Agr. Nat. Resour. 54 (2020) 17–24

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Longitudinal survey of *Campylobacter* and *Salmonella* isolates from free-grazing, laying duck flocks in lower central provinces, Thailand

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Article Info

Article history:
Received 21 February 2018
Revised 6 July 2018
Accepted 29 July 2018
Available online 28 February 2020

Keywords:
Campylobacter,
Free-grazing, laying duck,
Longitudinal survey,
Salmonella

Abstract

A longitudinal investigation was undertaken of the prevalence of *Campylobacter* and *Salmonella* in two flocks of free-grazing, laying ducks raised in the lower central region of Thailand. The ages of the ducks studied were 4–40 wk. During 4–28 wk, the ducks grazed in rice fields; afterwards, they were accommodated in open houses. In total, 1,262 samples were collected from the two flocks; 39.0% of all samples were positive to *Campylobacter* spp. of which 71.3% and 28.7% were *C. jejuni* and *C. coli*, respectively. For *Salmonella* spp., 10.2% of samples showed positive results with 22 serovars. Moreover, the three most isolated serovars were *S.* Mbandaka (27.1%), *S.* Typhimurium (17.8%) and *S.* Newport (14.0%). In summary, the 9 mth survey detected *Campylobacter* and *Salmonella* from the bodies and environment of the ducks at all ages. Consequently, duckling selection from parent stock should avoid selecting individuals with these pathogens. Moreover, biosecurity measures should be improved according to the farm standards issued by the Ministry of Agriculture and Cooperatives.

Introduction

In Thailand, diarrheal patients per annum numbered approximately one million during 1994–2015 (Bureau of General Communicable Diseases, Department of Disease Control, 2017), with the main cause of the diarrhea being pathogens that had contaminated food made from meats, also known as food poisoning. Globally, the major bacteria causing food poisoning are *Salmonella* spp. and *Campylobacter* spp., which mainly contaminate meats and eggs of animals used for human consumption (Luber, 2009). In 2014, approximately 88,000 and 236,000 people of the European Union suffered from food poisoning caused by Campylobacteriosis and Salmonellosis, respectively; however, Salmonellosis in European people declined during 2008–2014 because of the effective measures through the food chain, especially in chicken flocks from grandparent stocks of both broilers and layers (European Food Safety Authority and European Centre for Disease Prevention and Control, 2015). *Salmonella* contamination is mostly found in eggs and egg-derived products, while *Campylobacter* contamination is mainly found in chicken meat (European Food Safety Authority and European Centre for Disease Prevention and Control, 2015). Nowadays, ducks are one of the most favorite food animals and contamination with *Salmonella* spp. and *Campylobacter* spp. has been reported in the meat and eggs of ducks from many countries (Adzitey et al., 2012). During 1990–2011, a global survey revealed that the prevalence of ducks contaminated with *Campylobacter* spp. and their environment was approximately 53% (0.0–83.3%) and 94.4% (92.0–96.7%), respectively (Adzitey et al., 2012). The prevalence of ducks contaminated with *Salmonella* spp. varies by country. During 1997–2012, the prevalence of *Salmonella* spp. contamination in ducks and their environment from many countries was 3.3–56.9% and 10.5–82.6%, respectively (Adzitey et al., 2012).

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https://doi.org/10.34044/j.anres.2020.54.1.03

The present study was conducted to survey the prevalence of *Campylobacter* spp. and *Salmonella* spp. in two flocks of laying ducks and their environment in the age range 4–40 wk. The ducklings of both flocks were sourced from the same parent stock. The samples were first collected when the ducklings were aged 4 wk and had been raised on paddy and aquatic animals in the rice fields. Farmers from several provinces in central Thailand, in Nakhon Pathom, Ratchaburi, Kanchanaburi, Suphan Buri and Chai Nat provinces, normally move their ducks to different rice fields for grazing every 2–4 wk until the ducks start laying. After age 28 wk, the flocks are kept in separated, open houses. Raising ducks in these houses is convenient with regards to feeding and egg collection and farmers feed the ducks in the houses until they stop laying. Consequently, a longitudinal study during 4–40 wk of these two flocks could provide information on the horizontal transmission of the two pathogens from duck to duck, and between duck and environment, along with a vertical transmission to the eggs which could further affect consumers.

