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# Outbreaks of salmonellosis in three different poultry farms of Kerala, India

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PEER REVIEW

**Peer reviewer**

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**Comments**

This is a good study of three outbreaks of Avian salmonellosis in which the authors isolated/characterized the causative agent and compared their antibiogram profile. The results are significant in terms of antibiotic resistance profile which will prove useful in future outbreak cases.

Details on Page 500

ABSTRACT

Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries of Asia and Africa. Since no "effective" immunoprophylactic measures are available for the disease till date, strict biosecurity is the only alternative to preclude the disease. For formulating the control measures, an understanding of the epidemiology of the disease, proper diagnosis and identification of the causative agent is quintessential. This report sheds light on three different outbreaks of salmonellosis in three different farms in Kerala (India) describing the disease diagnosis, antibiotic resistance and the suggested control measures. All the three isolates were revealed to be *Salmonella gallinarum* and were resistant to at least three of the antimicrobial agents tested.

KEYWORDS

Avian salmonella, Biochemical identification, Antibiotic sensitivity testing, Drug resistance

## 1. Introduction

Salmonellosis caused by *Salmonella* sp., a Gram negative bacterium, is an important disease of chicken all over the world. Avian host specific salmonellae include *Salmonella gallinarum* (*S. gallinarum*) and *Salmonella pullorum* (*S. pullorum*) which causes fowl typhoid and pullorum disease respectively. Though they do not have zoonotic potential like *Salmonella typhimurium* or *Salmonella enteritidis*, they can cause severe mortality among chicken resulting in huge economic loss. Although, Indian poultry industry is evolving and emerging as the world's second largest market, fowl salmonellosis is increasingly rampant if not endemic, with a huge bearing on the economy as well as the future

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development of poultry sector.

From 1996 to 2008, typhoid was diagnosed several times in India but pullorum disease was reported only once during 2002[1]. Kumar *et al*. reported that out of 4672 isolates from different parts of India, 118 (6.5%) were *S. gallinarum*[2]. There are relatively less number of reports of salmonellosis from India despite its very high prevalence, which can be attributed to limited diagnostic facilities under field conditions and underreporting.

This report deals with three outbreaks of salmonellosis in three different Government owned poultry farms of Kerala State, India, *viz*., Regional Poultry Farm in Mundayad, Central Hatchery in Chengannur and University Poultry Farm in Mannuthy.

## 2. Case report

### *2.1 History and clinical signs*

The first outbreak occurred in the Regional Poultry Farm, Mundayad. Two live grower birds were presented to Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India during September 2005. The symptoms reported were drooping, reduced feed intake and death of grower birds. The second outbreak also took place during September 2005 at Central Hatchery, Chengannur. The authorities reported severe mortality among newly hatched chicks. Signs reported include reduced feed intake, ruffled feathers and diarrhoea. A few dead chicks of 5-6 days of age were presented for investigation. A third case was reported from Revolving Fund Hatchery at University Poultry Farm, College of Veterinary and Animal Sciences, Mannuthy during October 2005. It was reported that the day old chicks are weak and dying immediately after hatching along with an increased incidence of dead in shell. Two dead chicks were presented for bacteriological examination.

### *2.2 Isolation of the causative agent*

Post mortem examination was performed under aseptic conditions and organ samples from liver, spleen, gall bladder and heart blood were collected. The isolation of the causative agent was done using standard bacteriological methods with special emphasis on *Salmonella*[3]. The organ samples collected during post mortem examination were streaked onto brain heart infusion agar (BHIA) and to Mac Conkey lactose agar (MLA) (Himedia, Mumbai, India) for primary isolation. The gall bladder was gently cut using a flame sterilized scissors and the content was collected using a sterile cotton swab which was subsequently swabbed onto the surface of agars.

Intestinal contents were inoculated into 10 mL of buffered peptone water as pre-enrichment for *Salmonella*. A small portion of intestine (tied up on both ends with sterile cotton thread so that the contents will not leak out) was cut using sterile scissors and then immersed the tissue into buffered peptone water. Subsequent to an incubation period of 12 h at 37 °C, about 0.1 mL of the pre-enrichment broth was transferred to 10 mL of selective broth *viz*., Rappaport-Vassiliadis broth (Himedia, Mumbai, India) and it was then incubated at 42°C for 48 h. Following the incubation period, a loopful of inoculum was transferred to MLA; incubated for 24 h under aerobic condition in a bacteriological incubator (Cole-Parmer, India).

In the case of Mundayad birds, the post mortem revealed slight necrosis of liver and no other gross lesions. Soiled vent and liver necrosis were observed in case of birds from Chengannur. Birds from Mannuthy farm revealed necrosis of liver, air sacculitis, peritonitis and unabsorbed yolk.

