Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Research Article

Zoonotic potential of *Salmonella* and *Escherichia coli* isolated from ostrich eggs of a flock in a recreational park

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Abstract. Aim: This study investigated *Salmonella* sp. and *Escherichia coli* contamination of ostrich's eggs from an ostrich flock kept in a recreational park downtown of Giza city, Egypt by bacteriological and serological methods. Material and methods: A total of 20 ostrich eggs were submitted for detection of *Salmonella* and *E. coli* in the outer shell and egg content. The isolates were examined for *Salmonella* species, *S. enterica serovars typhimurium* and *enteritidis* using multiplex PCR. *E. coli* isolates were confirmed by PCR and surveyed for O157: H7 serotype by PCR reaction. Results: Results showed that the prevalence of *Salmonella* isolated from the outer surface of the eggshell was 25% and 70 % for *Salmonella* and *E. coli*, respectively. The isolation rate from egg contents was 30% and 20% for *Salmonella* and *E. coli* respectively. Two (10%) *Salmonella* isolates and 1 (5%) *E. coli* isolate were recovered from egg albumin, whereas 3 (15%) *Salmonella* isolates and 2 (10%) *E. coli* isolates were recovered from egg yolk only. All the examined eggs were negative for antibody to *S. enteritidis* in the egg yolk. *Salmonella sp. serovars* other than *S. enteritidis* and *S. typhimurium* were not identified among all *Salmonella* isolates whereas, *S. cerro* (O6, 14, 18 H1 Z4, Z23 H2) was identified by serotyping assay. Among 19 *E. coli* isolates, one (5.3%) isolate of *E. coli* from the outer eggshell surface was identified by PCR as serotype O157: H7. Serological examination of two *E. coli* isolates revealed that one isolate from the egg shell surface was identified as *E. coli* (2), monovalent 2; O: 55 and another isolate from egg albumin were identified as *E. coli* (1), monovalent 6; O: 169 serotypes. Conclusion: In conclusion, ostrich eggs could be sources of *Salmonella* and *E. coli* to egg handlers and consumers.

Key Words: Ostrich, Eggs, *Salmonella*, *Escherichia coli*, Zoo, Zoonoses.

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Introduction

The ostrich (*Struthio camelus*) is a species that aroused people's interest since ancient times. In recent years, ostrich farming has expanded worldwide, providing an economical trade of products such as feathers, meat, valuable skin and eggs that make them important alternative livestock in many countries (Cooper et al 2008). Ostrich is also exhibit animals in zoos and private parks. Direct contact with ostrich and ostrich products could represent a potential risk of transmission of many zoonotic diseases. The recent increase in ostrich farming and exhibition has led to an increasing demand for information about the zoonotic disease risk posed by this bird, its products, and by-products.

In Egypt, the African ostrich was widely distributed since ancient eras. However, because of climatic changes and intensive hunting by humans, ostrich numbers gradually declined until disappearing from Egypt by 1990s (Manlius 2001). At the beginning of 21 century, it reappeared again as a domestic farm species. The ostrich industry in Egypt is making progress and the number of farms is also increasing (Cooper et al 2008).

Egyptian ostrich farms recorded values for monthly egg production (7.27 eggs/hen/month) that exceeded mean values in South Africa (5.99 eggs/hen/month) (El-Maghawry et al 2006; Fair et al 2005). An ostrich egg weighs 1.5 kg and the mean weight of albumen in a 1500 g ostrich egg reaches 900 g, with a 317g yolk and a 296g voided shell. This renders the ostrich eggs a highly nutritious food (Cooper et al 2009). However, the ostrich egg could be a source of many bacterial agents. Microbiologic investigation showed bacterial isolation rate of 19.3% in ostrich eggs (Cabassi et al 2004).

Salmonella has been shown to cause clinical disease in ostrich (More 1997; Verwoerd 2000), and as a part of the flora in clinically healthy ostrich (de Freitas Neto et al 2009; Tully & Shane 1996). Many serovars of *Salmonella* were isolated from ratites reared in contact with other animal species (Vanhooser & Welsh 1995). Enterotoxigenic *E. coli* strains cause watery diarrhea in animals and birds worldwide (Neill et al 1994). The occurrence of *E. coli* in ostrich products could result in commercial restrictions in the trade of meat and other products. It is also a matter of concern to public health, being a widespread human foodborne pathogen (Foley et al 2008; Smith et al 2008).

