Scientific Report

Bacteriological study of dead-in-shell embryos of ostrich

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Summary

In order to study the bacterial flora of dead-in-shell ostrich chicks, twelve unhatched eggs which did not have external pipping during the hatching period were transferred to the laboratory of microbiology. The egg shells were accurately disinfected and the embryos were removed and placed in a sterile plate. The surface of each embryo was swabbed with a sterile swab which was also plunged through the yolk sac and the embryo contents and the swab were inoculated into tryptic soy broth (TSB) or nutrient broth. To enrich *Salmonella* spp., another swab was prepared as above and inoculated into Selenite-F broth. These media were incubated at 37°C for 24 h and then subcultured by streak plate method on solid media. Different bacterial colonies on solid media were isolated in pure cultures for further identification. The results of this study showed that the predominant bacterial flora of dead-in-shell embryos of ostrich were *Bacillus* spp. (45%) and *Staphylococcus* spp. (25%).

Key words: Bacterial isolates, Ostrich egg, Yolk sac infection

Introduction

The knowledge of basic biology of ostrich production under farming conditions is low. For example, despite being a well established industry, production of ostriches in South Africa is poor in comparison to more conventional domesticated stocks; on average, 50% of all eggs hatch and only 40% of hatchings survive to slaughter age (Deeming, 1995). Several researchers have emphasized the importance of yolk sac infection as a cause of mortality, even in the hatchery, which could increase the number of so-called dead-in-shell eggs (Dzoma and Dorrestein, 2001; Walker *et al*., 2002). Faecal contamination of eggs is considered to be the most important source of yolk sac infection (Rajesh *et al*., 2001). Bacteria may be acquired *in ovo* if the hen has oophoritis or salpingitis or *via* contamination following artificial insemination (Montgomery *et al*., 1999). Yolk sac infections can also result from translocation of bacteria from the chick's intestine or from the bloodstream (Saif *et al*., 2003). Because of the frequency of occurrence of yolk sac infection and little bacteriological investigation of dead-in-shell embryos of ostrich, this research was conducted to study the bacterial flora of dead-in-shell ostrich chicks.

Materials and Methods

A total of one hundred and twenty ostrich eggs in Zabol were disinfected by fumigation with formalin and potassium permanganate (1 g $KMnO_4$/2 mL formalin) and then stored at 15°C for a maximum 7 days. Each group of 40 eggs was warmed at room temperature, fumigated and incubated at 37°C and 20% humidity as one batch. After 39 days of incubation, the eggs were transferred to a hatcher for 2 days. Twelve unhatched eggs which did not have external pipping during the hatching period were transferred to the laboratory. The egg shells were accurately disinfected with 96% alcohol and after the shell crushing; the embryo was removed with sterile forceps and placed in a sterile plate. The surface of each embryo was swabbed with a sterile swab which was also plunged through the yolk sac and other embryo contents and the

swab was inoculated into TSB or nutrient broth. To enrich *Salmonella* spp., another swab was prepared as above and inoculated into Selenite-F broth. All cultured media were incubated at 37°C. After 24 h, from the TSB and nutrient broth, cultures were streaked on blood agar and MacConkey agar, but culture in Selenite-F were subcultured on the MacConkey, *Salmonella-Shigella* or brilliant-green agar. After the 24-48 h incubation of the solid media, the plates were observed for colony formation. The identification of the isolated colonies was performed using standard bacteriological and biochemical procedures as described by Quinn *et al*. (2002) and Swayne *et al*. (1998).

Results

Bacterial cultures of the sampled eggs which were fertile but the embryos died before hatching showed 100% bacterial contamination, and from each egg one or more organisms were recovered. From these samples, 20 isolates of bacteria comprising *Bacillus* spp. (9 isolates), *Staphylococcus* spp. (5 isolates), *Klebsiella* spp. (3 isolates), *Escherichia coli* (2 isolates) and *Proteus* spp. (1 isolates) were recovered (Table 1). However, *Salmonella* spp. was not isolated from any of the eggs.

Table 1: The results of bacterial cultures from dead-in-shell embryos of ostriches

No.	Bacterial isolate	Number of eggs with bacterial isolates*	Frequency of isolate (%)
1	*Bacillus* spp.	9/12	45
2	*Staphylococcus* spp.	5/12	25
3	*Klebsiella* spp.	3/12	15
4	*Escherichia coli*	2/12	10
5	*Proteus* spp.	1/12	5

*Number of eggs culture positive/number of eggs tested

As shown in Table 1, bacterial flora of dead-in-shell embryos of ostrich were *Bacillus* spp. (45%), *Staphylococcus* spp. (25%), *Klebsiella* spp. (15%), *Escherichia coli* (10%) and *Proteus* spp. (5%).

