DETECTION OF CAMPYLOBACTER AND SALMONELLA IN OSTRICH

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SUMMARY

Three ostrich show farms were visited to detect the presence of Campylobacter and Salmonella in the birds. Cloacal and skin swabs were taken from 31 ostriches which were clinically healthy. Campylobacter was isolated from one (1.6%) cloacal swab and Salmonella from two (3.2%) samples, a skin and a cloacal swabs; all were from different birds. Although the detection of the organisms was low, it could pose public health risk partly because of contact with the birds and the organisms in the faeces may contaminate the environment and it could be that they may be present in other birds but were not shed at the time of sampling.

Keywords: Salmonella, Campylobacter, ostriches

INTRODUCTION

Campylobacter and Salmonella are among the most important zoonotic pathogens causing acute gastroenteritis worldwide, particularly in industrialized countries (Cuomo et al, 2007). According to literature, Campylobacter species, primarily Campylobacter jejuni and C. coli, are reported to be commensals in many mammals and avian species which included chickens, ducks, turkeys, geese, ostriches and wild birds. Also, many animals in particular reptiles and birds are reported asymptomatic carriers of non-typhoidal Salmonella. These two organisms have been isolated in many outbreaks of campylobacteriosis and salmonellosis in humans with poultry meat and poultry products commonly implicated as the sources of the organisms.

Many studies have been carried out on the occurrence of Campylobacter and Salmonella in livestock and their products, pet and zoo animals and in wild birds; however, very little is known on the occurrence of the organisms in ostrich. Ostriches have been farmed since the middle of the nineteenth century, firstly in South Africa and subsequently in other countries for the principal purpose, until recently, for their feathers for use in the fashion industry and for industrial and household cleaning equipments (Gill et al., 2007). As the industry expands, more products have been produced from ostriches which include the hide (leather go and their products, pet and zoo animals, and in wild birds; however, very little is known on the occurrence of the organisms in ostrich. Ostriches have been farmed since the middle of the nineteenth century, firstly in South Africa and subsequently in other countries for the principal purpose, until recently, for their feathers for use in the fashion industry and for industrial and household cleaning equipments (Gill et al., 2007). As the industry expands, more products have been produced from ostriches which include the hide (leather goods), variety meats (liver and heart), fresh meat (steaks and roasts), processed meats (sausage and ham type products) and health care products (Harris et al., 1993) These zoonotic organisms in the ostriches can cause diseases in humans when they come in contact with live or dead birds or upon consuming meat from infected ostriches, although for the latter the risk is minimal (Huchzermeyer, 1997).

Leisure activities in the show farms may expose the zoonotic organisms to visitors, especially if they consume food without proper washing of the hands after touching the animals or upon consumption of undercooked or contaminated cooked meat.

In Malaysia, ostrich farming is at infant stage as compared to Asia-Pacific region (China, Australia, Indonesia, Tasmania) (Leong, 2010). Thus, the objective of the study was to detect the presence of Campylobacter spp. and Salmonella spp. in ostrich.

MATERIALS AND METHODS

Collection of samples

The samples were collected from three ostrich show farms, with eight ostriches from Farm A, 11 ostriches from Farm B and 12 ostriches from farm C, a total of 31 birds. The ostriches were restrained by experienced staff. The eyes and head of each ostrich were covered by a hood so as to block the vision; this would calm the bird and facilitate handling.

Four sterile swabs were used to collect samples which consisted of two cloaca and two skin swabs from each bird. One set of swabs was placed into individual Cary Blair transport medium (Oxoid) for the isolation of Campylobacter spp. and the other set was placed into individual Buffered Peptone Water (Oxoid) for the isolation of Salmonella spp. Then, the swabs were transported in ice-packed cool box to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, and were cultured within two to three hours.

Culture for isolation and identification

Campylobacter spp.

Each swab in the Cary Blair transport medium was streaked directly onto Campylobacter blood-free selective agar base (Modified CCDA- Preston, Oxoid CM0739) incorporated with CCDA selective supplement (Oxoid

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Salmonella spp.

Each swab in Buffered Peptone Water (Oxoid) was pre-enriched by incubating at 37°C for 24 hours under aerobic condition. Then, 1 ml of each pre-enriched broth culture was transferred into 10 ml of Rappaport Vassalidis broth (RV) (Oxoid) enrichment broth and was incubated at 42°C for 24 hours. One loopful of each enriched broth culture was streaked onto Xylose-Lysine-Tergitol-4® (XLT4) agar (Merck) and another loopful was streaked onto Salmonella Chromogenic agar incorporated with Salmonella selective supplement (Oxoid) and were incubated at 37°C for 24 to 48 hours under aerobic condition. The presumptive Salmonella colonies were gram-stained and subcultured. Biochemical tests which included Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) and urease tests were performed. The colonies that showed typical reactions of Salmonella spp. were then subjected to slide agglutination test (SAT) using Salmonella polyvalent ‘O’ antisera A-S.

RESULTS AND DISCUSSION

The Campylobacter colonies appeared as irregular shape, moist, slightly raised, grey, mucoid, discrete colonies and often as flat spreading colonies; the organism is Gram-negative, seen as slender, curved, S- or gull-wing shaped with corkscrew or darting movement. Only one sample (1.6%) from a cloacal swab of an ostrich in Farm A was positive for Campylobacter. Typical Salmonella spp. colonies appeared as red to yellow colonies with black centers on XLT4 agar and as magenta to purple colonies on the Chromogenic agar. They were seen as Gram-negative rods on Gram-staining. Two samples (3.2%) were positive, which were from a skin swab in Farm B and a cloacal swab in Farm C.

