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## A report on incidence of avian Salmonellosis in poultry farm

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#### Abstract

The objective of present study is to identify infectious agent responsible for mortality in white laying hens. Total five white leghorn layer birds were presented for postmortem examination with the history of reduced feed intake, diarrhea, ruffled feathers and respiratory distress. On necropsy, caseous exudation from ocular sinuses, consolidated lungs, enteritis, degenerated ovaries and distended uterus were observed suspecting salmonella infection. Samples were aseptically collected from visceral organs showing lesions and inoculated in to nutrient broth and tetrathionate broth. After incubation gram staining was done and later Isolation and identification, biochemical tests were carried out to confirm the infectious agent involved. Cultural characteristics and biochemical characterization reveals the presence of salmonella sps infection in poultry farm. Finally Antibiotic sensitivity test was carried out to choose a proper antibiotic for treatment. and it was found that salmonella isolates were sensitive to ciprofloxacin, gentamicin and resistant to other antibiotics included in the present study.

**Keywords:** Salmonella sps, Kirby Bauer disc diffusion method, antibiotic resistance

#### Introduction

Indian poultry industry is growing and emerging as it bearing second rank in the world. Avian Salmonellosis is one of the vertically transmitted bacterial disease which causes huge economic loss to the poultry farmer in the form of mortality as well as drop in egg production. Salmonellosis is a main food borne bacterial diseases in humans [1]. and majority of outbreaks are due to intake of poultry products [2]. Salmonellosis is caused by Salmonella species belonging to family *Enterobacteriaceae*. It is a gram-negative bacilli, facultative anaerobic bacteria. At present more than 2500 serotypes of salmonella were identified. Two species namely *S. bongori* and *S. enteric* were recognized and was accepted by Judicial Commission of the International committee on Systematics of prokaryotes in 2005 [3]. *S. bongori* contain 21 serotypes which commonly affects cold blooded animals where as *S. enteric* contain six subspecies which affects warm blooded animals. Serotypes such as *S. Pullorum*, and *Gallinarum*, are host specific where as *Enteritidis*, *enterica* subspecies *arizonae*, and *Typhimurium* are non-host specific. *S. pullorum* and *Gallinarum* produce Bacillary white diarrhoea, fowl typhoid and clinical infection produced by non specific serotypes is collectively known as fowl paratyphoid. Salmonella infection is associated with drop in egg production, infertility and mortality [1]. Septicaemic salmonellosis is associated with lesions on different visceral organs like heart, liver, spleen, and mostly caeca [1, 4]. Barrow, Infected birds will excrete salmonella through faecal droppings, which act as main source of infection. Once the farm is contaminated, it is very difficult to eliminate the *Salmonella* infection [5]. Thus, the control of *Salmonella* in poultry flocks is crucial for poultry industry success.

#### Material and methods

Total five white leghorn layer birds were presented to department of veterinary pathology, College of veterinary science, Proddatur, India during august 2017 for postmortem examination with the history of reduced feed intake, drop in egg production diarrhoea, and respiratory distress.

#### Postmortem examination

Systematic postmortem examination was conducted [6] and characteristic gross lesions were noticed.

**Sample collection**

Samples were collected aseptically from heart, liver, lungs and intestines in duplicates following standard procedures.

**Isolation and Identification**

Samples were collected aseptically and immediately inoculated in to selenite broth (for enrichment and incubated at 37°C for 24 hrs. Isolation and identification is carried out according to protocols by OIE manual [7]. After incubation gram staining was done and streaked on to Mac conkey agar, Brilliant green agar, Xylose lysine deoxycholate agar and Salmonella-shigella agar respectively. Finally biochemical tests such as Catalase test, Oxidase test, motility test, Indole

test, Methyl red test, Vogesproskauer test, citrate test and urease test were done as per the protocols mentioned in the text book of Clinical Veterinary Microbiology by Markey *et al.* (2013) [8] for further confirmation.

**Antimicrobial susceptibility test**

After isolation and biochemical characterization individual colonies were inoculated in to nutrient broth and ABST was done using standard Kirby Bauer disc diffusion method on Muller Hinton agar following guide lines given by Clinical and Laboratory Standards Institute (CLSI, 2014) [9]. Concentration of Antibiotics (Himedia) and Zone of inhibitions used in present are mentioned in Table -1

**Table 1:** Susceptibility zone diameter break points of antibiotics (CLSI, 2014)

Antibiotic disc	Symbol	Concentration µg/disc	Interpretive criteria		
			Sensitive	Intermediate	Resistant
Cefotaxime	CTX	30 µg	≥ 26 mm	23-25 mm	≤ 22 mm
Ceftazidime	CAZ	30 µg	≥ 21 mm	18-20 mm	≤ 17 mm
Ceftriaxone	CTR	30 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Amikacin	A	30 µg	≥ 18mm	15-17 mm	≤ 15mm
Ciprofloxacin	CIP	5 µg	≥ 21 mm	16-20 mm	≤ 15 mm
Gentamicin	GEN	10 µg	≥ 15	13-14 mm	≤ 12 mm
Tetracycline	TE	30 µg	≥ 15 mm	12-14 mm	≤ 11 mm

**Results**

Total five white leghorn layer birds came for postmortem with the history of diarrhoea and respiratory distress.