Although the commercial production of the ostrich is widespread, little research has been undertaken on the occurrence of pathogens in or on their eggs. Therefore, this study aimed to investigate *Salmonella sp*. and *E. coli* contamination of outer surface and contents of ostrich eggs collected from a flock of

ostrich kept at one recreational park located in a city from Giza governorate, Egypt.

Material and methods

Studied flock and sample collection

Twenty ostrich eggs were submitted for detection of *Salmonella* and *E. coli* in the outer shell and egg contents. These eggs were laid by domesticated ostriches belonging to one flock in a park located in downtown Giza governorate Egypt from December 2015 to February 2016. This flock suffered from infertility due to embryo death and dead-in-shell embryos. Immediately after laying, eggs were collected and chilled to 4 ◦C, transported to the laboratory within 48-72 h, and immediately examined. Eggs were in good condition in terms of shell and membrane integrity. The eggs were handled using aseptic techniques.

Egg preparation for bacteria isolation from the outer shell, egg yolk, and albumen

Eggs were prepared for bacteria isolation from the outer surface of the intact whole egg as well as inner egg contents (egg white and egg yolk, separately), following the protocols described by (ISO:6887-4, 2003). Briefly, without breaking, each egg sample was rinsed several times while turning it around, using 30 ml of buffered peptone water (HiMedia, Mumbai, India, 400086). For preparation for bacteria isolation from egg yolk and egg albumen, the whole surface of the shell was wiped using gauze soaked in 70% ethanol and allowed to dry completely. Afterward, eggs were aseptically opened using a sterile high-speed cutting disc. Yolk and white were separated aseptically and placed in sterile containers. Buffered peptone water was added to give a 1ml in 9 ml dilution for the yolk and 1ml in 40 ml for the white to dilute out the naturally occurring lysozyme inhibitor.

Isolation and identification of *Salmonella*

Salmonella sp. was isolated and identified according to (ISO:6579, 2002). One ml each of the rinse, diluted albumen, and diluted yolk suspensions were inoculated separately into 10 ml of buffered peptone water and incubated at 37 °C for 18 h. One hundred microliters each of incubated buffered peptone water cultures were inoculated into 10 ml of Rappaport-Vassiliadis medium with soya (RVS) (Oxoid, Hampshire, United Kingdom, RG24 8PW) and incubated at 41 °C for 24 h then incubated for 24 hr at 37 °C. Finally, plates of Xylose Lysine Deoxycholate agar (XLD) and *Salmonella-Shigella* (SS) agar were streaked each from the incubated (RVS), then incubated at 37°C for 24 h. Suspected colonies were identified using the biochemical examination.

Suspected *Salmonella sp.* and *E. coli sp*. based on colony morphology on the selective media, were identified by standard metabolic and biochemical tests as described previously (Ewing 1986).

Isolation and identification of *E. coli*

One ml of each of egg rinse, diluted yolk, and diluted white were aseptically dispensed into sterile Petri plates. Ten ml of Tryptone Bile X-glucuronide Agar (TBX) (Oxoid, Hampshire, United Kingdom, RG24 8PW) was poured and the contents were mixed and incubated at 40 °C for 24 hr. *E. coli* colonies are colored blue/green. The colony indicated for *E. coli* by TBX was further subjected to biochemical confirmation

Serotyping of *Salmonella*. Biochemically positive *Salmonella* colonies were serologically investigated using Polyvalent O and O1 antisera then with the respective mono-specific "O" antisera by slide agglutination test as described by the White-Kauffmann-Le Minor scheme (formerly known as Kauffmann-White scheme) (Popoff and Le Minor 1997). The same procedures were applied to "H" (phase 1 and phase2) antisera (Denka Sieken Co., LTD, Tokyo, Japan, 103-8338).

