BACTERIAL FLORA FROM DEAD-IN-SHELL JAPANESE QUAIL EMBRYOS AND THEIR DRUG RESISTANCE

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ABSTRACT

A study was carried out to determine the bacterial flora in dead in shell embryos from commercial quail hatcheries operating in Namakkal, Tamil Nadu. Among one hundred and twenty quail eggs failed to hatch at termination of incubation were collected, 58 isolates of bacteria comprising of *Escherichia coli* (29), *Klebsiella* spp (14), *Staphylococcus* spp (10), *Salmonella spp* (5) were cultured. The incubation period of Japanese quail eggs to hatch is 18 days. The level of bacterial contamination in quail eggs failing to hatch at 18th day of incubation was 70%. The antibiotic sensitivity pattern revealed that highest sensitivity were seen to Chloramphenicol (82.7%), Amoxycillin (70.6), Ciprofloxacin (60.3) and Gentamicin (48.27%) but were quite resistant to Oxytetracycline, Lincomycin and Co-trimaxazole.

KEY WORDS: Japanese quail, dead –in-shell embryos, bacterial flora, antibiotic sensitivity

INTRODUCTION

Japanese quail has been gaining popularity as a speciality food fetching a high premium. Japanese Quails have merits of short generation period, high rate of egg production, easy handling, disease resistant and less floor space. Hence, a sizeable number of commercial quail farming are being grown in India. (Anthony, *et al.,* 1996; Edwin, 2008). Hatchability is one of the important attributes for the success of poultry enterprises and is influenced by a number of factors like early embryonic mortality, dead in shell embryos etc. (Landauer, 1967).

Hatchery losses associated with embryonic mortality and dead in shell embryos in chickens due to pathogenic bacteria were well documented. (Harry,1957; McClenaghan, *et.al*., 1981; Orajaka & Mohan, 1985). However, bacterial flora associated with dead in shell embryos in Japanese quails is scanty. The present study was, undertaken to determine the bacterial flora in dead in shell embryos from commercial quail hatcheries operating in Namakkal, Tamil Nadu.

MATERIALS AND METHODS

Source of samples

One hundred and twenty Japanese quail eggs failed to hatch at 18th day of incubation were randomly collected from two commercial quail hatcheries in Namakkal. All samples were critically examined, and those with cracks and those from which embryos piped the shell but failed to emerge were discarded to minimize the incidence of extraneous contamination.

Processing of samples

The eggs were washed in warm water, dried with paper-towels and their shells were further disinfected by mopping with ethyl alcohol and allowed to dry. The eggs were then opened by making a circular cut with sterile scissors along the outline of the airspace. The infertile eggs were eliminated. In fully developed dead in shell embryos, the yolks were collected and homogenized aseptically.

Bacterial isolation and identification

The homogenized yolks were cultured to isolate bacteria by streaking 0.2 ml of the homogenized yolk on nutrient agar and inoculating in nutrient broth as well as selenite broth. The agar plates and broth tubes were incubated for 18 hrs at 37°C. The positive cultures were streaked onto bacteriological media purchased from Himedia, Mumbai such as Mannitol salt agar, MacConkey agar, Brilliant green agar and Eosin Methylene

blue agar for isolation, characterization and identification of the bacteria employing the standard bacteriological methods (Cowan and steel, 1974; Cruickshank,, et al., 1975).

The bacterial isolates were identified on the basis of their colonial, morphological, cultural, physiological and biochemical properties such as growth on MacConkey agar, Mannitol salt agar, Brilliant green agar, Triple sugar iron agar, IMViC test, catalase test, oxidase test, coagulase test as detailed by Cowan and Steel (1974)

Sensitivity testing

Antibiogram of bacterial isolates were determined by disc diffusion method (Bauer *et al*., 1966). The antibiotic discs used and their concentration were as follows. Amoxyxillin (10ug), Chloramphenicol (30ug), Ciprofloxacin (5ug), Co-trimaxazole (25ug), Gentamicin (10ug), Oxytetracycline (30ug) and Lincomycin (30ug). The criteria of Bauer, *et al*., (1966), based on the diameter of the zone of inhibition around a single high potency disc were used in classifying each culture as resistant, intermediate or susceptible.

