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Isolation of *Escherichia coli* from Ducks and Duck Related Samples

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ABSTRACT

The conventional method was used to isolate *Escherichia coli* from ducks and duck related samples. The samples were obtained from a wet market and two duck farms. The average occurrence of *Escherichia coli* was 78.00% and was highest in duck faeces (87.93%), followed by duck intestines (81.25%), soil (70.83%) and wash water (50.00%) samples. The prevalence in Farm B (90.45%) was higher than in Farm A (80.33%). The occurrence of *Escherichia coli* did not differ significantly ($p>0.05$) among the samples examined. In addition, most of the isolates belongs to the serotype 0517 (82.44%) and biotype 1 (82.44%). The study indicates that ducks like other farm animals are primary reservoirs for *Escherichia coli* including potential pathogenic types and the opportunity for cross contamination and consequently foodborne poisoning or illness exists through the consumption of contaminated food.

Key words: Conventional method, ducks, wet market, farm, *Escherichia coli,* occurrence

INTRODUCTION

The Gram negative facultative anaerobe bacteria, *Escherichia coli* are widely distributed in the gastro-intestinal tract of humans, poultry, ruminants, non-ruminants, pets and wild animals, where they are known to live as commensals (WHO, 2005; Feng and Weagant, 2009). They are also members of the family Enterobacteriaceae and ferments glucose and/or lactose (Feng and Weagant, 2009; Adzitey *et al*., 2011). Though most *Escherichia coli* live as commensals with their host, a number of strains possess certain genes that produce toxins making them pathogenic and thus can cause foodborne illnesses. For instance, El Metwally *et al*. (2007) reported on the detection of a Shiga-like toxin producing strains and the presence of the pathogenic genes *stx" stx*$_2$*elt, ESAT, bfp* and *eae* in *Escherichia coli* isolated obtained from clinical, marine water, river water, food and animal sources in Malaysia. Pathogenic *Escherichia coli* strains normally cause gastroenteritis or in severe cases cause hemolytic uremic syndrome and/or haemorrhagic colitis that can be fatal (Feng and Weagant, 2009). In recent times outbreak of *E. coli* have been associated to a number of deaths (WHO, 2005). Efficient method of isolating foodborne pathogens is therefore important for clinical and epidemiological studies (Adzitey and Corry, 2011; Frederick and Huda, 2011a, b). Molecular methods are thought to be rapid and a more efficient way of isolating and characterizing foodborne pathogens. Molecular methods such as multiplex PCR, RAPD and ERIC have been used to successfully identify and genotype *Escherichia coli* isolates (Ling *et al*., 2000; Gomes *et al*., 2005; Al-Haj *et al*., 2008).

Escherichia coli have been isolated in samples such as chickens, beef, pork, turkey, mutton, chevon, rodents, bats, vegetables, drinking water and may more (Zhao *et al*., 2001; Tambekar *et al*., 2006; El-Zubeir Ibtisam *et al*., 2006; Tambekar *et al*., 2007; Apun *et al*., 2008, 2011; AL-Haj *et al*., 2007; Adzitey *et al*., 2011; Halablab *et al*., 2011). Zhao *et al*. (2001) studied the prevalence of *Escherichia coli* in a variety of meat samples. They reported a prevalence of 38.7, 19.0, 16.3 and 11.9% for chicken, beef, pork and turkey samples, respectively. In wildlife animals, the occurrence of *Escherichia coli* was 43% in rodents, 18% in birds and 11% in bats (Apun *et al*., 2011). In human blood samples collected from hospital patients, 1% was positive for *Escherichia coli* (Tambekar *et al*., 2007). *Escherichia coli* were isolated from 42.30% of lettuce and 13.80% of parsley (Halablab *et al*., 2011). In another study conducted by Tambekar *et al*. (2006) on water samples, *Escherichia coli* were isolated in open wells 51 (60), 23 (23%) in tube wells and 11 (13%) from hotels and restaurants.

The importance of ducks to humans vary from the provision of food in the form of meat and eggs to serving as a source of employment and income to those involved in duck production. Duck meats and eggs can also be exported to other countries to earn foreign income. Duck farming has been integrated with rice farming to help control water snails and to provide manure for the rice plant. It has also been integrated with fish farming to provide manure for phytoplankton which is source of food for fishes. Since, ducks are source of food to humans, the presence of foodborne pathogens are concerns for human health. Thus this study was conducted to determine the occurrence of *Escherichia coli* in ducks and duck related samples.

MATERIALS AND METHODS

Sample collection: A total of 150 duck and duck related samples were collected from a wet market and two duck farms in Penang, Malaysia within a 4-month period. The samples were duck intestines (48), wash water (water use for washing duck carcasses) (20) from a wet market and duck faeces (58), soil (24) from duck farms. The samples were stored under 4°C and transported to the laboratory where analysis was carried out immediately upon arrival for the presence of *Escherichia coli*.