Serological identification of *E. coli*

The diagnostic O sera polyvalent and corresponding monovalent antisera (Denka Sieken Co., LTD, Tokyo, Japan, 103-8338) were used for serotyping of the isolates by slide agglutination technique according to Edwards and Ewing (1972).

Antibody detection of *S. enteritidis* using ELISA kits. Antibody to *S. enteritidis* in ostrich eggs yolk samples was detected by enzyme immunoassay (FlockCheck Se, IDEXX Laboratories Inc., Maine, USA, 04092) as per manufacturer's instructions.

DNA extraction and PCR amplification reaction from *Salmonella* and *E. coli* isolates. The bacteriologically positive strains were grown in 10 ml tryptic soya broth at 37°C for 24 h. The overnight cultures were centrifuged at 4◦C at 3,000 xg 10 min, and the pellet was washed twice with phosphate buffered saline pH 7.2 and resuspended in 500 µl Tris- ethylene diamine tetraacetic acid (EDTA) buffer (pH 8.0) and heated in water bath at 100°C for 20 min. The samples were then left to cool at room temperature and centrifuged at 3,000-x g for 10 min. An aliquot of 5 µl of the supernatant was used as template DNA in the PCR. Amplification reactions were carried out with 5 µl of boiled bacterial suspensions, 5 µl of 5X Taq Master/ high yield (Jena Bioscience, GMBH, Loebstedter Strasse Germany, 71D-07749) and two pairs of primers 50 pmol. Distilled water was added to bring the final volume to 25 µl. After PCR reactions, the reaction products were subjected to electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and visualized and photographed under UV light. Products of the appropriate sizes were identified by comparisons with a 100-bp DNA ladder (BioBasic Inc., Markham, Ontario, Canada, L3R 8T4).

PCR protocol for *Salmonella* consisted of the following steps: (i) an initial denaturation step of 2 min at 95°C; (ii) 30 cycles, with 1 cycle consisting of 1 min at 95°C, 1 min at 57°C, and 1 min at 72°C; and (iii) a final elongation step of 5 min at 72°C. Isolates were confirmed to *Salmonella* species, *S. enterica serovars typhimurium* and *enteritidis* were examined using multiplex PCR. *E. coli* isolates were confirmed by PCR and surveyed for O157: H7 serotype by PCR reaction. The primer sequences and the size of amplification products are shown in Table (1).

Results

Total prevalence of *Salmonella* and *E. coli* in ostrich egg surface and egg contents

Results shown in table 2 reveal that the total incidence of *Salmonella* isolated from the outer surface of eggshell was 25% and 70 % for *Salmonella* and *E. coli*, respectively. However, the isolation rate from egg contents was 30% and 20% for *Salmonella* and *E. coli*, respectively.

Regarding isolation from egg albumin and egg yolk contents, a high percentage of *Salmonella* and *E. coli* were isolated from yolk or albumen alone. Out of 20 eggs, 2 (10%) of *Salmonella*

Table 1. Primers sequences and amplicon sizes of targeted genes of *Salmonella* and *E. coli* isolates

Primer Name	Sequence (5'-3')	Amplicon size (bp)	Target	Reference
OMPC	F:5-ATC GCT GAC TTA TGC AAT CG-3	204	*Salmonella genus*	
	R:5-CGG GTT GCG TTA TAG GTC TG-3			Alvarez et al 2004
ENTF	F: 5-TGT GTT TTA TCT GAT GCA AGA GG-3	304		Alvarez et al 2004
	R:5-TGA ACT ACG TTC GTT CTT CTG G-3		*S. enteritidis*	
TYPHF	F:5-TTGTTCACTTTTTACCCCTGAA-3	401	*S. typhimurium*	
	R:5-CCCTGACAGCCGTTAGATATT-3			Alvarez et al 2004
E. coli 670-F	F: 5-ACCTGCGTTGCGTAAATA-3.	670	gadA/B	
	R:5-GGGCGGGAGAAGTTGATG-3.			McDaniels et al 1996
eaeAO157:F	F: 5-AAG CGA CTG AGG TCA CT-3	450	eaeAO15:7	
	R: 5-ACG CTG CTC ACT AGA TGT-3			Louie et al 1994

Table 2. The total prevalence of Salmonella and E. coli in outer shell and egg contents of ostrich eggs

