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Research Note

Occurrence of *Campylobacter* and *Salmonella* in ducks and duck eggs in Selangor, Malaysia

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Abstract. The importance of *Campylobacter* and *Salmonella* as foodborne pathogens is well recognised globally. A recent work in Penang found ducks in commercial farms were infected with these organisms. The aim of the study was to detect the presence of *Campylobacter* and *Salmonella* in ducks and *Salmonella* in duck eggs in farms in a small part of Selangor. Cloacal swabs were obtained from 75 ducks and 30 duck eggs from three farms. The isolation and identification of *Campylobacter* and *Salmonella* were done using conventional methods. Twelve percent of *Campylobacter* and 16.0% of *Salmonella* were isolated from the ducks sampled. *Salmonella* was absent on and in eggs. *Campylobacter* isolates consisted of 22% *Campylobacter jejuni* and the remaining was *Campylobacter coli*. Three *Salmonella* serovars identified were *Salmonella* Agona, *S.* Braenderup and *S.* Corvallis. The presence of *Campylobacter* and *Salmonella* in ducks may cause contamination of the meat during processing and handling which can constitute public health hazard. Moreover, the farm workers may be exposed to the organisms through contact with the infected animals.

Campylobacter and *Salmonella* are among foodborne pathogens commonly reported in poultry and poultry meat. Both the organisms are associated with causing gastroenteritis in humans worldwide, with *Campylobacter* the leading cause in industrialised countries while *Salmonella* are mostly encountered as the causative agent in industrialised and developing countries.

Countless studies reported human campylobacteriosis and salmonellosis are frequently due to consumption of poultry and poultry products as the main risk factor; other foods involved include consuming raw or unpasteurised milk, drinking untreated drinking water and handling of animals and pets may also be sources of infections in humans (Wingstrand *et al*., 2006). In several occasions, eggs and egg products have also been reported as sources of human infections with *Salmonella* Enteritidis (Martelli & Davies, 2012).

Poultry industries are at the forefront in Malaysia in meeting the demand of the country's populations. Poultry, especially chicken meat and eggs are popular and considered inexpensive, and they also fulfil the preference of all ethnic groups without religious restriction. Malaysia is reported as the third largest producer of duck after China and France and duck production has increased substantially in the last decade (Adzitey *et al*., 2012a). Duck meat is popular among the Malaysian Chinese. Duck eggs are often prepared as "century eggs" and those preserved in brine as salted eggs are popularly eaten with rice dishes. The studies on *Campylobacter* and *Salmonella* in chickens in Malaysia are plenty, however

very few in ducks. A recent study by Adzitey *et al.* (2012a,b) reported the occurrences of these organisms in ducks in farms and their processing places in Penang. Thus, the objective of this study was to isolate *Campylobacter* and *Salmonella* from ducks in farms and *Salmonella* from duck eggs purchased in a small part of Selangor.

The samples consisted of cloacal swabs from 75 ducks in three farms in Selangor. Thirty eggs were purchased from the farms. For *Campylobacter* isolation, each cloacal swab was placed in a bottle containing Cary Blair (Oxoid) as a transport medium. For *Salmonella* isolation, each swab was placed in a bottle containing Buffered Peptone Water (BPW) (Oxoid) as pre-enrichment medium. All bottles containing the cloacal swabs were placed in an ice box packed with ice and transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. All samples were analysed within 3-4 hours.

The isolations of *Campylobacter* and *Salmonella* were described previously (Choo *et al.*, 2011). Briefly for *Campylobacter* isolation from ducks: each swab was streaked directly onto the surface of *Campylobacter* Blood – Selective Agar (a modified charcoal cefoperazone deoxycholate agar or CCDA Medium) (Oxoid) supplemented with CCDA Selective Supplement (Oxoid). All plates were incubated at 42°C for 48 h under microaerophilic condition; this condition is generated in an anaerobic jar using a gas generating pack (CampyGen, Oxoid). Suspected *Campylobacter* colonies were subjected to hanging drop examination to observe motility and gram staining for cellular morphology examination. *Campylobacter* showed typical cockscrew movement and had a spiral, curved, S-shaped or gull-wing shaped. Two to three of suspected *Campylobacter* colonies were subcultured onto Columbia Blood Agar (Oxoid) supplemented with 5% defribinated horse blood; all plates were incubated at 37°C for 24 h microaerobically. Biochemical tests were used to identify and speciate *Campylobacter*, which include catalase, oxidase, urease, indoxyl acetate hydrolysis and hippurate hydrolysis tests.

For *Salmonella* isolation from ducks: the bottles containing swabs in BPW were incubated aerobically at 37°C for 24 h. One ml of each pre-enriched culture were transferred into Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid) and incubated aerobically at 42°C for 48 h. Then, two to three loopfuls of each enriched broth culture was streaked onto the surface of a selective medium, Xylose Lysine Tergitol 4 Agar (XLT-4) (Merck), then all plates were incubated at 37°C for 24 to 48 h.

The isolation of *Salmonella* from ducks' eggs followed the method described by Hassan *et al.* (2005). Briefly the shell of each egg was swabbed with a sterile swab moistened with BPW, then the swab was placed in a bottle containing BPW and was cultured for *Salmonella* isolation as described above. The shell of the entire egg was cleaned and then immersed in 70% alcohol solution for 1 min and dried before it was cracked open aseptically using a sterile forceps. The whole content of an egg was placed in a sterile stomacher bag and homogenized. One ml of the egg homogenate was pipetted out and placed in a bottle containing BPW and cultured for *Salmonella* isolation as described above.

