Amsterdam, *S.* Cerro, *S.* Chester, *S.* Chicago, *S.* Cubana, *S.* Hvittingfoss, *S.* I 4,5,12:i:-, *S.* Kentucky, *S.* Mbandaka, *S.* Stanley, *S.* Tennessee, *S.* Typhimurium, and *S.* Weltevreden. The most common serotypes were *S.* Mbandaka (17.5%) and *S.* I 4,5,12:i:- (15.0%) as shown in Table 5.

The cloacal swab of laying ducks from seven free-grazing system flocks showed positive results of *Salmonella* spp. by 10.7% (75/700). In the environment, *Salmonella* spp. were detected from the feed, soil, and water by 25.0% (4/16), 70.0% (7/10), and 47.1% (8/17), respectively. Furthermore, 20 different serotypes were found: *S.* Agona, *S.* Bangkok, *S.* Bareilly, *S.* Corvallis, *S.* Dublin, *S.* Eastbourne, *S.* Hvittingfoss, *S.* I 4,5,12:i:-, *S.* Javiana, *S.* Mbandaka, *S.* Newport, *S.* Paratyphi B Var. Java, *S.* Ramatgan, *S.* Saintpaul, *S.* Schwarzengrund, *S.* Stanley, *S.*Thompson, *S.* Typhimurium, *S.* Verchow, and *S.* Weltevreden. The most common serotypes were *S.* Hvittingfoss (28.4%) and *S.* Mbandaka (10.8%), together with *S.* Stanley (10.8%) as displayed in Table 6.

Only 7 serotypes of *Salmonella* were found in the ducks raised both in confinement and free-grazing systems: *S.* Agona, *S.* Hvittingfoss, *S.* I 4,5,12:i:-, *S.* Mbandaka, *S.* Stanley, *S.* Typhimurium, and *S.* Weltevreden. A survey of the prevalence of *Salmonella* spp. in ducks from 1997 to 2012 revealed that the prevalence in meat-type ducks, duck meat and parts, and rearing and processing environment were 19.9% (3.3-56.9%), 28.4% (4.4-75.6%), and 32.5% (10.5-82.6%), respectively. The prevalence from such survey was higher than that of the present study, except for those raised in the USA (3.3%) and China (5.3%) (Adzitey et al., 2012). In the current study, the prevalence of *Salmonella* in the environment of grazing ducks was higher than that of the ducks accommodated in confinement systems since the environment at the grazing area were more at risk of *Salmonella* distribution by local birds' feces. Therefore, because these rice fields were the regular place for raising the grazing ducks, they were the considerable reservoir and distributing port of *Salmonella* from local birds.

Between 1997 and 2012, a survey of serotypes in ducks from several regions of the world reported that *S.* Typhimurium was the most popular one in human health in most countries, except for Vietnam and Egypt (Adzitey et al., 2012a). The present study demonstrated that *S.* Typhimurium was found in the ducks both in confinement and free-grazing system flocks by 7.5% and 2.0%, respectively. Owing to the study in China, Taiwan and Brazil by Adzitey et al. (2012a), the most common serotypes were *S.* Typhimurium (26.7%), *S.* Potsdam (31.9%), and *S.* Saintpaul (29.8%). In this study, the most common serotypes found in the laying ducks from confinement and free-grazing system flocks were *S.* Hvittingfoss, *S.* Mbandaka, and *S.* Stanley, respectively.

The *Salmonella* serotyping from cloacal swabs and environment of ducks in confinement systems showed the same serotype at least 1 sample from 4 out of 7 houses (57.1%). For instance, the *Salmonella* from water samples had the same serotype as that from cloacal swab from these three confinement systems: *S.* Mbandaka of a farm in Suphanburi province, *S.* Tennessee of a farm in Phra Nakhon Si Ayutthaya province, and *S.* I4,5,12:i:- of a farm in Nakhon Pathom province. This is because they used water pipeline for consumption and as the residence of ducks. In addition, not only in water, *S.* Mbandaka was also found in the concentrate feed in a farm in Suphanburi province. It is possible that both concentrate feed and water in pipeline were the major source for continually dispersing *S.* Mbandaka to other ducks. In Song Phi Nong district of Suphan Buri province, *S.* Mbandaka was detected both in the soil and cloacal swab. In general, confinement systems use only soil as house bedding, except for laying areas which are made of hay. This causes some ducks to carry *S.* Mbandaka from defecation and transmit it to the soil directly. When concentrate feed falls on the soil, the ducks might, afterwards, acquire *Salmonella* from the infected feed and soil.

