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Multivariate logistic regression analysis of risk factors for *Salmonella* spp. among ducks in selected villages in Baybay City, Leyte, Philippines

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ABSTRACT

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Salmonella contamination in poultry and poultry products is a threat to public health and food safety. This study aimed to assess the carriage rate and the associated risk factors of *Salmonella* spp. among ducks in selected villages in Baybay City, Leyte, Philippines. *Salmonella* was identified using conventional culture methods. On-farm survey interviews were carried out with 400 duck farmers to generate information on the risk factors associated with *Salmonella* spp. Univariate analysis was performed to screen potential risk factors and multivariate logistic regression analysis to identify significant risk factors. The overall carriage rate of *Salmonella* in ducks was 22.75% ± 4.11. Multivariate logistic regression analysis identified the incorporation of snails into the diet (OR=5.212; 95% CI:1.374 to 19.765) and sources of water from rivers (OR=2.823; 95% CI:1.273 to 6.264) and ponds (OR=6.413; 95% CI:2.827 to 14.550) as significantly associated with *Salmonella* spp. in ducks in the sampled farms. The use of antibiotics (OR=0.022; 95% CI:0.003 to 0.196) and flooding in the rearing area (OR=0.485; 95% CI:0.242 to 0.971) indicated lower chances of *Salmonella* infection. The data suggested that *Salmonella* spp. does infect ducks in the target population. It is recommended that randomized field testing be carried out to validate these findings.

Keywords: carriage rate, demographics, farm management, odds ratio

INTRODUCTION

For more than two decades, *Salmonella* spp. remain the leading microbiological cause of foodborne disease outbreaks in the Philippines (Azanza 2006, Azanza et al

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2019). *Salmonella* can be found in various foods of both animal and plant origin (CDC 2020). In particular, contaminated poultry products (eggs and meat) have been considered the main vehicles of *Salmonella* infection that are clearly associated with human outbreaks (Antunes et al 2016). *Salmonella* is a group of gram-negative bacteria that causes food poisoning in humans (Rodriguez et al 2015). There are currently more than 2500 serotypes distributed around the world (Gong et al 2014) with non-typhoidal *Salmonella* as the leading cause to about 93.8 million illnesses and 155,000 deaths each year worldwide (Majowicz et al 2006).

Duck raising is a lucrative livestock industry in the Philippines due to high demand for balut (boiled incubated egg) and salted egg production (DA-BAR 2012). Duck inventory in 2019 had expanded by 3% (Bautista 2019) as government programs promoted raising of native ducks to uplift farmers from poverty and promote the localization of the food supply (Sinon 2019). With ducks becoming a popular alternative source of meat and eggs in developing countries, it is imperative to look into the quality and safety of these products. According to Chang and Dagaas (2004), one of the issues of the duck industry in the Philippines is the lack of product standards that may compromise food safety. In 2014, for example, a foodborne outbreak in Pangasinan was attributed to salted eggs suspected to be contaminated with *Salmonella* spp. (Azanza et al 2019). The traditional methods of raising ducks ie, free-ranging and foraging around rivers, ponds, lakes, and rice fields expose ducks to an array of microbial contaminants from these environments. With minimal attention given to biosecurity, ducks raised in the field or open houses may shed enteric pathogens such as *Salmonella* that may be acquired by other species including humans. *Salmonella* infection in poultry can be less apparent and may only be seen as pasting of the vent but the organism is already being shed in the feces (Demirbilek 2018). Because of this, it is believed that the incidence of human infection arising from the consumption of duck meat and eggs is likely to be much greater, than human infections arising from chickens (Barrow et al 1999). Outbreaks of human salmonellosis due to contact with ducks have been reported in Australia, United States, United Kingdom, and Denmark as well as in Italy and Thailand due to consumption of contaminated duck eggs (Ribeiro et al 2006). Data similar to this are limited in the Philippines and very few epidemiological studies have been carried out to investigate the risk factors associated with ducks. In Baybay City, duck farming is one of the means of livelihood for the agricultural populace. We surveyed duck farms in 11 barangays of Baybay City to assess the carriage rate and risk factors associated with *Salmonella* spp. One limitation of the study is the non-probability sampling methodology; thus, the findings are deemed appropriate for the sample population. Nonetheless, the information could help describe the role that each risk factor plays in the infection cycle of *Salmonella* and could be an essential consideration in improving farm management and food safety.

