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Research Article

EPIDEMIOLOGICAL INVESTIGATION ON POOR EGG ATCHABILITY IN AN OSTRICH *(STRUTHIO CAMELUS)* FARM - A CASE REPORT OF EGG PERITONITIS

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ABSTRACT
From March 2008 to December 2010, 206 ostrich eggs were investigated. The eggs were laid by 95 ostriches of Livestock Research Station, Kattupakkam that suffered poor egg hatchability disorders and stray mortality caused by egg peritonitis. In this farm, epidemiological investigation showed bacterial isolation in 35 (16.9 %) of 206 eggs examined with a high prevalence of *Enterobacteria* from yolk and albumen. In few cases only did bacterial isolation from yolk and albumen alone. The antibiotic susceptibility test conducted on isolates by standard disc diffusion method. This is the first report of epizootiology of bacteriological agents established from this Ostrich unit.

Key Words: *Ostrich, Egg, Hatchability, Peritonitis and Antibiotic Sensitivity*

INTRODUCTION
In the past several years, interest has led to a demand for information about ostrich and emu farming in Tamilnadu, particularly Namakkal, Erode, Palladam and Puducherry. In Tamilnadu since the beginning of the 1990's ostrich chicks have been imported from Malaysia. Ostrich farm in Tamilnadu numbered only one with a total of 95 chicks in reproductive activity. Data exist concerning the farm management and infectious diseases of the bird in Tamilnadu and elsewhere, but the lack of knowledge concerning the microbial agents involved in reproductive failure is noteworthy. This study investigated the epizootiology of infectious agent status of eggs from an ostrich farm suffering infertility problems, beginning with the clinical needs of producing diagnostic findings and therapeutic indications.

MATERIALS AND METHODS
From March 2008 to December 2010, 206 ostrich eggs laid by 89 domesticated ostrich belonging to Livestock Research Station, Kattupakkam, Tamilnadu. Epidemiological investigation was made to find out the solution for embryo death, dead-in-shell embryos and egg peritonitis.
From the moment of laying eggs immediately examined, the eggs were in good condition in terms of shell and membrane integrity, as assessed by candling. The egg shells were accurately disinfected with 95% alcohol and sterilized by flaming under a Biosafety hood. The eggs were opened using a sterile high-speed cutting disc. Albumin and yolk were examined separately. 3 ml each of albumin and yolk were inoculated in 30 ml of buffered peptone water separately as well as Brain Heart Infusion broth(BHI). After 24 hrs of incubation at 37°C, BHI broth cultures were placed on to Tryptose agar (Himedia) plus 5% bovine red blood cells and on MacConkey agar(Himedia) and incubated for 48 hrs at 37°C in aerobic, microaerophilic and anaerobic environments (Balows et al., 1991). The egg shells were used for ornamental purpose, designed and exhibited in Science Exhibition. In addition, for Salmonella spp. Isolation, after incubation at 37°C for 24 hrs, 0.5 ml of buffered peptone water culture was inoculated in to 10 ml of Selinite-F broth and incubated for 24 hours at 43°C in a water bath. Finally this medium was placed on to MacConkey agar.

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Case Observation

One adult ostrich (5 years old) was died suddenly and brought to hospital section for post mortem examination. Ruptured yolk and egg shells were observed in the peritoneal cavity during postmortem examination. Aseptic swabs were taken from the peritoneal cavity and immediately inoculated in to 30 ml of buffered peptone water as well as BHI broth culture. After 24 hrs of incubation at 37°C, the cultures were placed on to Tryptose agar (Himedia) plus 5% bovine red blood cells on to MacConkey agar and incubated for 48 hrs at 37°C in aerobic, microaerophilic and anaerobic environments.

RESULTS AND DISCUSSION

Bacterial isolates were identified by morphologic (Gram's staining) and antibiotic sensitivity test. The number of isolates and each bacterial infection is depicted in Table 1.