Typical colonies of *Salmonella* appeared as pink to red colonies with black centres. Two to three colonies were picked and subcultured to obtain pure cultures; biochemical tests which included urease test and inoculation onto Triple Sugar Iron (TSI) agar (Oxoid) and Lysine Iron Agar (LIA) (Oxoid) were performed to identify *Salmonella*. The presumptive *Salmonella* isolates were then subjected to slide agglutination test (SAT) using *Salmonella* agglutinating antisera (Serotest) to confirm at genus level. All *Salmonella* isolates were cultured on nutrient agar slants and sent to Veterinary Research institute (VRI), the country National Reference Laboratory for veterinary isolates, for serotyping. The serotyping in VRI is done according to the Kauffmann-White Classification Scheme.

Overall, *Campylobacter* was isolated from 12% (9/75) ducks and of these isolates 22% were *Campylobacter jejuni* and the remaining were *Campylobacter coli*.

Salmonella was isolated from 16% (12/75) of ducks from two farms; none of the eggs were positive for *Salmonella*. Three *Salmonella* serovars were identified, *S.* Agona (16.7%), *S.* Braenderup (50.0%) and *S.* Corvallis (33.3%). The farm with the least occurrence of *Campylobacter* (6.6%) and negative (0%) for *Salmonella* was observed to have dry floor and clean environment compared to the other farms with rather wet and muddy floors.

The on-farm occurrence of *Campylobacter* among domestic ducks ranged from 2.5% to 60% and in one study found 100% colonization, while 9.2 to 52.2% was observed among wild ducks (Colles *et al.*, 2011). The studies by Tran *et al.* (2004) in Vietnam, Pan *et al.* (2010) in China and Tsai & Hsiang (2004) in Taiwan reported the occurrence of *Campylobacter* in ducks at 8.7%, 5.3% and 43.5% respectively. In a previous study in different farms in Selangor, Lim (1996) found 45% of ducks carried campylobacters, ranging from 18.4 to 75.0% and Adzitey *et al.* (2012b) detected 7% in cloacal swabs from ducks at the farms in Penang with a higher rate of 62% in intestinal contents and 85% in caecal contents from ducks at wet markets. Adzitey *et al.* (2012a) isolated 39% salmonellae from ducks in the farms and 20% and 28% from cloaca swabs and intestinal contents respectively from ducks at wet markets. At wet markets, ducks are slaughtered, dressed and eviscerated before they are sold to consumers.

The occurrence of *Campylobacter* and *Salmonella* in poultry at the farms may be due to a number of potential sources, which include old litter, untreated drinking water, presence of other farm animals, pet animals and wildlife animals on the farms, insects, equipment, transport vehicles and farm workers (Sahin *et al.*, 2002). Flying birds and houseflies have been shown to carry *Campylobacter* and *Salmonella* which should be considered as potential risks for the spread of the organisms to the flocks and the environment (Choo *et al.*, 2011; Sahin *et al.*, 2002; Saleha *et al.*, 2001). Lim (1996) had noted that in farms with muddy ground and dirty pond for ducks to waddle was dirty and had suggested it could have probably led to the high occurrence of *Campylobacter* in the farms compared to the others which had drier sandy ground.

During processing and handling, duck carcases and meat may become contaminated. Studies reported 50.7% of duck meat in UK, 6–36% in Egypt, 31% in Thailand and 45.8% in Ireland were contaminated with *Campylobacter* (Colles *et al.*, 2011). Similarly, processing practices and handling of *Salmonella*-infected ducks may cause contamination of carcasses and meat with the organisms. Adzitey *et al.* (2012a) showed 10% of the duck carcass rinse was positive for *Salmonella* in Penang, Malaysia.

In poultry, *C. jejuni* is said to be the most predominant species as reported in most studies; however in this study, *C. coli* was more frequently isolated. *Salmonella* Typhimurium is the predominant serovar in the UK (Martelli & Davies, 2012), Penang, Malaysia (Adzitey *et al.*, 2012a), Eastern China (Pan *et al.*, 2010) and Mekong Delta, Vietnam (Tran *et al.*, 2004); in Taiwan, S. Potsdam (Tsai & Hsiang, 2004) and in this study, *S.* Braenderup were frequently isolated.

Although two studies showed the presence of *Campylobacter* in 1% of eggs of colonised hens, several studies failed to isolate the organisms from eggs (Newell & Fearnley, 2003). Hassan *et al.* (2005) found *Salmonella* sp. in 7.5% and 10.2% of the contents of eggs from commercial layer chickens and free-range chickens respectively and 2.6% of the shell of eggs of free-range chickens while Loongyai *et al.* (2011) detected the presence of salmonellae in 5% of chicken egg shells but none in egg contents. The study by Adzitey *et al.* (2012a,b) reported the shells of duck eggs (n=10) were negative for the presence of salmonellae and campylobacters; this study too found no salmonellae on and in duck eggs.

The presence of *Campylobacter* and *Salmonella* in ducks which were apparently healthy showed that ducks can act as reservoirs for these organisms and spread them in the environment. Their presence in the animals may also lead to the contamination of the carcases and meat which are of public health significance. Kessel *et al.* (2001) reported that 2% of

foodborne infections in England and Wales in 1992 – 1999 was associated with the consumption of duck meat.

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