Materials and Methods

Sampling

Sampling was conducted from cloacal swabs, eggs and the environment (water, soil and feed) as pooled samples in the two laying duck flocks from June 2011 to May 2012 in seven provinces located in lower central Thailand. Flock A contained samples from Kanchanaburi, Chai Nat and Suphan Buri provinces, while flock B comprised samples from Nakhon Pathom, Ratchaburi and Suphan Buri provinces. Ducks from both flocks were produced from the same parent stock. Cloacal swabs were taken in ducks aged 4 wk, 10 wk, 16 wk, 22 wk, 28 wk and 40 wk. In total, 1,171 samples (573 from flock A and 598 from flock B) were investigated. Samples from the environment (n = 59; 34 from flock A and 25 from flock B) were pooled from the soil, water and feed sources. In addition, samples (n = 32; 15 from flock A and 17 from flock B) were taken from egg contents and egg shells from ducks aged 22 wk and older.

Identification of Campylobacter

The genus and species of *Campylobacter* were identified using conventional methods and multiplex polymerase chain reaction (mPCR), respectively (Denis et al., 1999). The conventional method involved the collection of feces using a cloacal swab and the environmental samples were placed in Preston broth 9 ml [Nutrient broth No.2 (Oxoid, CM 67; Basingstoke, UK) composed of Lab-Lemco meat extract 10 g/L, peptone 10 g/L and sodium chloride 5 g/L; 5% (volume per volume) lysed horse blood, prepared according to Hunt et al. (2001); *Campylobacter* growth supplement (Oxoid, SR232; Basingstoke, UK) composed of sodium pyruvate 0.25 g/L, sodium metabisulphite 0.25 g/L and ferrous sulphate 0.25 g/L; modified Preston *Campylobacter* selective supplement (Oxoid, SR0204; Basingstoke, UK) composed of polymyxin B 5000 international units/L, trimethoprim 10 mg/L, rifampicin 10 mg/L and amphotericin B 10 mg/L]. and were incubated for 48 hr at 42°C under microaerophilic conditions in anaerobic jars using gas-generating kits (Oxoid; Basingstoke, UK). For environmental samples, 25 mL/g each of water, soil, egg shell, egg content or feed samples were cultured in 225 mL Preston broth at 42°C for 48 hr under microaerophilic conditions. Following enrichment in Preston broth, the samples were streaked on charcoal-cefoperazone-deoxycholate agar (CCDA) (*Campylobacter* blood-free selective agar base [Oxoid; Basingstoke, UK] with CCDA selective supplement [Oxoid; Basingstoke, UK], which contained 32 µg/mL cefoperazone and 10 µg/mL amphotericin B.) at 42°C for 48 hr under microaerophilic conditions. Afterward, dark-gray colonies were harvested and stained with Victoria blue to investigate their gull wing morphology.

The mPCR was undertaken by picking up one cultured colony of *Campylobacter* to extract deoxyribonucleic acid (DNA) using a commercial DNA extraction kit (Promega; Madison; WI, USA). The mPCR increased *16S* rRNA to confirm the results at the generic level of *Campylobacter*. In addition, the *mapA* and *ceuE* genes were amplified to confirm the results at the specific level of *C. jejuni* and *C. coli*, respectively. The genus-specific primers selected to amplify only the *Campylobacter 16S* rRNA gene were 5′-ATC TAA TGG CTT AAC CAT TAA AC-3′ (MD16S1) and 5′-GGA CGG TAA CTA GTT TAG TAT T-3′ (MD16S2). In order to subtype *C. jejuni*, the primer pair, 5′-CTA TTT TAT TTT TGA GTG CTT GTG-3′ (MDmapA1) and 5-GCT TTATTT GCC ATT TGT TTT ATT A-3′ (MDmapA2) was used to amplify its *mapA* gene. A third pair of primers, 5′-AAT TGA AAA TTG CTC CAA CTA TG-3′ (COL3) and 5′-TGA TTT TAT TAT TTG TAG CAG CG-3′ (MDCOL2), was used to identify the *C. coli* subtype based on the amplification of its *ceuE* gene. Amplification generated 857 bp, 589 bp and 462 bp DNA fragments corresponding to the genus *Campylobacter* and the species *C. jejuni* and *C. coli*, respectively (Fig. 1). The polymerase chain reaction procedures were mainly performed using a T-Gradient thermocycler (Whatman Biometra; Gottingen, Germany) according to the method