RESULTS

Among one hundred and twenty quail eggs failed to hatch at termination of incubation were collected, thirty-two numbers of infertile eggs were discarded. Remaining eighty-eight fully developed dead in shell embryos were investigated for bacterial contamination. From these samples, 58 isolates of bacteria comprising of *Escherichia coli* (29 isolates), *Klebsiella* spp (14 isolates), *Staphylococcus* spp (10 isolates), *Salmonella spp* (5 isolates) were cultured. The predominant groups of organism present belonged to Enterobacteriacea and *Staphylococcus aureus.* Table-1 shows the number of bacterial isolates from dead in shell Japanese quail embryos.

The antibiotic sensitivity pattern of all isolates are shown in table-2. Among the 58 isolates, the most common pattern of sensitivity was Chloramphenicol (82.7%) and the lowest was Oxytetracycline.

DISCUSSION

As assessment of the incidence and type of bacterial contamination occurring in the eggs produced and hatched in commercial hatcheries is essential for understanding the role that micro-organisms play in influencing hatchability. Of the bacterial isolates recovered from the samples, the predominant species belong to Enterobacteriacea particularly *Escherichia coli*, which comprised 50% of the bacterial isolation. These results are largely in agreement with those of Bruce and Johnson (1978) with respect to the groups of bacteria recovered from dead-in-shell chicken embryos. But the relative proportion of the various groups differed considerably.

Elucidation of the role of microorganisms in influencing hatchability of eggs is not an easy task. Factors other than the presence of contaminating organisms can influence hatchability. However, *Escherichia coli*

Table.1.Bacterial isolates from dead in shell Japanese quail embryos

Bacteria	Bacterial isolates on the 18th day of incubation	
	Number isolated	% of species total
Escherichia coli	29	50.00
Salmonella sp.	5	8.62
Klebsiella sp.	14	24.13
Staphylococcus aureus	10	17.24
Total	58	

are known to cause yolk sac infection in embryos which results in embryonic mortality. (Saif, 2003). Also *Staphylococcus aureus* which inhabits the skin surface of the birds and then litters, has been reported to be capable of gaining access to the yolk sac through shell penetration and causing embryonic death. (Harry, 1957).

Salmonella organisms can gain entrance into the egg either through direct ovarian transmission or eggshell contamination and penetration. They are able to multiply in the yolk rapidly and subsequently infect the developing embryo, which may die. (Taylor, 1976).

In the present study, the level of bacterial contamination in quail eggs failing to hatch at 18th day of incubation was 70%. A higher proportion of bacterial contamination with Enterobacteriacea and *Staphylococcus aureus* assumed that infection of embryos with these organisms might have contributed to the embryonic mortality. This indicates that appropriate sterile precautions of incubators are demanded for getting good hatchability.

The antibiotic sensitivity pattern revealed that highest sensitivity were seen to Chloramphenicol (82.7%), Amoxycillin (70.6), Ciprofloxacin (60.3) and Gentamicin (48.27%) but were quite resistant to Oxytetracycline, Lincomycin and Co-trimaxazole. These findings correlated with the findings of Nazer and Safari (1994), Batabyal, *et.al*. (2003) and Bhattacharya (2005). Development of resistance to antibiotics may be due to indiscriminate use of these antibiotics as feed additive and prophylaxis as well as therapeutic agent.

Table.2. In vitro drug sensitivity pattern in bacteria isolated from dead-in-shell Japanese quail embryos

Organisms	No. tested	Drug -sensitivity pattern*						
		Am	C	Cf	Co	G	L	O
E.coli	29	20 (68.96)	28 (96.55)	18 (62.05)	7 (24.13)	15 (51.72)	9 (31.03)	-
Salmonella sp.	5	4 (80)	4 (80)	3 (60)	1 (20)	3 (60)	2 (40)	-
Klebsiella sp.	14	10 (71.42)	12 (85.71)	5 (35.71)	5 (35.71)	7 (50)	1 (7.14)	-
S. aureus	10	7 (70)	4 (40)	9 (90)	-	3 (30)	-	3 (30)
Total	58	41 (70.68)	48 (82.7)	35 (60.34)	13 (22.41)	28 (48.27)	12 (20.68)	3 (5.17)

* - Figures in parenthesis indicate percentage sensitivity

Am, Amoxycillin; C, Chlorampenicol ; Cf, Ciprofloxacin;

Co, Co-trimaxazole; G, Gentamicin; L, Lincomycin; O, Oxytetracycline

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