MATERIALS AND METHODS

Study Area and Target Population

The study was carried out in Baybay City, Leyte, a coastal component city situated at approximately 10°41' North, 124°48' East, on the island of Leyte

(PhilAtlas 2020). It has a mountainous range in the eastern portion as it slopes down west towards the shore line. The climate is generally wet due to evenly distribution of rainfall throughout the year. The target population comprised of ducks raised in 11 *barangays* (villages) with duck farms based on the records of the Baybay City Agriculturist Office (Figure 1).

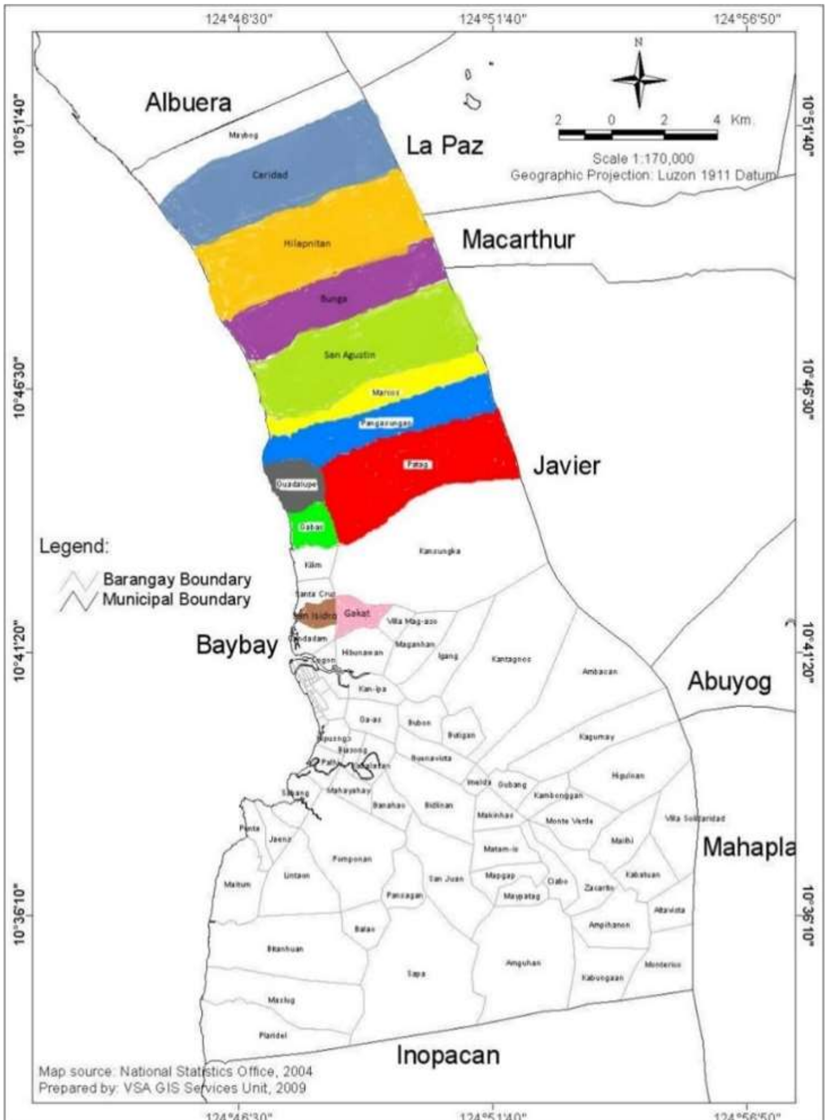

Figure 1. Map of Baybay City, Leyte showing the 11 study areas

Study Design and Sampling Method

The study was a cross-sectional approach to measure the carriage rate of *Salmonella* spp. in ducks. The targeted sample size (384 adjusted to 400) was calculated based on a 50% prevalence from unknown population at 95% confidence interval by Martin et al (1998). Survey interview using semi-structured questionnaires was conducted to gather the farm information, farmer's demographic background, duck information, and farm management practices. Informed consents were obtained from the respondents prior to the administration of on-farm interview that was delivered in Cebuano or English language. Purposive sampling was employed in the selection of survey respondents to include duck farmers who can and were willing to provide the needed information on account of their knowledge and experiences (Bernard 2002 as cited by Tongco 2007, Robinson 2014). In addition, the accessibility of farms that raised ducks near rice fields was also considered to ensure the safety of the researchers. To evaluate more farms in each barangay, only one duck was sampled per farm. Flock infection was established when at least one duck is found positive to *Salmonella* (Betancor et al 2010). Fecal swab samples were collected by holding the duck by its body with wings folded and inserting a swab into the vent. Swabs were placed into individual transport medium and transported in an ice bucket to the Microbiology Laboratory of the College of Veterinary Medicine, Visayas State University, Baybay City, Leyte for bacteriological examination.