**Postmortem examination**

On necropsy, caseous exudation from ocular sinuses, consolidated lungs (Pneumonia), catarrhal enteritis, degenerated, congested ovaries and distended ureters were observed. In bird hypoplasia and compensatory hypertrophy of kidneys were noticed. No characteristic lesions were founded in liver.



**Fig 1:** Swelling of eyes, accumulation of caseous material



**Fig 2:** Congested & Consolidated lungs



**Fig 3:** Distended Uterus



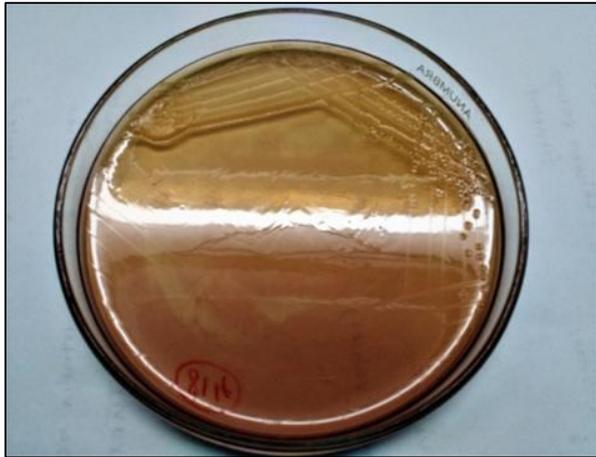
**Fig 4:** Complete degenerated ovarian follicles

**Isolation and Identification**

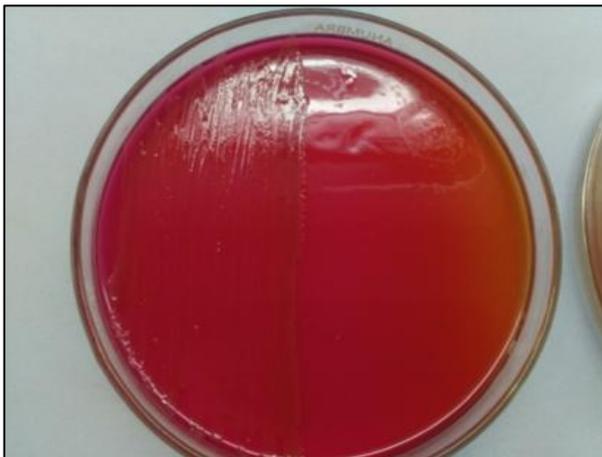
On grams staining pink color rods were observed indicating gram negative bacteria. Colorless colonies on MCA agar (Fig-1), red color colonies on brilliant green agar (Fig-2), red color colonies with black centers on XLD (Fig-3), and colorless colonies with black center on SS agar (Fig-4), were observed indicating salmonella sps. Biochemical characteristics and their results are mentioned in Table-2 & Fig-5, 6, 7, 8, 9, 10, 11x', 12.

**Table 2:** showing results of biochemical tests

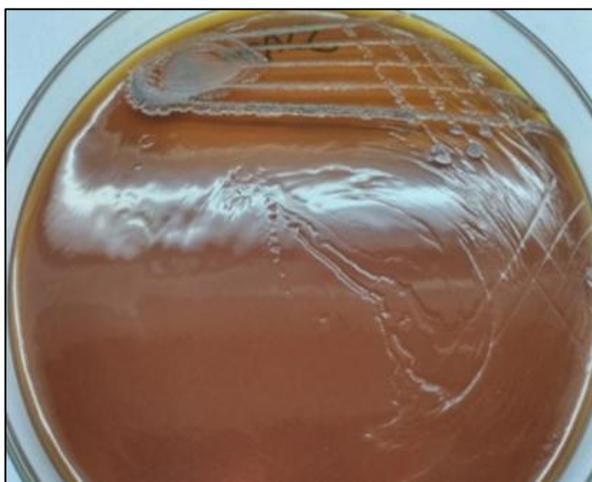
S. No	Name of the test	Result	Interpretation
1	Indole test	Colorless ring on top	Negative
2	Methyl red test	Bright red color	Positive
3	Voges proskeur test	colorless	Negative
4	Citrate test	Blue color	Positive
5	Urease test	Light pink color	Negative



