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Journal Article

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 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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SR0155E). The plates were incubated at 42°C for 48 hours under microaerophilic condition (5% oxygen, 10% carbon dioxide and 85% nitrogen) generated using a gas generating pack (BD Campy Pak) in an anaerobic jar. Each typical colony of *Campylobacter* was subjected to Gram-staining for cellular morphology and 'hanging drop' technique for motility. The presumptive colonies of *Campylobacter* spp. were subcultured on Columbia Blood agar (Oxoid) with 5% defibrinated horse blood added, and incubated at 42° C for 48 hours to obtain pure culture for biochemical tests. The tests included catalase, oxidase, urease, indoxyl acetate hydrolysis and hippurate hydrolysis tests. The isolated *Campylobacter* spp. was then kept in Brucella broth (Oxoid) with 10% glycerol added and stored at -20 ^{0}C.

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 Vassidalis 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. can 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 be 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^{0}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^{0}C but this species is more commonly found in ruminants. According to Bartelt (2000), the growth of non-thermophilic campylobacters is inhibited at 42^{o}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 a*ccording 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 caeca 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. in

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.

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REFERENCES

Aspinall S. T., Wareing, D. R. A., Hayward, P. G. and Hutchinson, D. N. (1993). Selective medium for thermophilic *Campylobacter* including *Campylobacter upsaliensis*. *Journal of Clinical Pathology*, **46**: 829–831

Bartel,t M.A. (2000). Diagnostic Bacteriology, a Study Guide. F.A. Davis Company. pp 168-172

Cuomo, A., Dipineto, L., Santaniello, A., Matteoli, G., Sarli, T., Vecchia, D.D., Fioretti, A. and ,Menna L.F. (2007). Detection of thermotolerant *Campylobacter* in ostriches (*Struthio camelus*) in Italy. *Veterinary Journal*, 174(2): 439-441

Freitas Neto, O.C. de, Silva Lages, S.L., Torres Carrasco, A.O. and Berchieri Junior, A. (2009). Search for *Salmonella* spp. in ostrich productive chain of Brazilian southeast region. *Tropical Animal Health and Production*. **41(8)**: 1607-1614

Friedman, C.R., Torigian,c., Shilliam, P.J., Hoffman, R.E., Heltzel, D., Beebe, J.L., Malcolm, G., DeWitt, W.E., Hutwagner, L. and Griffin, P.M. (1998). An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. *The Journal of Pediatrics* **132**: 802-807

Gaedirelwe, O.G. and T.K. Sebunya, (2008). The prevalence and antibiotic susceptibility of *Salmonella* sp. in poultry and ostrich samples from slaughter houses in Gaborone, Botswana. *Journal of Animal and Veterinary Adances*, **7**: 1151-1154

Gill, C.O. (2007). Review: Microbiological conditions of meats from large game animals and birds., *Meat Science*, **77(2)**: 149−160

Gopo J.M. and Banda G.N. (1997). Occurrence of *Salmonella* on meat and products in an ostrich abattoir as determined with a DNA probe. *South African Journal of Animal Science.* **27(1)**: 1-6

Harris, S.D., Morris, C.A., May, S.G., Lucia, L.M., Jackson, T.C., Hale, D.S., Miller, R.K., Keeton, J.T., Savell, J.W. and Acuff, G.R. (1993). Ostrich Meat Industry Final Report. American Ostrich Association, Forth Worth, Texas. pp. 3-40

Higgins, R., Desilets, A., Cantin, M., Messier, S., Khakhria, R., Ismaïl, J., Mulvey, M.R., Daignault, D. and Caron, H. (1997). Outbreak of *Salmonella give* in the province of Quebec. *Canadian Veterinary Journal*, **38(12)**: 780–781

Huchzermeyer, F.W. (1997). Human health risks of ostrich and crocodile meat. *Revue Scientifique et Technique de l'Office International des Epizooties*, **16:** 599–604.

Leong, C. O. (2010) Ostrich Farming in Malaysia. Website : http://ebookbrowse.com/ostrich-pdf-d35983264. Accessed on 18th November 2010.

Mahon, C.R. Lehman, D.C. and Manuselis, G. (2007). Textbook of Microbiology, 3rd ed., Saunders; pp. 518 - 521

Vanhooser, S.L. and R. D.Welsh. (1995). Isolation of *Salmonella* species from ratites. *Journal ofVeterinary Diagnostic Investigation.* 7: 268–269

Xu, Y.M., Pearson, G.R., and Hinton, M.H. (1988). The colonization of the alimentary tract and visceral organs of chicks with salmonellosis following caecal challenge via feed: bacteriological findings. *British Veterinary Journal*, **144**: 403-410

Zurina Ramli (2005). The pathology of *Salmonella potsdam* infection in yolk membraneand embryonated chicken eggs. A Master of Veterinary Science thesis in Universiti Putra Malaysia.