Salmonella Isolation and Identification

Salmonella spp. were isolated using the conventional culture method described in ISO-6579: 2002 standard (ISO 2004) with minor modifications. This involved culture of samples in pre-enrichment and enrichment media, and plating on selective media. Briefly, swab samples were inoculated in buffered peptone water (Oxoid, Basingstoke, UK) and incubated for 18-24h at 37°C, enriched in selenite broth (Oxoid, Basingstoke, UK) and incubated for 48h at 42°C and streaked on xylose-lysine-deoxycholate agar (Oxoid, Basingstoke, UK) and incubated for 18-24h at 37°C. Pink colonies with black centers on XLD agar were selected for further screening. A thin film of bacterial smear was produced from the isolated colony and gram-stained to determine the homogeneity of the bacteria. Putative *Salmonella* colonies were then confirmed through observation of characteristic growth and reaction in Mac Conkey Agar, Triple Sugar Iron Agar, Urease Broth, Lysine Decarboxylase, Ornithine Decarboxylase, and Indole.

Statistical Analysis

Data from the survey were tabulated in Microsoft Excel and analyzed in Epi Info version 3.3.2. The carriage rate which is the total number of positive ducks divided by total number of ducks examined was computed at 95% confidence interval. Independence of categorical variables was first tested using Chi-square test or univariate logistic regression analysis to explore the potential risk factors ($p \le 0.20$). Multivariate logistic regression analysis was done using backward-elimination where only the variables with $p \le 0.05$ were considered significantly associated with *Salmonella* spp. All analyses were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Demographic Characteristics of Duck Farmers

Ducks raised in the 11 barangays of Baybay City were owned by farmers with an age range between 32-76 years old (mean=54.19). The majority of the interviewed farmers were male (84.75%), married (91.25%) and had finished or reached at least secondary education (63.0%). Ducks were primarily raised as sources of meat and eggs (52.75%) of which, eggs were processed for *balut,* fertile egg, (44.75%) and salted egg production (1.25%). Ducks were acquired through purchase from known suppliers (51.50%), although some farmers were also engaged in contract growing (35.25%).

Characteristics of Ducks Raised and Description of Farm Management Practices

The number of ducks raised by farmers ranged from 12-620 (mean=101.51). Of the 400 ducks sampled, 299 (74.75%) were Mallard and 101 (25.25%) were Muscovy. Most of the ducks sampled were female (89.75%) and above six months of age (85.25%). They were kept in a semi-confinement shelter (93.25%) with shed-type roofing (50.25%) and, since the ducks were let loose, without any litter on the floor (83.0%). Ducks were raised with access to other animals (44.75%), with dogs (83%) as the most predominant animal. Cats (25%), ruminants (7%) and other animals (chicken, geese and pigs, 28.25%) also had farm access. Most of the ducks were pastured daily (92%) from morning until 4:00 o'clock in the afternoon, preferably in the rice fields (97.5%). Ducks in the farms were of variable ages and culled stocks were replaced once every two years (74.0%).

Ducks were given wet method (94.25%) of feeding with a diet consisting of 'kuhol' or snail (*Pomacea* spp.) (84.0%), *palay*, unmilled rice, (45.0%), rice bran (32.75%), commercial feeds (27.5%), and others (*sapal,* grated coconut residue, and '*gabi*', taro) (15.25%). Drinking water was obtained from the river (64.5%), tap water supplied by the City Water District (28.25%), pond (22.25%), and wells (1.25%). A handful of farmers (16%) rarely, if at all, provided vitamin supplements, to ducklings (5.75%) and growers (5.75%) using commercially available vitamin-mineral supplements fortified with antibiotics. Less than 50% (41.50%) of the farmers encountered disease occurrence in ducks with cramps and diarrhea being the most common manifestations. The majority of the farmers (81.75%) used no preventive measures, but a few practiced vaccinations (4.50%) or resorted to antibiotics (10.5%) to treat disease. Manure was rarely used as fertilizer (1.25%) but more often disposed of into vacant lots (62.25%) or bodies of water (24.95%). Flooding, however, was observed in more than 50 percent of the areas (69%) where ducks were raised.

Isolation of Salmonella spp.