As for the ducks in free-grazing system, five of seven flocks had the same *Salmonella* serotype at least one sample between the cloacal swabs and environment. *Salmonella* from five flocks had the same serotypes between the cloacal swabs and soil samples. In addition, *Salmonella* found in four flocks from the water samples from field or water in residing crate near the field had the same serotypes as that from cloacal swabs. *S.* Mbandaka and *S.* Hvittingfoss were the major serotypes found in both cloacal swabs and field for raising these ducks.

The detection of *Salmonella* in the environment from water, soil, and feed of both confinement and free-grazing system flocks revealed that the environment was the significant source of transmitting *Salmonella* to other ducks in the flock. The ducks might acquire both *Campylobacter* and *Salmonella*, and spread to other ducks in the flock eventually.

Antimicrobial susceptibility of Salmonella: In the confinement systems, only one sample, which accounts for 2.6% (1/38), showed antimicrobial resistance. It was *S.* Typhimurium from the cloacal swab which was resistant to only Cephalothin. In the free-grazing system, the samples with antimicrobial resistance were 5.3% (5/94) and derived from 5 flocks. They were four serotypes of *Salmonella*: *S.* Agona, *S.* Stanley, *S.* Thompson, and *S.* Corvallis. In addition, all samples were resistant to more than 2 antimicrobial agents. *S.* Agona, was the most resistant serotype; it resisted to seven antimicrobial agents. In addition, *S.* Agona from the cloacal swab and water in rice field was resistant to the same two antimicrobial agents, Gentamicin and Streptomycin.

Antimicrobial resistance of *Salmonella* in grazing ducks might be caused by drug administration by farmers without any recommendations from veterinarians. The present study demonstrated that rearing grazing ducks might cause a risk of transmitting *Salmonella*, especially drug-resistant strain, to the environment. Since the field for raising these ducks adjoins water source for the community or other animals raised, *Salmonella* could be widely spread to other animals and humans.

The study related to antimicrobial susceptibility of *Salmonella* in ducks from the United Kingdom, China, Taiwan, and Malaysia between 2000 and 2011 revealed that the highest resistance of *Salmonella* was to Erythromycin (100.0%). Moreover, *Salmonella* was resistant to Amoxicillin-clavulanic acid

(89.6%), and Tetracycline (70.6%). Nevertheless, *Salmonella* was 100.0% susceptible to Ceftriaxone, Kanamycin, Ciprofloxacin, Ofloxacin, and Polymyxin B (Adzitey et al., 2012a), which was different to the present study. The *Salmonella* found in the laying ducks from confinement system, in the present study, was 100% susceptible to 15 antimicrobial agents: Ampicillin, Amoxicillin-clavulanic acid, Nalidixic acid, Kanamycin, Ciprofloxacin, Norfloxacin, Ofloxacin, Chloramphenicol, Streptomycin, Tetracycline, Trimethoprim-sulfamethoxazole, Gentamicin, Enrofloxacin, Ceftiofur, and Nitrofurantoin. Besides, the *Salmonella*, in this study, was resistant to only Cephalothin (2.6%). In the grazing ducks, *Salmonella* was resistant to Ampicillin (4.2%), Gentamicin, Streptomycin, and Cephalothin (3.2%), Amoxicillin-clavulanic acid (2.1%), and Tetracycline, Chloramphenical, Nalidixic acid, and Trimethoprim-Sulfamethoxazole (1.1%). In addition, *Salmonella* in the laying ducks from free-grazing system was 100.0% susceptible to Kanamycin, Ciprofloxacin, Norfloxacin, Ofloxacin, Enrofloxacin, Ceftiofur, and Nitrofurantoin.

According to the bacterial identification from cloacal swab, the association between *Campylobacter* and *Salmonella* was observed (Table 7). The laying ducks in confinement system had more *Campylobacter* than *Salmonella*, whereas those in free-grazing system had more *Salmonella* than *Campylobacter*. These imply that the confinement systems have higher risk of having *Campylobacter* than *Salmonella*, resulting in the contamination of *Campylobacter* in fresh eggs more than that of *Salmonella*. In contrast, the free-grazing systems provide higher risk of transmitting *Salmonella* than *Campylobacter* to consumers. Consequently, further comparative studies on both raising patterns should be performed in terms of contamination and methods in order to reduce the transmission of *Campylobacter* and *Salmonella* from egg to consumers since former studies reported that both were commonly found in human in Europe, North America, and Australia, including Thailand.