Discussion

In this research, the examined eggs belonged to an ostrich breeder farm that was suffering from infertility problems, and yolk sac infection was an important problem of the hatched chicks as well. Swab cultures of unhatched eggs which did not have external pipping during the hatching period showed 100% bacterial contamination and from each egg at least one organism was recovered (Table 1). Bacterial contamination is an important factor for ostrich egg infertility, but it is not the only factor of early embryonic mortality (Deeming, 1995). Microbiologic investigation from ostrich eggs that suffered fertility disorders have shown bacterial isolation in 19.3% of examined eggs. Additionally, in these farms, infertility was mainly due to embryo death and dead-in-shell embryos (Cabassi *et al*., 2004). Dzoma and Dorrestein (2001) have reported that 42% of dead-in-shell ostrich embryos contained bacteria, and that the most common isolate was *E. coli*. However, Deeming (1995) has demonstrated that 22.8% of all eggs and 36.3% of fertile eggs which failed to hatch were infected with bacteria and/or fungi.

In the present study, *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Bacillus* spp. were the isolated bacteria of dead-in-shell embryos. With the exception of *Bacillus* spp. the other isolated bacteria have been reported by Cabassi *et al*. (2004), however, *Bacillus* spp. was isolated from the contaminated eggs by Deeming (1995). Cabassi *et al*. (2004) have reported that *E. coli* was the most isolated bacterium in ostrich eggs, but in our study, *Bacillus* spp. was the most isolated bacterium of dead-in-shell embryos. The laying ostriches of this study were on the sandy litter contaminated with faecal matter. Faecal contamination of the surface of eggs leads to penetration of organisms through the shell and shell membrane, particularly if the shell is damaged. It seems that there is no transovarial transmission for the isolated bacteria of the dead-in-shell embryos of the ostriches. However, egg shell contamination leads to bacterial penetration through the shell and shell membrane and subsequently infects the developing embryo.

References

Cabassi, CS; Taddei, S; Predari, G; Galvani, G; Ghidini, F; Schiano, E and Cavirani, S

(2004). Bacteriologic findings in ostrich (*Struthio camelus*) eggs from farms with reproductive failures. Avian Dis., 48: 716-722.

Deeming, DC (1995). Factors affecting hatchability during commercial incubation of ostrich (*Struthio camelus*) eggs. Br. Poult. Sci., 36: 51-65.

Dzoma, BM and Dorrestein, GM (2001). Yolk sac retention in the ostrich (*Struthio camelus*): histopathologic, anatomic, and physiologic considerations. J. Avian Med. Surg., 15: 81-89.

Montgomery, RD; Boyle, CR; Lenarduzzi, TA and Jones, LS (1999). Consequences to chicks hatched from *Escherichia coli*-inoculated embryos. Avian Dis., 43: 553-563.

Quinn, PJ; Markey, BK; Carter, ME; Donnelly, WJ and Leonard, FC (2002). *Veterinary microbiology and microbial disease.* 1st Edn., Cornwall, Great Britain, Blackwell Science Ltd., PP: 43-122.

Rajesh, C; Rao, VDP; Gomez-Villamondos, JC; Shukla, SK and Banerjee, PS (2001). *Diseases of poultry and their control*. 1st Edn., Uttar Pradesh, India, International Book Distributing Co., PP: 68-70.

Saif, YM; Barnes, HJ; Glisson, JR; Fadly, AM; McDougald, LR and Swayne, DE (2003). *Diseases of poultry*. 11th Edn., Ames, Iowa, Iowa State University Press. PP: 631-644.

Swayne, DE; Glisson, JR; Jackwood, MW; Pearson, JE and Reed, WM (1998). *A laboratory manual for the isolation and identification of avian pathogens.* 4th Edn., Pennsylvania, USA, American Association of Avian Pathologists. University of Pennsylvania. PP: 4-16.

Walker, SE; Sander, JE; Cline, JL and Helton, JS (2002). Characterization of *Pseudomonas aeruginosa* isolates associated with mortality in broiler chicks. Avian Dis., 46: 1045-1050.