Salmonella spp. were initially identified by their typical red, transparent, yellow edges with black center colonies on XLD agar (Figure 2a) and pale colonies in MacConkey agar.

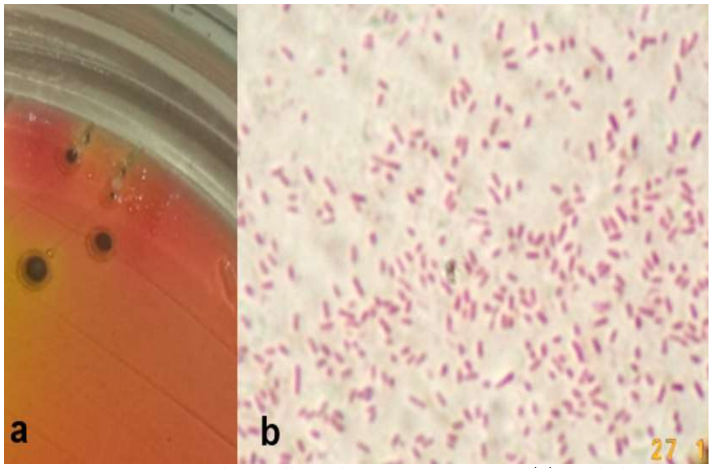

Figure 2. Putative *Salmonella* colony on XLD agar (a) and the gram reaction of the colonies (b)

Isolates had negative reaction to indole and urease tests, positive reaction to lysine decarboxylase and variable to ornithine decarboxylase, and growth reaction (alkaline/acid with H_2S production) in Triple Sugar Iron Agar. Gram staining revealed pink colored coccobacilli (Figure 2b).

Salmonella Carriage

The potential of foodborne pathogens like *Salmonella* to cause illness or even death in consumers underlines its importance and consequent need for monitoring and prevention (Schirone et al 2019). Complementary information obtained from prevalence studies are therefore needed to assess the economic impact of an intervention before it is implemented in a group or population (Harder 2014).

Table 1 shows the carriage rate of *Salmonella* spp. from the fecal swabs of ducks in the selected barangays in Baybay City. The data showed that regardless of origin, *Salmonella* spp. were isolated in ducks with an overall carriage rate of 22.75%±4.11 (91/400). The proportion of ducks positive per source was variable and ranged from 3% to 100% with farms in Caridad having the highest carriage (100.00; 95% CI:61.00-100.00). The difference was not statistically significant due to the nature of nonprobability sampling. The inherent bias of purposive sampling, however, contributed to its efficiency, particularly in the synthesis of qualitative evidence where knowledgeable farmers shared what they knew and had experienced in duck raising (Tongco 2007, Ames et al 2019). Nonetheless, the method provided robust information about the extent of *Salmonella* contamination in ducks among the sampled population.

The carriage rate of 22.75%±4.11 was close to the 25% prevalence of *Salmonella* in duck meat in the UK between 2003 and 2005 (Cha et al 2013), and 23.5% among ducks in Penang, Malaysia (Adzitey et al 2012). These findings clearly demonstrate that ducks could be potential sources of *Salmonella* and monitoring should be implemented to prevent its spread into the food chain. Variations in the occurrences of *Salmonella* may be due to the differences in the sample size and geographic location. Ducks could act as natural reservoir of various *Salmonella*, such as *S. gallinarum*, *S. pullorum*, *S. typhimurium*, *S. enteritidis,* and *S. anatum* (Barrow et al 1999), without exhibiting clinical signs (Zhang et al 2019). This asymptomatic state poses a serious concern for environmental contamination as the pathogen is continually shed via the feces and triggers rapid horizontal spread among flocks (Barrow et al 1999, Ribeiro et al 2006).

Table 1. Carriage rate of *Salmonella* spp. in ducks from the 11 the barangays of Baybay City

Barangay	Number of cloacal swabs collected (N)	Number of cloacal swabs positive for *Salmonella* spp.	Carriage rate (%, at 95% CI)
Marcos	72	27	37.50 (27.22-49.05)
Gabas	13	4	30.77 (12.68-57.63)
Pangasugan	34	5	14.71 (6.45-30.13)
Guadalupe	32	8	25.00 (13.25-42.11)
Patag	45	8	17.78 (9.29-31.33)
Bunga	30	1	3.33 (0.59-16.67)
Hilapnitan	10	2	20.00 (5.67-50.98)
San Agustin	5	1	20.00 (3.62-62.45)
Caridad	6	6	100.00 (61.00-100.00)
San Isidro	113	20	17.70 (11.76-25.76)
Gacat	40	9	22.50 (12.32-37.50)
Total	400	91	