Campylobacter colonizes the intestinal mucus found on the outer layer and in the crypts as a commensal organism (Beery et al., 1988) and the principal site of Salmonella colonization is the caecum (Xu et al., 1988). Hence, both bacteria are most likely to be shed in the faeces. These bacteria may contaminate farm surfaces and environment which in turn may contaminate the feathers and skin of the birds which can contribute to the contamination of the carcasses at processing. Humans may come in contact with the organisms such as upon handling and touching the animals. Salmonella spp. cause mortality in ostrich chicks (de Freitas Neto et al., 2009). The first isolation of Salmonella potsdam in Malaysia from ostrich chicks with yolk sac infection was reported by Jasni et al. in 1998 (Zurina, 2005).

The low detection of Campylobacter in the study may reflect the true status in ostrich in Malaysia. However, it may also be due to failure to isolate the organisms as they were not shed at the time of sampling. Being fastidious organisms that require microaerophilic condition and capnophilic environment to survive (Mahon et al., 2007), Campylobacter spp. may resort to ‘viable but non-culturable form’ if they were in adverse or stress condition. Another possibility is that the number of organisms on the swabs was too small, thus enrichment broth should have been used prior to plating (Bartelt, 2000). Enrichment stage is also important to revive the injured bacteria cells. Incubation temperature may also affect the number of Campylobacter spp. isolated. Incubation temperature used in this study was 42°C which is usually used to incubate enteric Campylobacter which are thermophilic such as C. jejuni subspecies jejuni. However, this is not suitable for non-thermophilic Campylobacter such as C. fetus subspecies fetus which are usually incubated at 37°C but this species is more commonly found in ruminants. According to Bartelt (2000), the growth of non-thermophilic campylobacters is inhibited at 42°C and thus no growth on the agar was observed. Some Campylobacter were susceptible to cefoperazone which was present in the CCDA agar, such as Campylobacter upsaliensis according to Aspinall et al., (1993), however this species is seldom isolated from avian species. Very few studies were available regarding the prevalence of Campylobacter in ostrich. Cuomo et al (2007) reported 40% of the clinically healthy ostriches in four farms in Italy were infected with Campylobacter.

There were a number of studies on Salmonella in ostrich that were accessible. The low presence of Salmonella in this study was similarly shown by Harris et al. (1993), who isolated Salmonella from 5.6% of the surface and 20% of the faecal or caecal swabs of the ostriches in slaughterhouses in Texas, US. On the other hand, Vanhooser and Welsh (1995) isolated a higher number of Salmonella spp in the intestines and caecae of ostriches at 18.5% (46/248); Salmonella were isolated from clinically infected ostriches and those with subclinical infection and these birds had fence-to-fence contact with other animal species. In this study, the ostriches were clinically healthy and had almost no contact with other animals as they were kept in separate areas in the farm. de Freitas Neto et al. (2009) did not detect Salmonella spp.
any droppings, eggs, caecal contents, swabs of carcases, spleens and livers from ostriches in production chain in Brazil; nevertheless, *Salmonella* Javiana and *Salmonella enteric* subsp. *enterica* 4, 12: i- were isolated from 2 out of 30 feed samples. Feed may be an important source of *Salmonella* spp. in ratite production which may be contaminated during processing or storage, and, as a result, it could play an important role in the introduction or maintenance of *Salmonella* spp. in ratite farms (Gopo & Banda, 1997; Higgins et al., 1997). There were studies that found high occurrences of *Salmonella* in ostriches. The study by Gaedirelwe and Sebunya (2008) in Botswana found the highest isolation of *Salmonella* in ostrich at 51.6% (16/31) was from the cloacae, while 13%, 16% and 29% from the livers, small intestines and large intestines respectively. Gopo and Banda (1997) found *Salmonella* on 51% of the ostrich’s surfaces (feathers) on arrival at the abattoir and on 8.3% of the skins. According to Friedman et al. (1998), the direct or indirect contact with animals colonized with *Salmonella* is another source of infection in humans, including contact during visits to petting zoos and farms. A report on an outbreak of salmonellosis among children was caused by an exposure to a reptile exhibit at a zoo; the visitors had touched the barriers which had been touched by the *Salmonella*-infected reptile and that they had placed their contaminated hands in their mouth or cross contaminated the food eaten (Friedman et al., 1998). As in the case of *Campylobacter*, the low presence of *Salmonella* spp. in the study may indicate the true status in the farm or the organisms were not shed at the time of sampling.

The comparison in the occurrences of pathogens among studies is not truly possible even if similar samplings and methods of isolation and identification are used; this is because the exposures to the organisms and risk factors involved may differ among animals, farm environment and locations. It is well recognised that molecular methods such as PCR assay are said to be better in detection of pathogens compared to conventional microbiological method even though very selective and chromogenic agar are used, however at times the results obtained are often comparable. According to Gopo and Banda (1997), molecular methods are more reliable and specific as they do not show any false negatives, but they can be expensive as special equipment and reagents and trained personnel are required to carry out the work.

**REFERENCES**


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