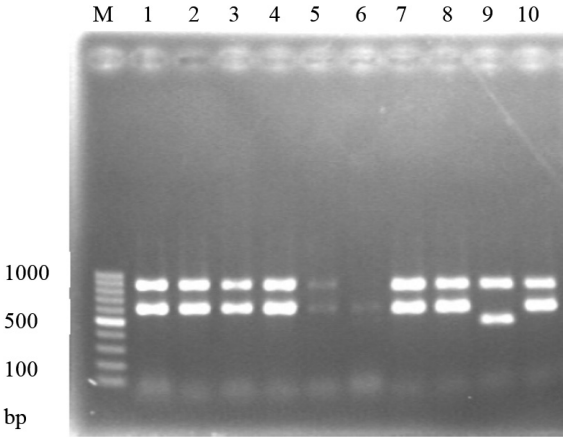

Fig. 1 Multiplex polymerase chain reaction results showing the amplification-generated DNA fragments corresponding to *Campylobacter* spp. (857 bp), *C. jejuni* (589 bp) and *C. coli* (462 bp), where M = DNA marker, Lane 1–4, 7–8, 10 = *C. jejuni*, Lane 9 = *C. coli*.

described by Denis et al. (1999) with modification. Differences were the use of dNTPs (Fermentas; Hanover; MD, USA) concentration of 200 μM, the concentrations of MD16S1 and MD16S2 primers were both 0.5 μM and the use of *Taq* DNA polymerase (Invitrogen; Carlsbad; CA, USA) 1.2 U. The examination of DNA products used gel electrophoresis with 1% (weight per volume) agarose gel (Seakem LE agarose; Rockland; ME, USA), 100 V for 30 min, staining with ethidium bromide (Amresco; Solon; OH, USA) and applied ultraviolet light to investigate the DNA size.

Identification of Salmonella

Conventional methods and serotyping were used to determine species of *Salmonella* according to the method of the Kauffmann-White classification (Grimont and Weill, 2007). Cloacal swabs, egg shell, egg content, water, feed and soil samples were taken to culture on the criterion of ISO 6579:2002 (annex D) (International Organization for Standard [ISO], 2007); cloacal swabs were cultured in 25 mL buffer peptone water (BPW; Oxoid; Basingstoke, UK), while 25 mL/g of water, feed, egg shell, egg content and soil samples were cultured in 225 mL BPW. All samples, thereafter, were incubated at 37°C for 18 hr. After incubation, 3 drops from 0.1 mL were transferred to modified semi-solid Rappaport-Vassiliadis (MSRV, Difco; Becton Dickinson; Franklin Lakes, NJ, USA) agar plate with novobiocin 0.01 g/Ll at 42°C for 24 hr. The growth on MSRV plates suspected to be *Salmonella* spp. was streaked on brilliant-green phenol-red lactose sucrose (BPLS; Oxoid; Basingstoke, UK) and xylose lysine deoxycholate agars (XLD; Oxoid; Basingstoke, UK). After incubating at 37°C for 24 hr, 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 using serological testing on the basis of slide agglutination with polyvalent anti-specific O antisera and specific flagellar H antisera (S.A.P. Laboratory; BKK, Thailand). Finally, the antigen pattern was compared with the Kauffmann-White classification using antigenic formulas of the *Salmonella* serovars (Grimont and Weill, 2007).

Results and Discussion

Campylobacter spp. and *Salmonella* spp. Were surveyed in 1,262 free-grazing, laying ducks from two flocks during age 4–40 wk and 39.0% of the samples were positive to *Campylobacter* spp. (Table 1). Of those, 71.3% were *C. jejuni* and 28.7% were *C. coli* (Table 2).

Table 1 Prevalence of *Campylobacter* spp. and *Salmonella* spp. from ducks, eggs and environmental samples