CI, confidence interval

Univariate Analysis

In the univariate analysis of farm data, 17 independent variables were identified as potential risk factors ($p \leq 0.20$) for *Salmonella* spp. in ducks (Table 2). These variables were elementary education of the farmer, source of meat and eggs as purpose of raising, ducks purchased from known source, mallard breed, replacement once every two years, age of stocks replaced is less than 2 months, litter flooring, access of other animals to ducks, dogs raised with ducks, flooding in the duck rearing area, use of antibiotics, practice of manure disposal, incorporation of snail in the diet, river and pond as sources of water, daily pasturing of ducks, and rice field as pasture area. These factors were further analyzed in multivariate logistic regression analysis (MLRA) to establish their significant association.

Table 2. Univariate analysis of risk factors associated with *Salmonella*

Variable	Category	Number of Positive Samples (%)	*p*-value
Farmer's education	Elementary level	42 (10.50)	0.0008
	Above elementary level	49 (12.25)	
Purpose of raising ducks	As source of meat and eggs	34 (8.50)	0.0013

Table 2. continued

Variable	Category	Number of Positive Samples (%)	*p*-value
	Other purposes	57 (14.25)	
Source of ducks	Purchased from known source	35 (8.75)	0.0067
	Other sources	56 (14.00)	
Breed	Mallard	77 (19.25)	0.0200
	Muscovy	14 (3.50)	
Frequency of replacement	Once every two years	74 (18.50)	0.0939
	Other frequency of replacement	17 (4.25)	
Age of stock replaced	Less than 2 months	65 (16.25)	0.0002
	Older than 2 months	26 (6.50)	
Type of flooring	Litter	10 (2.50)	0.1146
	Soil	81 (20.25)	
Access of other animals to ducks	Yes	28 (7.00)	0.0023
	No	63 (15.75)	
Animal raised with ducks	Dogs	81 (20.25)	0.1146
	Other animals	10 (2.50)	
Flooding in duck rearing area	Yes	17 (4.25)	0.0057
	No	74 (18.50)	
Use of antibiotics	Yes	4 (1.00)	0.0307
	No	87 (21.75)	
Practice manure disposal	Yes	89 (22.25)	0.1163
	No	2 (0.50)	
Presence of snail in the diet	Yes	85 (21.25)	0.0087
	No	6 (1.50)	
Sources of water	River	75 (18.75)	0.0001
	Tap water	16 (4.00)	
	Pond	33 (8.25)	0.0004
	Well	58 (14.50)	
Frequency of pasturing ducks	Daily	89 (22.25)	0.0356
	Not daily	2 (0.50)	
Pasture area	Rice field	91 (22.75)	0.1751
	Other areas	0 (0.00)	

Multivariate Logistic Regression Analysis

Results of the MLRA (Table 3) identified the incorporation of snail in the diet (OR=5.212; 95% CI:1.374 to19.765), river (OR=2.823; 95% CI:1.273 to 6.264) and pond (OR=6.413; 95% CI:2.827 to 14.550) as sources of water as significantly associated with *Salmonella* spp. infection in ducks. The odds were five times greater if snails are part of the duck's diet and two to six times greater if ducks were given or had access to water coming from river or pond. However, the use of antibiotics (OR=0.022; 95% CI:0.003 to 0.196) and flooding in the rearing area (OR=0.485; 95% CI:0.242 to 0.971) were likely to protect ducks from infection.

Multivariate logistic regression analysis of risk factors for *Salmonella* spp.

Table 3. Multiple logistic regression model for *Salmonella* spp. infection in ducks

Variable		Coefficient	SE	OR (95% CI)	*p*-value
Presence of snail in the diet					
	Yes	1.651	0.680	5.212 (1.374-19.765)	0.0152
	No	-	-	1.00	-
Source of water - River					
	Yes	1.038	0.407	2.823 (1.273-6.264)	0.0107
	No	-	-	1.00	-
Source of water - Pond					
	Yes	1.858	0.418	6.413 (2.827-14.550)	0.0000
	No	-	-	1.00	-
Use of antibiotics					
	Yes	-3.814	1.115	0.022 (0.003-0.196)	0.0006
	No	-	-	1.00	-
Flooding in duck rearing area					
	Yes	-0.725	0.354	0.485 (0.242-0.971)	0.0409
	No	-	-	1.00	-