Bacteriological analysis: Analysis for *Escherichia coli* was done using a modified method according to the Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM). One gram each of intestinal, faecal and soil samples were enriched in 9 mL EC broths. For wash water sample, 10 mL were enriched in 90 mL EC broth. Enriched EC broths were incubated for 24±2 h at 45.5°C. After which 10 jiL aliquots of EC broths were streaked onto Levine's eosin-methylene blue and Eosin-methylene blue agars. The plates were then and incubated for 24±2 h at 37°C. Presumptive *Escherichia coli* colonies appear as dark centered and flat, with or without metallic sheen. One to three colonies showing such characteristic nature were picked from each plate and purified on pfate count agar slants. They were identified and confirmed using Gram staining and biochemical tests such as Indole production, Voges-Proskauer (VP), Methyl red and Citrate reactions (popularly known as IMViC reaction). All media were purchased from Merck, Germany.

Statistical analysis: The data obtained were analyzed using Chi-square test for goodness of fit to determine whether significant variations existed between the samples examined for

Escherichia coli. Chi-square ($/^2$) was defined as: = $(o-e)^2/e$ where *o* is the observed result, e is the expected result and the data obtained were interpreted using Chi-square distribution table at 5% significant level (Fisher and Yates, 1963).

RESULTS AND DISCUSSION

The result for the occurrence of *Escherichia coli* in the duck and duck related samples examined are presented in

Table 1. Of the 150 samples tested, 117 (78.00%) were positive for *Escherichia coli*. The occurrence of *Escherichia coli* was highest in duck faeces 87.93% (51/58), followed by duck intestines 81.25 (39/48), soil 70.83% (17/24) and washes water 50.00% (10/20) samples. However, statistical analysis using chi-square indicated no significant difference ($p>0.05$) among the samples. It is possible that, *Escherichia coli* in soil samples might have resulted from the defaecation of birds or the pathogen may be harboring naturally in the soil. For *Escherichia coli* to be present in wash water samples it is feasible that contamination might have taken place during carcass processing. This is because portable and hot water used for carcass scalding is not known to be a major reservoir for *Escherichia coli*. *Escherichia coli* were also found more in farm B, 90.45% (19/21) than farm A, 80.33% (49/61). This suggests that farm B was more contaminated with *Escherichia coli,* compared to farm A. Moreover, the percentage *Escherichia coli* positive for both farms were higher than in the wet market (72,06%) but did not differ significantly from each other. In general the prevalence of *Escherichia coli* in the samples examined were relatively high. Thus healthy ducks like other animals may carry *Escherichia coli* in their intestines which they may share during defaecation. These pathogens can survive in the soil or faeces and cross contaminate other samples or equipments on the farm. Under poor or faulty processing conditions *Escherichia coli* can be transferred from duck intestines to wash water and other food samples.

Escherichia coli O157:H7 was determined using the ability and inability of the *Escherichia coli* isolates to ferment sorbitol. Biotype 1 gave ++- and biotype 2 gave -+-- for the IMViC reaction. Thus biotype 2 *Escherichia coli* strains were negative for indole production. Twenty three (17, 56%) of the isolates belonged to the biotype 2 and 108 (82.44%) were of the biotype 1. Similarly, out of the 131 *Escherichia coli* types observed, 82.44 and 17.56% were serotype 0517 and O517:H7, respectively. Sixteen *Escherichia coli* O517:H7 was isolated from intestinal samples, four from faeces, two from soil and one from wash water samples. The identification and differentiation of *Escherichia coli* types 0517 and O517:H7 is important because the presence of *Escherichia coli*

Table 1: Occurrence of *Escherichia coli* in ducks and duck related samples

				E. coli type		*B iotype	
Duck sample	No. of tested	No. of positive	Prevalence (%)	O157:H7	0157	Type 1	Type 2
Wet market							
Intestines	48.00	39.00	81.25	16.00	37.00	37.00	16.00
Wash water	20.00	10.00	50.00	1.00	9.00	9.00	1.00
Farm A							
Faeces	46.00	40.00	86.96	4.00	36.00	37.00	3.00
Soil	15.00	9.00	60.00	1.00	8.00	8.00	1.00
FarmB							
Faeces	12.00	11.00	91.67	0.00	11.00	10.00	1.00
Soil	9.00	8.00	88.89	1.00	7.00	7.00	1.00
Overall	150.00	117.00	78.00	23.00	108.00	108.00	23.00

* Number of positive isolates

O517:H7 suggests that pathogenic or diarrheagenic *Escherichia coli* may be present in the ducks we sampled. Pathogenic *Escherichia coli* O517:H7 are threats to public health and Feng and Weagant (2009) indicated that, the analysis for pathogenic *Escherichia coli* requires that the isolates should first be identified as *Escherichia coli* before testing for their virulence markers. The pathogenic groups includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), entero aggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and others that are not yet well characterized; and O157:H7 is the prototypic EHEC most often implicated in illness worldwide (Nataro and Kaper, 1998; WHO, 2005; Feng and Weagant, 2009).

CONCLUSION

In general, the prevalence of *Escherichia coli* in the samples analyzed was relatively very high. It ranged from

50.00 to 88.89%. Most of the *Escherichia coli* isolates were of the type 0157 which are usually non-pathogenic and belong to the biotype 1. Duck faecal samples had the highest occurrence for *Escherichia coli* while wash water samples showed the least contaminated. There is the potential for contamination and cross contamination of *Escherichia coli* from ducks to farming and processing equipments and to other food samples.

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