Bacteriological culture from all the three cases revealed round translucent smooth convex colonies on BHIA after 24 h from spleen and liver. Yellow colonies were obtained on Mc Conkey agar which were suggestive of *Salmonella* sp. Pre-enrichment broth culture of intestinal contents on (brilliant green agar ) BGA revealed pink colonies with pink colouration of the surrounding media as well and some green colonies which revealed to be *Escherichia coli*. Gram staining of a single pink colony from BGA and BHIA revealed small Gram negative bacteria arranged singly or in pairs. The pure cultures of the isolates were stored in nutrient agar slants at 4 °C, until further characterization. For convenience, the isolates were named after the places from where they originated and were designated as Mundayad isolate (*Mund 1*), Chengannur isolate (*Cgnr 1*) and Mannuthy isolate (*Mthy 1*).

### *2.3 Biotyping*

Biochemical identification was done as described by OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Volume 1[3]. Briefly, the tests employed were catalase, oxidase, O/F test, motility test using motility medium, tripple sugar iron agar (TSI), urease, nitrate reduction, indole, methyl red, Voges Proskauer, citrate (IMVC), ornithine decarboxylase (ODB), lysine decarboxylase, growth on BGA, growth on MLA agar and various sugar fermentation tests. All the media used were procured from M/s Himedia (Mumbai, India).

The biochemical characterization revealed similar reactions for all three strains excepting the sugar reactions which were slightly varied for some sugars. All the three isolates were catalase negative, oxidase positive, O/F test fermentative, motility negative, TSI reaction showing alkaline slant, acid butt with black colouration due to production of hydrogen sulphide, urease negative, nitrate reduction positive, indole negative, methyl red positive, Voges Proskauer negative, Simmond's citrate positive (IMVC reaction pattern as - + - +), pink coloured colonies

**Table1**
Biochemical reactions of the *Salmonella* isolates.

| Test | Results | | |
|---|---|---|---|
| | *Mund 1* | *Cgnr 1* | *Mthy 1* |
| Gram Staining | Gram negative | Gram negative | Gram negative |
| Arrangement | Singly or in pairs | Singly or in pairs | Singly or in pairs |
| Oxidase | Negative | Negative | Negative |
| Catalase | Positive | Positive | Positive |
| Motility | Negative | Negative | Negative |
| O/F | Negative | Fermentative | Fermentative |
| ODB | Negative | Negative | Positive |
| Lysine decarboxylase | Positive | Positive | Positive |
| Urease | Negative | Negative | Negative |
| TSI | Alkaline slant, acid butt | Alkaline slant, acid butt | Alkaline slant, acid butt |
| H2S production | Positive | Positive | Positive |
| Gas production | Negative | Negative | Negative |
| Indole | Negative | Negative | Negative |
| MR | Positive | Positive | Positive |
| VP | Negative | Negative | Negative |
| Citrate | Negative | Negative | Negative |
| Nitrate Reduction | Positive | Positive | Positive |
| MLA | Yellow colonies | Yellow colonies | Yellow colonies |
| BGA | Opaque Pink colonies | Opaque Pink colonies | Opaque Pink colonies |
| Sugar utilization | | | |
| a) Lactose | Negative | Negative | Negative |
| b) Maltose | Positive | Positive | Positive |
| c) Fructose | Positive | Positive | Positive |
| d) Sucrose | Negative | Negative | Negative |
| e) Dextrose | Positive | ND | Positive |
| f) Galactose | Positive | Positive | Positive |
| g) Trehalose | Positive | Positive | Positive |
| h) Adonitol | Negative | Negative | Negative |
| i) Xylose | Positive | Positive | Positive |
| j) Mannitol | Positive | Positive | Positive |
| k) Dulcitol | Positive | Positive | Positive |
| l) Inocitol | Negative | Negative | ND* |
| m) Mannose | Positive | Positive | ND |
| n) Salicin | Negative | Negative | ND |
| o) Arabinose | Negative | Negative | ND |
| p) Raffinose | Negative | Positive | ND |
| Biotype | *S. gallinarum* | *S. gallinarum* | *S. gallinarum* |

* ND denotes not done.

on BGA and yellow coloured colonies on MLA. The entire biochemical reactions for the isolates are presented in Table 1.

### *2.4 Antibiotic sensitivity testing*

Antibiotic sensitivity testing was done using disc diffusion technique[4]. The following antibiotic discs are used: ciprofloxacin (10 μg), ampicillin (30 μg), gentamicin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), oxytetracycline (30 μg), cotrimoxazole (25 μg), streptomycin (10 μg), cloxacillin (10 μg), benzyl penicillin (10 IU) and erythromycin (15 μg). All the antibiotic discs were procured from M/s Himedia (Mumbai, India). The growth inhibition zones were measured and the degree of sensitivity was interpreted using National Committee for Clinical Laboratory Standards (1990) chart, provided along with the antibiotic discs.

*In vitro* antibiotic sensitivity of the isolates showed different patterns. All the three isolates showed complete

resistance to erythromycin, two isolates showed resistance to benzyl penicillin, at least one isolate showed resistance to cotrimoxazole, cloxacillin and ampicillin. All the strains were sensitive to at least four antibiotics and resistant to three, among the panel which we tried. The detailed sensitivity and resistance patterns are presented in Table 2.