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บทคัดย่อ

ความชุกและการดื้อยาของซัลโมเนลลาและแคมไพโลแบคเตอร์ในฝูงเป็ดไข่ที่เลี้ยงแบบโรงเรือนเปิดและแบบไล่ทุ่ง

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สำรวจความชุกและการดื้อยาของ *Salmonella* spp. และ *Campylobacter* spp. ของเป็ดระยะให้ไข่ที่เลี้ยงแบบโรงเรือนเปิด 7 ฝูงและเลี้ยงแบบไล่ทุ่ง 7 ฝูง พบว่า *Campylobacter* spp. ที่แยกได้จาก cloacal swab ของเป็ดระยะให้ไข่ในโรงเรือนเปิดทุกฝูงรวมกันเป็น 13.8% (88/639) โดยแยกได้เป็น *C. jejuni* 72.7% และ *C. coli* 27.3% และพบ *Campylobacter* spp. เฉพาะน้ำที่เป็ดกินและอาศัยเป็น 60.0% โดยทุกตัวอย่างเป็น *C. jejuni* สำหรับตัวอย่างจาก cloacal swab ของเป็ดระยะให้ไข่ในทุ่งทุกฝูงรวมกันพบ *Campylobacter* spp. เป็น 0.3% (2/700) โดยพบว่าทั้ง 2 ตัวอย่างเป็น *C. jejuni* และในสิ่งแวดล้อมพบ *Campylobacter* spp. เฉพาะน้ำที่เป็ดกินและอาศัยเป็น 52.9% โดยทั้ง 9 ตัวอย่างเป็น *C. jejuni* การดื้อยาของเชื้อ *C. jejuni* จากทุกฝูงของเป็ดระยะให้ไข่ในทุ่งและในโรงเรือนเปิดต่อ Streptomycin, Nalidixic acid, Ciprofloxacin และ Levofloxacin อยู่ระหว่าง 67.9% ถึง 81.0% ส่วนการดื้อยาของเชื้อ *C. coli* จากทุกฝูงของเป็ดระยะให้ไข่ในทุ่งและในโรงเรือนเปิดต่อ Streptomycin, Nalidixic acid, Ciprofloxacin และ Levofloxacin อยู่ระหว่าง 50.0% ถึง 87.5% ส่วน *Salmonella* spp. ที่แยกได้จาก cloacal swab ของเป็ดระยะให้ไข่ในโรงเรือนเปิดทุกฝูงรวมกันเป็น 4.2% และจากตัวอย่างของอาหารเป็ด ดินที่เลี้ยงเป็ด และน้ำที่เป็ดกินและอาศัยในโรงเรือนเปิดทุกฝูงรวมกันเป็น 13.3%, 57.1% และ 33.3% ตามลำดับ และพบซีโรไทป์ที่แตกต่างกันทั้งหมด 14 ซีโรไทป์ ส่วนเชื้อ *Salmonella* spp. ที่แยกได้จาก cloacal swab ของเป็ดระยะให้ไข่ในทุ่งทุกฝูงรวมกันเป็น 10.7% และพบ *Salmonella* spp. จากตัวอย่างของอาหารเป็ด ดินที่เลี้ยงเป็ด และน้ำที่เป็ดกินและอาศัยในทุ่งทุกฝูงรวมกันเป็น 25.0%, 70.0% และ 47.1% ตามลำดับ และพบซีโรไทป์ที่แตกต่างกันทั้งหมด 20 ซีโรไทป์ การดื้อยาของของ *Salmonella* spp. ในการเลี้ยงแบบโรงเรือนเปิดทุกฝูงนั้นดื้อต่อยาเพียงชนิดเดียว คือ cephalothin และพบเพียง 1 ตัวอย่าง คือ *S.* Typhimurium ส่วนตัวอย่างจากการเลี้ยงเป็ดในทุ่งทุกฝูง พบเชื้อดื้อยา 5 ตัวอย่างจาก 5 ฝูง โดยพบ *Salmonella* ทั้งหมด 4 ซีโรไทป์ คือ *S.* Agona, *S.* Stanley, *S.* Thompson และ *S.* Corvallis สำหรับผลการตรวจแยกเชื้อจาก cloacal swab พบว่าความสัมพันธ์ระหว่าง *Campylobacter* spp. และ *Salmonella* spp.ที่แยกได้มีความแตกต่างกันในรูปแบบการเลี้ยงแบบโรงเรือนเปิดและแบบไล่ทุ่ง โดยเป็ดระยะให้ไข่ในโรงเรือนเปิดตรวจพบ *Campylobacter* spp. มากกว่า *Salmonella* spp. ส่วนเป็ดระยะให้ไข่ในทุ่งนั้นตรวจพบ *Salmonella* spp. มากกว่า *Campylobacter* spp.

คำสำคัญ: แคมไพโลแบคเตอร์ การเลี้ยงแบบไล่ทุ่ง การเลี้ยงแบบโรงเรือนเปิด เป็ดไข่ ซัลโมเนลลา

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