Isolates	*Salmonella sp.*	*E. coli sp.*	*Salmonella and E. coli*	Negative
Site of isolation				
	No (%)	No (%)	No (%)	No (%)
Egg surface	5 (25%)	14 (70%)	4 (20%)	6 (30%)
Egg content	6 (30%)	4 (20%)	1 (5%)	10 (50%)
White only	2 (10%)	1 (5%)	0 (0%)	
Yolk only	3 (15%)	2 (10%)	1 (5%)	
white and yolk	1 (5%)	1 (5%)	0 (0%)	

isolates and 1 (5%) of *E. coli* isolates were recovered from egg albumin. However, 3 (15%) of *Salmonella* isolates. and 2 (10%) of *E. coli* isolates. were recovered from egg yolk only. Mixed *Salmonella* and *E. coli* contamination of outer shell surface was detected in 4 (20%) of the examined eggs whereas one (5%) egg showed *Salmonella* and *E. coli* isolation from both the albumen and yolk.

Serological examination of *S. enteritidis* antibodies in ostrich egg yolk

Serological examination revealed that all examined eggs were negative to detection of antibody to *S. enteritidis* in the egg yolk.

Serovars identification of *Salmonella sp.* by molecular typing and serotyping

Molecular serotyping of five isolates from outer egg shell and 6 isolates from egg contents were all serovars other than *S. enteritidis* and *S. typhimurium*. *Salmonella cerro* (O6, 14, 18 H1 Z4, Z23 H2) was identified by serotyping of one isolate from the outer shell.

E. coli serovar **identification by molecular typing and serotyping**

All *E. coli* isolates were confirmed to be *E. coli* by PCR evaluation. Among 19 *E. coli* isolates, one (5.3%) isolate of *E. coli* from the outer eggshell surface was identified by PCR as serotype O157:H7. Serological examination of two *E. coli* isolates revealed that one isolate from the egg shell surface was identified as *E. coli* (2) monovalent 2; O: 55 and another isolate from egg albumin was is identified as *E. coli* (1) monovalent 6; O: 169 serotypes.

Discussion

Microbial contamination of outer shell surface and spoilage of ostrich eggs contents causes low reproduction rate and poses health hazards to the egg handlers and consumers (Cooper et al 2009; Cabassi et al 2004). The incidence of microbial spoilage of ostrich eggs with bacteria and fungi has been reported as high as 21% (Deeming 1996a).

This study searched for *Salmonella* and *E. coli* in the eggs laid by an ostrich flock kept in a recreational park downtown of Giza city, Egypt. The birds were used for exhibit and to produce meat, eggs, feathers and leather. Moreover, the untreated dropping of this flock was used as a fertilizer for the gardens in the park area. There is wide dissemination of *Salmonella* and *E. coli* isolates in different ecosystems, including captive ostriches that could be transferred to humans through the food chain (Carneiro et al 1994). Therefore, this flock of captive ostrich could be a potential source of *E. coli* and *Salmonella* food poisoning to the park workers, visitors, the consumers in case of ingestion of raw or undercooked contaminated food.

Results of this study revealed high incidences of *E. coli* and *Salmonella* contamination of the outer shell surface of ostrich eggs. Based on current data, it is not possible to establish the origin of the bacterial isolates. One potential cause of the high rate of *Salmonella* and *E. coli* contamination of eggs in this flock is the lack of strong sanitation protocols. Outer shells could be inoculated in contaminated nests and inadequate egg cleaning could contribute to transmission. In addition, infectious agents have been implicated in ostrich infection from multispecies collections, animal dealers, and similar 'zoo type' situations which are predisposed to cross-species transmission of pathogens under highly stressful conditions (Vanhooser & Welsh 1995). In

this study, some other zoo animals and birds were housed with the ostrich which was constituting a risk of interspecies transmission. Contamination of the outer shell by *Salmonella* and *E. coli* is a matter of concern in incubation of eggs in hatcheries and a public health concern during handling and preparing eggs for consuming (Foley et al 2008; Rasschaert et al 2008; Smith et al 2008).