Flock and Location	Sample location (duck age)	*Campylobacter* spp.	*Salmonella* spp.
Flock A			
Kanchanaburi	Ducks (4 wk)	56.3% (45/80)	2.5% (2/80)
	Environment (4 wk)	14.3% (1/7)	57.1% (4/7)
Kanchanaburi	Ducks (10 wk)	30.3% (30/99)	4.0% (4/99)
	Environment (10 wk)	40.0% (2/5)	40.0% (2/5)
Suphan Buri	Ducks (16 wk)	44.0% (44/100)	0.0% (0/100)
	Environment (16 wk)	16.7% (1/6)	66.7% (4/6)
Chai Nat	Ducks (22 wk)	7.4% (7/94)	2.1% (2/94)
	Environment (22 wk)	14.3% (1/7)	28.6% (2/7)
	Eggs (22 wk)	0.0% (0/3)	33.3% (1/3)
Suphan Buri	Ducks (28 wk)	14.0% (14/100)	2.0% (2/100)
	Environment (28 wk)	25.0% (1/4)	25.0% (1/4)
	Eggs (28 wk)	0.0% (0/6)	100.0% (6/6)
Suphan Buri	Ducks (40 wk)	45.0% (45/100)	3.0% (3/100)
	Environment (40 wk)	60.0% (3/5)	60.0% (3/5)
	Eggs (40 wk)	33.3% (2/6)	100.0% (6/6)
	Total flock A	31.5% (196/622)	6.8% (42/622)
Flock B			
Nakhon Pathom	Ducks (4 wk)	62.2% (61/98)	5.1% (5/98)
	Environment (4 wk)	50.0% (2/4)	75.0% (3/4)
Suphan Buri	Ducks (10 wk)	38.0% (38/100)	51.0% (51/100)
	Environment (10 wk)	25.0% (1/4)	100.0% (4/4)
Ratchaburi	Ducks (16 wk)	69.0% (69/100)	0.0% (0/100)
	Environment (16 wk)	25.0% (1/4)	25% (1/4)
Ratchaburi	Ducks (22 wk)	35.0% (35/100)	0.0% (0/100)
	Environment (22 wk)	50.0% (2/4)	0.0% (0/4)
	Eggs (22 wk)	0.0% (0/5)	40.0% (2/5)
Suphan Buri	Ducks (28 wk)	16.0% (16/100)	5.0% (5/100)
	Environment (28 wk)	25.0% (1/4)	25.0% (1/4)
	Eggs (28 wk)	0.0% (0/6)	83.3% (5/6)
Suphan Buri	Ducks (40 wk)	69.0% (69/100)	3.0% (3/100)
	Environment (40 wk)	20.0% (1/5)	20.0% (1/5)
	Eggs (40 wk)	0.0% (0/6)	100.0% (6/6)
	Total flock B	46.3% (296/640)	13.6% (87/640)
	Total flock A and B	39.0% (492/1262)	10.2% (129/1262)

Table 2 Campylobacter spp. isolated from ducks and environmental samples

Flock	Sample location (duck age)	*C. jejuni*	*C. coli*
A	Ducks (4 wk)	55.6% (25/45)	44.4% (20/45)
	Environment (4 wk)	100.0% (1/1)	0.0% (0/1)
	Ducks (10 wk)	96.7% (29/30)	3.3% (1/30)
	Environment (10 wk)	50.0% (1/2)	50.0% (1/2)
	Ducks (16 wk)	100.0% (44/44)	0.0% (0/44)
	Environment (16 wk)	100.0% (1/1)	0.0% (0/1)
	Ducks (22 wk)	100.0% (7/7)	0.0% (0/7)
	Environment (22 wk)	100.0% (1/1)	0.0% (0/1)
	Eggs (22 wk)	0.0% (0)	0.0% (0)
	Ducks (28 wk)	71.4% (10/14)	28.6% (4/14)
	Environment (28 wk)	100.0% (1/1)	0.0% (0/1)
	Eggs (28 wk)	0.0% (0)	0.0% (0)
	Ducks (40 wk)	53.3% (24/45)	46.7% (21/45)
	Environment (40 wk)	66.7% (2/3)	33.3% (1/3)
	Eggs (40 wk)	0.0% (0/2)	100.0% (2/2)
	Total	74.5% (146/196)	25.5% (50/196)
B	Ducks (4 wk)	67.2% (41/61)	32.8% (20/61)
	Environment (4 wk)	50.0% (1/2)	50.0% (1/2)
	Ducks (10 wk)	39.5% (15/38)	60.5% (23/38)
	Environment (10 wk)	0.0% (0/1)	100.0% (1/1)
	Ducks (16 wk)	98.6% (68/69)	1.4% (1/69)
	Environment (16 wk)	100.0% (1/1)	0.0% (0/1)
	Ducks (22 wk)	97.1% (34/35)	2.9% (1/35)
	Environment (22 wk)	100.0% (2/2)	0.0% (0/2)
	Eggs (22 wk)	0.0% (0)	0.0% (0)
	Ducks (28 wk)	50.0% (8/16)	50.0% (8/16)
	Environment (28 wk)	0.0% (0/1)	100.0% (1/1)
	Eggs (28 wk)	0.0% (0)	0.0% (0)
	Ducks (40 wk)	50.7% (35/69)	49.3% (34/69)
	Environment (40 wk)	0.0% (0/1)	100.0% (1/1)
	Eggs (40 wk)	0.0% (0)	0.0% (0)
	Total	69.3% (205/296)	30.7% (91/296)