SE, standard error
OR, odds ratio

The involvement of snails (OR=5.212; 95% CI:1.374 to19.765) as a risk factor could be due to the wide range of ecosystems where these species are found including swamps, marshes and ponds where they may accumulate a diverse array of microorganisms (Serrano et al 2004). Ducks acquire snails as they forage for food off the ground surface in open water ponds and wetlands (Downs et al 2017). Inaddition, local farmers deliberately raise ducks, particularly Mallard, on the same piece of land where rice is grown because they are used as a biological control against the highly destructive rice pest, golden apple snail (*Pomacea* spp.) (Escobin et al 2008). This increases the chances of ducks to feed on snails contaminated with *Salmonella* on their shell surface or within the flesh (Andrews et al 1975). In Itu Creek, Niger Delta, Nigeria, *Salmonella typhi* was isolated from freshwater snails, *Pomacea bridgesii* and *Achatina fulica* (Adebayo-Tayo et al 2011). Previous studies showed that *Salmonella* had been isolated from 62% of *Achatina achatina* (edible land snail) in Nigeria (Obi and Nzeako 1980), 43% of *H. aspersa* in Morocco (Andrews et al 1975) and 27% of *Ampullaria* spp. (aquarium snails) in Canada and Florida (Bartlett and Trust 1976). *Salmonella* spp. were also detected in the irrigation water, especially after rainfall events (Gerba and Rock 2014).

The odds of contracting *Salmonella* spp. were two to six times greater if the ducks were given water from or had access to a river (OR=2.823; 95% CI:1.273 to 6.264) or pond (OR=6.413; 95% CI:2.827 to 14.550). This event is hard to avoid since ducks naturally spend 69% of their time in the water (Downs et al 2017) thereby predisposing them to *Salmonella* contamination. Many pathogens, *Salmonella* as one, survive and persist in the various types of natural water including river, lakes, ponds and contaminated ground water (Levantesi et al 2012). *Salmonella* continuously contaminates the aquatic environment through the direct entry of feces from infected humans and animals or indirectly via sewage discharge or agricultural land runoff. *S. newport* was isolated from pond water (Levantesi et al 2012) and *S. poona* from fields irrigated with contaminated sewage waters (Greene et al 2008). Moreover, unhygienic conditions, inappropriate water supply structure and improper disinfection are significant factors contributing to water contamination in developing countries (Akinyemi et al 2006).

Ducks treated with antibiotics were 98% (OR=0.022; 95% CI:0.003 to 0.196) protected from bacterial infection. According to the farmers, vitamin-mineral packs incorporated with doxycycline (HCl) and tiamulin (hydrogen fumarate) were commonly given to ducks during disease occurrences. The treatment was given twice a day for five days via drinking water. Antibiotics, in general, are intended to treat and prevent many of the avian diseases although they are also added in the feeds at sub-therapeutic levels to improve production. Tiamulin and doxycycline are both inhibitors of protein synthesis but bind differently in the ribosomes. It is believed that the combination of their modes of action is responsible for their synergistic effects (Islam et al 2009, Garmyn et al 2017).

The study also showed that occurrence of flooding within the vicinity of duck farms decreases the chance of *Salmonella* infection in ducks by 52% (OR=0.485; 95% CI:0.242 to 0.971). Although more evidence is needed to elucidate this effect, studies have shown that, in rice fields, flooding accelerates the decomposition of residual rice straw on the surface (Pernolett et al 2015). This process is found to be beneficial for the soil as nutrients are exuded from the decomposed straw to provide a complex network of elements that can influence the microbial community structure. In effect, there is a preponderance of bacterial classes that are able to decompose plant residues using cellulolytic enzymes (Koeck et al 2014, Pittol et al 2018). It can be speculated that a possible microbial shift takes place after flooding that reduces the number of *Salmonella* or affects its ability to thrive in the soil.

Overall, it appears that the diet preference and foraging behavior of ducks in aquatic environments impart some risks to *Salmonella* infection. Thus, the role of ducks in the transmission cycle of this foodborne zoonosis should be given high attention.

CONCLUSION AND RECOMMENDATION

The findings of this study suggest that there were some management and environmental factors that contributed to the infection and transmission of *Salmonella* in ducks. Corrective interventions should be pursued to improve the management practices of domestic waterfowl to reduce exposure to *Salmonella*. Modernizing farm practices and instituting product standards for duck derivatives may be considered to improve production yield as well as food safety. It is further recommended that a randomized field investigation of *Salmonella* infection among ducks be carried out to validate our initial findings.

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