In the current study, *Salmonella* was isolated from albumin and yolk at a fairly high prevalence. These findings disagree with Cabassi et al (2004) who did not find *Salmonella sp.* in 543 unhatched eggs from ostriches of Italian farms. Additionally, *Salmonella sp.* was not isolated from ostrich eggs on farms in Brazil (de Freitas Neto et al 2009). Another study showed only 1 of 152 carcass samples was found positive for *Salmonella* (Ley et al 2001). The variation of isolation of *Salmonella* from ostrich eggs could be attributed to the differing quality of quarantine and husbandry practices on different ostrich farms around the world (de Freitas Neto et al 2009).

Many serovars of *Salmonella* were isolated from ratites reared in contact with other animal species such as *S. typhimurium*, *S. copenhagen*, *S. muenchen*, and *S. Pomona* (Verwoerd 2000; Vanhooser & Welsh 1995). However, this study presents the first record of *Salmonella serovar cerro* in ostrich egg samples. All of the *Salmonella* isolates in this study were serovars other than *S. typhimurium* and *enteritidis*. Serological examination revealed the absence of antibodies to *S. enteritidis* in egg yolk. These findings disagreed with Cadman et al (1994) who detected a prevalence of 8% of *S. enteritidis* antibodies by ELISA in ostrich in Zimbabwe. The absence of *S. typhimurium* could be explained by the age factor. *S. typhimurium* is common in multispecies collections and causes mortality in chicks younger than three months on commercial farms, but is rarely found in chicks older than six months (Verwoerd 2000). It also suggests that higher storage temperature causes greater *S. enterica serovar typhimurium* internal penetration of artificially contaminated, commercially available, washed free range eggs (Whiley et al 2016).

E. coli contamination of food is considered a potential cause of food poisoning. *E. coli* is a common pathogen in the large intestine of ostrich, indicating a contamination of eggs after laying (Ley et al 2001). In this study, a high prevalence of *E. coli* was detected in the outer surface of ostrich eggs. A lower isolation rate of *E. coli* from ostrich eggs has been reported (15%) (Knobl et al 2012). High eggshell porosity favors the contamination of the eggs by microorganisms present in the faeces. Results of this study demonstrated that one isolate was present only in the internal egg structure, suggesting the possibility of vertical infection. *E. coli* enterohaemorrhagic serotype O157: H7 was identified from the outer surface of one egg which might be external cross-contamination from other sources such as flies or contaminated sand. A previous study revealed absence of *E. coli* serotype O157:H7 from ostrich carcasses (Ley et al 2001). On the basis of the current data, it is not possible to establish the origin of the bacterial isolates. Bacteria isolated in albumen only could be related to high shell porosity, so that they may arise from extra body contamination (Deeming 1995). However, the infection of both yolk and albumen could also indicate that both the yolk and albumen can be potentially contaminated before the egg is laid and could be related to infections of the ovary and/or of the oviduct (Gast & Beard 1990). Moreover, a long holding period at room temperature before sampling could lead to an increase in the possibility of cross contamination between the yolk and albumen (Gast & Beard 1990). Therefore, quick collection prevents microbial spoilage and loss from theft or predation. The maintenance of nest hygiene is considered the simplest way of reducing microbial contamination (Deeming 1996 a & b). Moreover, the egg should be carefully gathered and wiped with a dry cloth. Holding the eggs with sterile toweling helps to prevent possible contamination from the worker's hands.

Conclusion

It could be concluded that the wide dissemination of *Salmonella* and *E. coli* isolates in farmed ostrich eggs could pose a zoonotic threat to humans through the food chain.

Acknowledgements

The authors are very grateful for Dr. Amani Lotfi, the Animal Health Research Institute, Food Hygiene Department, Dokki, Giza, Egypt for serotyping of the isolates.

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Citation	Youssef AI and Afifi RA. Zoonotic potential of Salmonella and Escherichia coli isolated from ostrich eggs of a flock in a recreational park. HVM Bioflux 2017;9(3):71-75.
Editor	Ştefan C. Vesa
Received	6 March 2017
Accepted	28 May 2017
Published Online	28 July 2017
Funding	None reported
Conflicts/ Competing Interests	None reported