Furthermore, 10.2% of the samples were positive to *Salmonella* spp. categorized into 22 serovars: *S.* Agona, *S.* Amsterdam, *S.* Bangkok, *S.* Bovismorbificans, *S.* Chester, *S.* Dublin, *S.* Enteritidis, *S.* Hadar, *S.* Hvittingfoss, *S.* I 4,5,12:i:, *S.* I 4, 12:i:-, *S.* IV 43:Z4Z23:-, *S.* Mbandaka, *S.* Montevideo, *S.* Newport, *S.* Orion, *S. Paratyphi B var. Java*, *S.* Poona, *S.* Stanley, *S.* Thompson, *S.* Typhimurium and *S.* Weltevreden. Of these, the three most commonly isolated serovars were *S.* Mbandaka (27.1%), *S.* Typhimurium (17.8%) and *S.* Newport (14.0%) as shown in Table 3. Based on the cloacal swabs from both flocks, the prevalence of *Campylobacter* spp. was higher than that of *Salmonella* spp., except in the ducks aged 10 wk in flock B where the opposite result was recorded (51% *Salmonella* spp. versus 38% *Campylobacter* spp.) as shown in Fig. 2 and 3. This corresponded with the retrospective study performed during 1990–2012 on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in ducks from several regions of the world by Adzitey et al. (2012) who reported that the average prevalence of *Campylobacter* spp. was higher than that of *Salmonella* spp. (53.0% versus 19.9%, respectively).

The coexistence of *Campylobacter* spp. and *Salmonella* spp. in the same sample was found both in cloacal swabs and environmental samples from both flocks (Table 4). In flock A, this coexistence was apparent in ducks of all ages (2.41%). The highest positive results were detected in ducks aged 40 wk from both the cloacal swabs and the environmental samples (soil, drinking water, pond water and egg shell). In flock B, the coexistence was 4.22% for all duck ages, except in ducks aged 16 wk and 22 wk. The highest positive results were recorded in ducks aged 10 wk from both cloacal swabs and the environmental sample using water from the rice field. Interestingly, the coexistence of the two pathogenic bacteria demonstrated the danger from contamination in ducks, eggs and the environment. Moreover, this indicated the broad, common environment of these two pathogenic bacteria. In Thailand, Saengthongpinit et al. (2015) reported the prevalence of *Salmonella* and *Campylobacter* in free-grazing, laying ducks from seven flocks. In addition, the cloacal swabs showed positive results for *Campylobacter* spp. (0.29%), while for the environmental samples, positive results were only recorded in the drinking water and the water in the duck houses (52.94%). *Salmonella* spp., were isolated from the cloacal swabs of laying ducks in the rice fields (10.71%). In addition, *Salmonella* spp. were found in the feed, soil and water for drinking in the duck houses (25.0%, 70.0% and 47.06%, respectively).

Table 3 *Salmonella* serotypes isolated from ducks and environmental samples

Flock	Sample (duck age)	*Salmonella* serotype (no. of positive sample)	Environmental samples (no. of positive sample)
A	Ducks (4 wk)	Enteritidis (1), Thompson (1)	Amsterdam (3), Thompson (1)
	Ducks (10 wk)	Bovismorbificans (1), Enteritidis (1), I 4,12 :i:- (1), Typhimurium (1)	Paratyphi B var. Java (2)
	Ducks (16 wk)		Newport (1), Stanley (3)
	Ducks (22 wk)	I 4,5,12:i:- (1), Typhimurium (1)	Mbandaka (1), Dublin (1)
	Eggs (22 wk)	Stanley (1)	
	Ducks (28 wk)	Mbandaka (2)	Mbandaka (1)
	Eggs (28 wk) *	Mbandaka (6), Montevideo (1)	
	Ducks (40 wk)	Mbandaka (2), Orion (1)	Mbandaka (1), Orion (2)
	Eggs (40 wk)	Amsterdam (1), Mbandaka (4), Montevideo (1)	
B	Ducks (4 wk)	Chester (1), Dublin (1), Hvittingfoss (1), Mbandaka (1), Thompson (1)	Amsterdam (1), Thompson (2)
	Ducks (10 wk)	Chester (1), Bangkok (4), Hadar (1), Hvittingfoss (1), I 4,12 :i: (1), Mbandaka (3), Newport (17), Paratyphi B var. Java (3), Poona (2), Typhimurium (18)	Typhimurium (3), Paratyphi B var. Java (1)
	Ducks (16 wk)		Weltevreden (1)
	Ducks (22 wk)		
	Eggs (22 wk)	Amsterdam (1), Hvittingfoss (1)	
	Ducks (28 wk)	IV 43:Z4Z23:- (1), Mbandaka (3), Montevideo (1)	Amsterdam (1)
	Eggs (28 wk) *	Mbandaka (4), Montevideo (2)	
	Ducks (40 wk)	*S.* Mbandaka (1), *S.* Montevideo (2)	Agona (1)
	Eggs (40 wk) *	*S.* Mbandaka (6), *S.* Montevideo (1)	