Table 1: Isolation of Bacteria and Their Antibiotic Susceptibility in 15 Drugs

S.No	No. of eggs with bacterial isolate[a]	Isolate	Antibiotic susceptibility[b]
1.	3/15	Escherichia coli (y/a)	AN AMC CN CEF CIP ENO GM
2.	1/13	Enterobacter sakazakii (a)	CIP OT ENO
3.	4/10	Escherichia coli (y/a)	AMC D OT ENO
4.	1/15	Acromonas hydrophila (y)	ENO AMC CTX
5.	3/17	Klebshiella ornithinolytica (y/a)	ENO OT AMC
6.	2/10	Pasturella hemolytica (y)	RF CX AMC D SXT
7.	1/17	Acinobacter lwoffii (a)	AMC OT D
8.	3/10	Escherichia coli (y/a)	AN ENO CIP AMC
9.	1/5	Enterobacter ayglomeranes, (y)	OT D AN
10.	5/12	Enterobacter sakazakii (y/a)	D ENO CIP
11.	4/20	Escherichia coli (y/a)	CIP AMC ENO CTX
12.	2/10	Enterococcus aerogenus (y)	AN OT ENO
13.	1/15	Yersinia enterocolitica (y/a)	D AMC OT ENO
14.	1/10	Proteus mirabilis (a)	ENO K AN NOR CTX
15.	1/15	Pseudomonus luteola (y)	NOR DFX ENO AMC
16.	2/12	Staphylococcus aureus (y/a)	CX AMC CN CEF

Number of eggs culture positive/Number of eggs tested

Abbreviations:

AN*= amikacin;* ***AMC****= amoxicillin clauvulanic acid; CN= gentamicin ;* ***CEF****= ceftrioxone;* ***CIP:****= ciprofloxacin;* ***ENO****= enrofloxacin;* ***D****=doxicyclin;* ***OT****= oxytetracyclin;* ***CTX****= cefatoxime;* ***RF****= rifampicin;* ***CX****= cloxacillin;* ***SXT****= trimethoprim- sulphamethoxazole;* ***K****= kanamycin;* ***NOR****= norfloxacin;* ***DFX****= danofloxacin*.

Specifically, 35 (16.9%) ostrich eggs showed the presence of bacteria in yolk and albumen. *Escherichia coli* was the most frequent isolate (n=14 isolates) followed by strong prevalence of other *Enterobacteria* (Table 1). More over no multiple infections with different bacterial species were noticed for any egg,

which was well corroborated with the results of Deeming (1995). On the basis of data obtained from laboratory it is not possible to establish the origin of bacterial isolates. Bacteria isolated from the albumen could be related to high shell porosity, so that they may come from extra body contamination. However, the infection of both yolk and albumen could also indicate that both yolk and albumen can be potentially contaminated before the egg was laid and could be related to infection of the ovary, which was in concurrent with reported data of Huchzermeyer (1998). In this study the chilling of samples and the immediate processing at the laboratory was aimed at limiting cross contamination.

Infection of the oviduct by various microorganism decreases fertility. Antibiotic treatment was recommended in infected birds on the basis of an antibiotic susceptibility test. The bacterial isolates were tested for susceptibility to antibiotic discs according to the standard bacteriological disc diffusion method. For each bacterial strain 15 antibiotic drugs have been tested. Hicks (1993) and Huchzermeyer (1998) who reported that multiple etiological agents have been established from the oviduct, which causes oophoritis, salpingitis and metritis. However in the present study, *Escherichia coli* was the most frequent isolate (n=14), followed by the strong prevelance of other enterobacteria such as *Enterobacter sakazakii* (n=6), *Klebshiella ornithinolytica* (n=3), *Enterococcus aerogenus* (n=2), *Pasturella hemolytica* (n=2) and each one isolate of *Enterobacter ayglomeranes, Acromonas hydrophila, Acinobacter lwoffii, Proteus mirabilis and Pseudomonus luteola* respectively. This findings is well corroborated with reported data of Hawkins et al. (1991) and Gonzales et al. (1999). In this report, the presence of different bacteria in eggs from ostriches belonging to the same bird could be considered an indirect transmission of the origins of the bacterial agents from different species of animals reared in this farm. However, different antibiotic susceptibility patterns shown by strains belonging to the some bacterial species and isolated in the same flock could be a further indication of the different origin of the animal species.

In conclusion, that our evidence does not allow us to elucidate the pathogenic role of *Enterobacteria* isolated from ostrich eggs. However, the established potential role of many isolates in eliciting epidemiological surveillance on domesticated ostriches is of concern with regard to the environment.

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