**Fig 5:** Colorless colonies on Mac Conkey agar indicating Non-lactose fermenters (Salmonella sps)



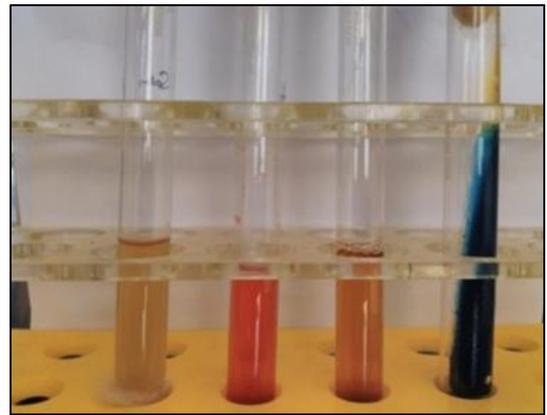
**Fig 6:** Pink color colonies on Brilliant green agar



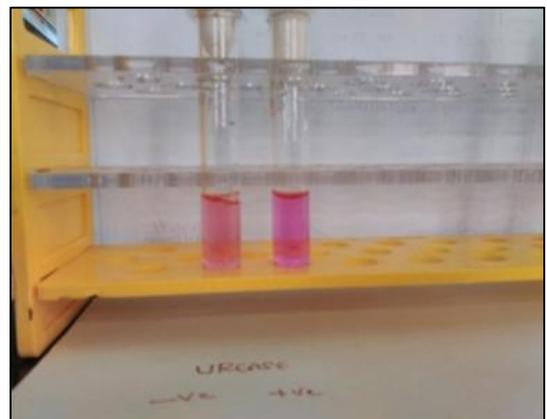
**Fig 7:** Colorless colonies with black centres of salmonella spp on SS agar



**Fig 8:** Pink color colonies with black centres of salmonella spp on XLD agar indicating H<sub>2</sub>S positive



**Fig 9:** ++-+ IMViC pattern indicative of *Salmonella sps* isolate.



**Fig 10:** light pink color (Left) indicating urease negative



**Fig 11:** Oxidase test negative (right)



**Fig 12:** Gas bubble formation indicating Catalase positive

**Table 3:** Results of Antimicrobial Suseptibility test

Antibiotic disc	Symbol	Concentration $\mu\text{g}/\text{disc}$	Diameter of Zone of inhibition in mm	Interpretation
Cefotaxime	CTX	30 $\mu\text{g}$	14mm	Resistant
Ceftazidime	CAZ	30 $\mu\text{g}$	18mm	Intermediate
Ceftriaxone	CTR	30 $\mu\text{g}$	15mm	Resistant
Amikacin	A	30 $\mu\text{g}$	10 mm	Resistant
Ciprofloxacin	CIP	5 $\mu\text{g}$	27 mm	Sensitive
Gentamicin	GEN	10 $\mu\text{g}$	20 mm	Sensitive
Tetracycline	TE	30 $\mu\text{g}$	5 mm	Resistant

## Discussion

Salmonellosis is one of the economically important infectious disease of poultry with worldwide in distribution. Several outbreaks of salmonellosis have been reported from India over the years <sup>[10, 11]</sup>. The gross lesions observed were similar to those described by Y.M Saif <sup>[12]</sup>. On initial isolation pure cultures are obtained from heart blood, liver, spleen and mixed cultures from intestinal contents. Isolation of infectious agent from parenchymatous organs is confirmatory for identification causative agent responsible for disease. The cultural characteristics and biochemical reactions are in accordance with reactions mentioned for salmonella sps <sup>[7]</sup>. In the present study, all the salmonella isolates showed complete resistance against cefotaxime, ceftriaxone, tetracycline, moderately sensitive to ceftazidime, and highly sensitive to ciprofloxacin followed by gentamicin. Singh *et al.* <sup>[13]</sup> in his study reported that salmonella sps is highly resistant to penicillin and highly susceptible to chloramphenicol followed by ciprofloxacin, cephalosporins and gentamicin. Rajagopal *et al.* <sup>[14]</sup> in his study reported that salmonella isolates are highly sensitive to ciprofloxacin followed by gentamicin, ceftriaxone, oxytetracycline and resistant to penicillin, erythromycin, ampicillin and cloxacillin. In the present study patterns of susceptibility and resistance to majority of antibiotics are in accordance with Singh *et al.*, 1999 and Rajagopal *et al.*, 2013 <sup>[13,14]</sup>. But in present study, salmonella isolates are showing resistance towards cefotaxime and ceftriaxone which was not much reported in previous studies.

## Conclusion

With the gross lesions, cultural and biochemical characteristics Salmonellosis was suspected. Based on antimicrobial sensitivity test, ciprofloxacin and gentamicin are suggested for treatment. Strict quarantine measures and disinfection procedures are suggested to control further spread of infection to other birds in poultry farm. Molecular diagnosis, serotyping are required for further confirmation and to identify the serotypes involved in outbreak.

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