*Found 2 serovars in one sample

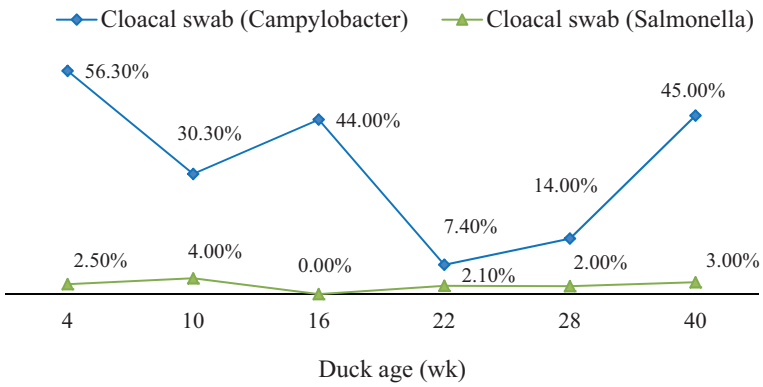

Fig. 2 Prevalence of *Campylobacter* spp. and *Salmonella* spp. from ducks in flock A

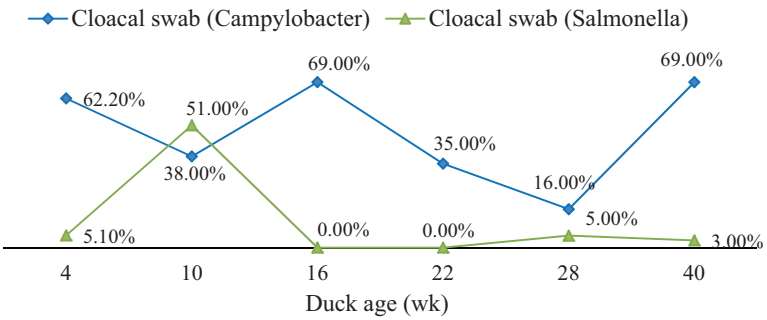

Fig. 3 Prevalence of *Campylobacter* spp. and *Salmonella* spp. from ducks in flock B

Table 4 Coexistence of *Campylobacter* and *Salmonella* in the same sample from ducks, eggs and environmental samples

