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## Isolation and identification of *Salmonella enterica* in commercial layer chicken affected with egg peritonitis

### Udhayavel Shanmugasundaram, Gopala Krishna Murthy TRG, Gowthaman V, Senthilvel K, Saranya S and Naagarjun A

### Abstract

The objective of the present study is to isolate *Salmonella enterica* associated with egg peritonitis in commercial layer birds and to determine their *in vitro* sensitivity to various antibacterial agents. Samples were collected from the birds which showed egg peritonitis lesions during necropsy carried out in Poultry disease diagnosis and surveillance laboratory, Namakkal from the period of May 2019 to February 2020. *Salmonella enterica* was isolated and identified by standard bacteriological techniques. The *in vitro* antibiotic sensitivity test with different antibiotic discs was carried out. Out of 50 samples screened, 4 isolates of *Salmonella enterica* were isolated. The *in vitro* antibiotic sensitivity test indicated that all the isolates (100%) were sensitive to Levofloxacin, Ciprofloxacin and Co-Trimoxazole. All the isolates showed resistance to Tylosin, Gentamicin and Chlortetracycline. This study revealed that *Salmonella enterica* also associated with egg peritonitis in commercial layer birds and the drug Levofloxacin and Ciprofloxacin are highly effective.

Keywords: egg peritonitis- Salmonella enterica antibiotic sensitivity

### 1. Introduction

Egg peritonitis is one of the major causes of morbidity and mortality for commercial layer chicken during laying period and gives the appearance of contagious disease. Egg peritonitis can be classified as non septic egg peritonitis and septic egg peritonitis. In non septic egg peritonitis there is no involvement of bacterial infections where as septic egg peritonitis is the most common type occurs due to contamination with bacteria like *E. coli* and others (Zanella *et al.*, 2000: Barnes *et al.*, 2008)<sup>[9, 2]</sup>. It is mainly diagnosed by observing the gross lesions in necropsy examination. It is characterized by fibrin or albumen like material with a cooked appearance among the abdominal viscera (Ate *et al.*, 2009)<sup>[1]</sup>.

Egg peritonitis (12.2%) was the high prevalent disease among all the poultry diseases recorded at Poultry disease Diagnosis and surveillance Laboratory, Namakkal during the period of 2011-2018 (Udhayavel *et al.* 2020)<sup>[8]</sup>. Bur, reports on the association of *Salmonella enterica* with Egg peritonitis in commercial layer birds in India are very scanty. In this study *Salmonella enterica* has been isolated from commercial layer chicken affected with egg peritonitis.

### 2. Materials and Methods

Layer birds of different age groups (21 weeks to 72 weeks) in the layer flocks located in and around Namakkal district of Tamil Nadu were brought to Poultry disease Diagnosis and surveillance Laboratory, Namakkal. During necropsy examination the birds showed characteristic fibrin or albumen like material with a cooked appearance among the abdominal viscera. Fifty peritoneal Samples were collected from the birds showed characteristic postmortem lesions and subjected to bacteriological isolation. The peritoneal swab samples were inoculated into Brain heart infusion agar and incubated overnight at 37 <sup>o</sup>C. Growth characteristics of the isolate were recorded. Then, two loopful of cultures from brain heart infusion agar were streaked onto the Brilliant Green agar (BGA) (Himedia) and incubated at 37 <sup>o</sup>C for 24 to 48 hours. Pink colour colonies from BGA agar suspected for Salmonella were inoculated into Triple sugar iron (TSI) slope and Salmonella differential agar (Himedia). Then biochemical tests like Indole, Methyl red, Voges proskauer's, Citrate utilization test, Urease test, Lysine utilization, ONPG and sugar fermentation tests were carried out for confirmation (Quinn *et al.*, 1994)<sup>[6]</sup>.

Finally, *In vitro* antibiotic sensitivity pattern of the bacterial isolates to various antibiotics has been detected by disc diffusion method as described by Bauer *et al.*, 1966<sup>[3]</sup>.

### 3. Results and Discussion

Out of 50 samples examined, 4 samples showed smooth transparent colonies in Brain heart infusion agar. These colonies revealed Gram negative coccobacillary organisms in Gram staining. In Brilliant green agar (BGA) the colonies from brain heart infusion agar showed typical pink colonies. In TSI slope the reaction observed as R/Y/H2S <sup>+</sup>. The characteristic Pink color colonies were also noticed in Salmonella differential agar. When the suspected Salmonella isolates were subjected to biochemical tests it produced positive reaction for Methyl red test; utilized Citrate and Lysine. These isolates were negative for Urease, Indole test, Voges proskauer's test and ONPG. The isolates fermented arabinose, maltose, sorbitol and dulcitol and not fermented lactose. Thus, four isolates were confirmed as *Salmonella enterica* by biochemical tests.

This study revealed that *Salmonella enterica* was isolated in 8% of the total samples collected from commercial layer birds affected with egg peritonitis. Srinivasan *et al.*, 2014 also isolated *Salmonella enterica* in commercial layer birds in India.

The *in vitro* antibiotic sensitivity test indicated that the all the isolates (100%) were sensitive to Levofloxacin, Ciprofloxacin and Co-Trimoxazole. Two isolates were Sensitive to Ampicillin (50%) and All the isolates showed resistance to Neomycin, Tylosin, Lincomycin, Erythromycin, Bacitracin, Colistin and Chlortetracycline. Khan *et al.*, 2005 <sup>[5]</sup> also observed the similar kind of sensitivity pattern. This kind of antibiotic resistance might be due to arbitrary use of antibiotics in poultry industry (Bonnet *et al.*, 2009) <sup>[4]</sup>.



Fig 1: Bird showing egg peritonitis lesion



Fig 2: Salmonella enterica in Brilliant Green Agar



Fig 3: Salmonella enterica in Salmonella Differential Agar



Fig 4: Antibiotic sensitivity pattern

### 4. Conclusion

It is concluded that *Salmonella enterica* has also been associated with egg peritonitis in commercial later birds. This study revealed that prevalence of *Salmonella enterica* in commercial layer birds might be a serious hazard for the poultry industry.

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