Flock	Sample	Duck age (wk)	*Campylobacter*	*Salmonella*
A	Cloacal swab	4	*C. jejuni*	*S.* Enteritidis
	Soil from rice field	4	*C. jejuni*	*S.* Amsterdam
	Cloacal swab	10	*C. jejuni*	*S.* Enteritidis
	Cloacal swab	10	*C. jejuni*	*S.* Bovismorbificans
	Water from rice field	10	*C. coli*	*S.* Paratyphi B var. Java
	Feed (paddy)	16	*C. jejuni*	*S.* Stanley
	Water from rice field	22	*C. jejuni*	*S.* Dublin
	Drinking water	28	*C. jejuni*	*S.* Mbandaka
	Cloacal swab	40	*C. jejuni*	*S.* Mbandaka
	Cloacal swab	40	*C. coli*	*S.* Mbandaka
	Soil	40	*C. coli*	*S.* Orion
	Drinking water	40	*C. jejuni*	*S.* Mbandaka
	Pond water	40	*C. jejuni*	*S.* Orion
	Egg shell	40	*C. coli*	*S.* Mbandaka
	Egg shell	40	*C. coli*	*S.* Amsterdam
	Total			15/622 (2.41%)
B	Cloacal swab	4	*C. coli*	*S.* Mbanaka
	Cloacal swab	4	*C. coli*	*S.* Chester
	Watercourse	4	*C. jejuni*	*S.* Thompson
	Water from rice field	4	*C. coli*	*S.* Amsterdam
	Cloacal swab	10	*C. jejuni*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Newport
	Cloacal swab	10	*C. coli*	*S.* Newport
	Cloacal swab	10	*C. coli*	*S.* Newport
	Cloacal swab	10	*C. jejuni*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Newport
	Cloacal swab	10	*C. jejuni*	*S.* Newport
	Cloacal swab	10	*C. jejuni*	*S.* Bangkok
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. jejuni*	*S.* Newport
	Cloacal swab	10	*C. coli*	*S.* Mbandaka
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	10	*C. coli*	*S.* Mbandaka
	Cloacal swab	10	*C. jejuni*	*S.* Paratyphi B var. Java
	Cloacal swab	10	*C. coli*	*S.* Paratyphi B var. Java
	Water from rice field	10	*C. coli*	*S.* Typhimurium
	Cloacal swab	28	*C. coli*	*S.* Mbandaka
	Cloacal swab	40	*C. coli*	*S.* Mbandaka
	Cloacal swab	40	*C. jejuni*	S. Montevideo
	Total			27/640 (4.22%)

In flock A, 31.5% of all samples were positive for *Campylobacter* spp. Of those, 74.5% were *C. jejuni* and 25.5% were *C. coli*. Furthermore, *C. jejuni* was more commonly found in cloacal swabs than *C. coli* in all ages of duck. Moreover, *C. jejuni* was found in 100% of the cloacal swabs of ducks aged between 16 wk and 22 wk (Table 2). *Campylobacter* spp. were detected from only two samples of eggs and egg shells of ducks aged 22–40 wk, with both being *C. coli* and isolated from egg shells of ducks aged 40 wk. Water for drinking and general use by the ducks was considered as one of sources of the spread of *Campylobacter* spp. to other ducks. *C. coli* was found in water from rice fields with ducks aged 10 wk, *C. jejuni* was found in water from rice fields with ducks aged 22 wk, in watercourses with ducks aged 28 wk and in watercourses and general water for living with ducks aged 40 wk. These results implied that *Campylobacter* spp. could, to some extent, survive in the environment and could have been spread from domestic or wild birds living in that area. The study of Pitkänen (2013) revealed the relationship among agricultural areas, populous water birds and *Campylobacter* existence. A natural water source, therefore, serves as a reservoir for *Campylobacter* and a shedding site for local birds. Correspondingly, the survey of *Campylobacter*

spp. in water used by domestic and wild ducks in Alberta, Canada, found that 26.6% of the samples were positive for *Campylobacter* spp., 60% for *C. jejuni*, 28% for *C. coli* and 12% for other species. Moreover, samples collected from duck feces in the same area had 42.1% positive for *Campylobacter* spp., composed of *C. jejuni* (81.25%), *C. coli* (12.5%) and other species (6.25%). These results demonstrated the relationship between *Campylobacter* spp. detected from fecal and water samples (Jokinen et al., 2011), indicating the risk of spreading *Campylobacter* spp. to the broader duck environment. Contamination of *Campylobacter* spp. was also detected in soils from the duck houses, especially from age 28 wk onward, as they were kept in the houses until the end of the laying period. There was extensive contamination of *Campylobacter* spp. in duck feces, since there was little cleaning in the houses during this period. In the ducks aged 16 wk, contamination of *C. jejuni* was found in paddy used as a supplementary area for the ducks. Consequently, the tendency of contamination between ducks and environment was clear, especially in the feed and water. Once the ducks had contaminated their feed and water with *Campylobacter* spp., these species were then dispersed to the environment so that ducks in each flock acquired *Campylobacter* without clinical signs through a rearing cycle.

In flock B, 46.3% of all samples were positive for *Campylobacter* spp with *C. jejuni* (69.3%) and *C. coli* (30.7%). Of those, *C. jejuni* was highest from cloacal swab samples of ducks aged 16 wk (98.6%) and 22 wk (97.1%) as shown in Table 2. *Campylobacter* spp. were not detected in any egg and egg shell samples from growing ducks at any age. *Campylobacter* spp. were not found in any soil and feed samples, but were found in the drinking water and in pond water used by the ducks. There was evidence of contamination of *Campylobacter* spp. in water from the environment for all duck ages. In the ducks aged 4 wk, both *C. jejuni* and *C. coli* were found in water from the rice field. In ducks aged 10 wk, *C. coli* was found in water from the rice field. In ducks aged 16 wk and 22 wk, *C. jejuni* was found in water from the rice field. In ducks aged 28 wk, *C. coli* was detected from water in watercourses, while for the ducks aged 40 wk, *C. coli* was found in canals where these ducks swam.

The present study found *Campylobacter* spp. from cloacal swab samples from ducks of all ages in the range 7.4–69.0%. Tsai and Hsiang (2005) 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. Moreover, Colles et al., (2011) studied domesticated and wild ducks in the United Kingdom and found *Campylobacter* spp. in the feces of domesticated ducks aged 25–56 d (93.3–100.0%), but only 9.2–52.2% in the feces of wild ducks. This indicated that intensive farming requires good management to reduce the spread of *Campylobacter* spp. to other ducks and the environment.

The results indicated that 6.8% of the samples were positive for *Salmonella* spp. in flock A. The highest *Salmonella* population in ducks aged 22–40 wk was for *S.* Mbandaka (40.5%), especially between 28 wk and 40 wk. Moreover, *S.* Mbandaka was found in all samples from cloacal swabs and the environmental samples, such as drinking water, laying tray, eggs and egg shells. This indicated both horizontal and longitudinal transmissions. Longitudinal contamination was found in ducks aged 22–40 wk; the contamination to egg and egg shells was found in ducks aged 28–40 wk. Furthermore, the present study found *S.* Enteritidis in a cloacal swab sample from two ducks aged 4 wk and 10 wk, respectively. Two cloacal samples of *S.* Typhimurium were individually found in ducks aged 10 wk and 22 wk, respectively, as shown in Table 3. Both *S.* Enteritidis and *S.* Typhimurium have been globally considered the first two pathogenic serovars in human which are mostly found in egg or egg products from food animals (European Food Safety Authority and European Centre for Disease Prevention and Control, 2015). For this reason, eggs from ducks in the present study might transmit both serovars of *Salmonella* spp. to consumers.

In flock B, 13.6% of samples were positive for *Salmonella* spp., with most being *S.* Typhimurium (24.1%). In addition, *S.* Typhimurium was found only in ducks aged 10 wk. Furthermore, cloacal swab examination of those ducks revealed 10 serovars of *Salmonella*. *S.* Typhimurium and *S.* Newport were the most two serovars detected from the samples from ducks aged 10 wk, as shown in Table 3. *S.* Typhimurium was a common serovar between cloacal swabs and environmental samples (paddy and water). In the present study, *S.* Paratyphi B var. Java was detected using both cloacal swabs and soil samples from the rice fields, showing that this contamination might transmit such a bacterium to humans because paddy and water are a common part of everyday rural life. The water also contained nutrients and other factors supporting the growth of bacteria to disperse along public waterways. Moreover, *S.* Typhimurium has been considered one of the most important serovars impairing human health worldwide (European Food Safety Authority and European Centre for Disease Prevention and Control, 2015).

In addition to *S.* Typhimurium, *S.* Mbandaka was another outstanding serovar (20.7%). Longitudinal contamination was found in cloacal swab samples of ducks aged 4 wk, 10 wk, 28 wk and 40 wk, as well as in eggs and egg shells of ducks aged between 28 wk and 40 wk as shown in Table 3. Thus *S.* Mbandaka could be found in almost all ages of ducks, implying that *S.* Mbandaka could have become well-adjusted to living in the gastrointestinal tract of ducks and had high resistance to the environment outside the duck's body. In addition, the consumption of eggs contaminated with *S.* Mbandaka might affect human health, especially where the eggs were consumed raw or even when contaminated and then cooked.

Campylobacter spp. and *Salmonella* spp., which have been considered pathogenic bacteria in humans, were found in ducks raised both in rice fields and houses at any age. Those bacteria could be detected in the duck carcass, eggs and the environment at almost all duck ages sampled. To control these bacteria, duckling selection should be conducted from parent stock without *Campylobacter* spp. and *Salmonella* spp.. Moreover, biosecurity according to farm standardization issued by the Ministry of Agriculture and Cooperatives should be strictly applied to reduce the risk of contamination by pathogenic bacteria and the incidence of diarrhea in humans.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The present study was financially supported by the Center of Duck Health Science, Kasetsart University, Bangkok, Thailand and the Thailand Research Fund.

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