**Table 2**
Antibiotic sensitivity and resistance profile of the *Salmonella* isolates.

| Isolate | Resistant | Moderately sensitive | Sensitive | Most sensitive |
|---|---|---|---|---|
| *Cgnr1* | P, E, S | CIP, CO | G, AMP, CTX, C | CTX |
| *Mund1* | E, CO, CX | P | CIP, G , AMP, CTX, OTC | AMP |
| *Mthy1* | P, E, AMP | G | C, CTX, CIP, CO | CTX |

P (penicillin), CIP (ciprofloxacin), G (gentamicin), C (chloramphenicol), CTX (ceftriaxone), OTC (oxytetracyclin), CO (co-trimoxazole), E (erythromicin), S (streptomycin), CX (cloxacillin), AMP (ampicillin)

## 3. Discussion

Salmonellosis is a very important disease of avian species because of its huge economic impact, worldwide distribution and difficulty posed in the control of the disease. Barrow *et al*. opined that *Salmonella* remain as a serious economic problem to livestock in countries where measures of control are not efficient or in those where the climatic conditions favour the environmental spread of these microorganisms[1]. India qualifies for both of the above descriptions.

Several outbreaks of salmonellosis have been reported from India over the years[5,6]. Fowl typhoid caused by *S. gallinarum* is a disease affecting grower birds than older birds and much less prevalent among chicks. The differentiation of *S. pullorum* and *S. gallinarum* cannot be made clearly from the disease symptoms and lesions as lesions produced by certain strains of *S. gallinarum* in chicks are indistinguishable from those produced by *S. pullorum*. The gross lesions observed were similar to those described by Shivaprasad[7].

Rappaport-Vassiliadis broth is considered superior for pre-enrichment of intestinal contents[8]. From liver and spleen, we obtained pure cultures. But mixed cultures were obtained from heart blood and intestinal contents even after pre-enrichment. *S. gallinarum* and *S. pullorum* strains have very similar biochemical reactions, but they can be differentiated by their ability to decarboxylase ornithine and metabolize sugars like dulcitol, maltose and rhamnose[3]. Isolates typical of *Salmonella* not fermenting dulcitol and decarboxylating ornithine were considered *S. pullorum*. *S. gallinarum* mostly ferments dulcitol and maltose but not rhamnose. *S. pullorum* ferments dulcitol and rhamnose, but most strains give a negative reaction to maltose. The biochemical reactions obtained for our isolates were in accordance with the classical reactions reported for *S. gallinarum*. In the present study, all the three isolates fermented dulcitol and maltose. Rhamnose was not included in the panel of sugars we tested. *Mund 1* and *Cgnr 1* showed a negative ODB but *Mthy1* decarboxylated ornithine. Such an aberrant reaction for *S. gallinarum* was previously reported by Crichton and old[9], where one out of 50 of their *S. gallinarum* strains showed a positive reaction for ODB.

Epidemiology of salmonellosis is complex. Kumar *et al*. established the role of hatcheries in spreading the *Salmonella* infection[5]. More than one third (37.8%) of hatcheries they studied were found to be infected with *Salmonella*. The maximum number of outbreaks (n=96) was recorded in the age group of 7-9 d while the maximum mortality was found in chickens of 1-2 weeks of age, which was true in our case also. Vertical transmission cannot be ruled out atleast in case of Chengannur farm, as this outbreak coincided with the import of new stock. First indication of salmonellosis in a farm is an excessive number of dead-in-shell chicks and deaths shortly after hatching as observed in the case of Mannuthy farm. The control of *Salmonella* is difficult as *Salmonella* can remain in the environment. Rodents also play an important role in the persistence of *salmonella* in poultry farms[10].

Various antimicrobial agents have been used for curbing the mortality. The antibiotic resistance of *Salmonella* strains of avian origin is attributed to chromosomal mutation, gene transfer mechanisms like conjugation, transduction and transformation. Avian *Salmonella* shows resistance against many antimicrobials; tetracycline, oxy tetracycline, penicillin, aminoglycosides, sulpha drugs and fluoroquinolones[6,11,12]. In this study, all the strains showed complete resistance against at least three antibiotics and some were only moderately sensitive which will become resistant in near future. Singh and Gupta observed considerable variation in the resistance pattern of different isolates of *Salmonella* and the isolates showed 100% sensitivity to chloramphenicol, ciprofloxacin, cephazoline, gentamicin and 100% resistance to penicillin G; which correlated with our results[13]. With the bacteria gaining antimicrobial resistance over time, the situation is alarming in that the scope for treatment is getting limited and narrowed down to currently sensitive antibiotics, for which the bacteria will eventually acquire resistance since they will be used excessively and indiscriminately. The prophylactic use of many antimicrobials in poultry feed can also lead to acquired antibiotic resistance[14].

The birds were treated with ceftriaxone and it contained the infection in all the farms. In addition, disinfection of the entire farm by formaldehyde spraying and fumigation also helped in control of the disease. The farm authorities were advised to stop all hatching operations, screen all birds with on-the-spot slide agglutination test for salmonellosis, cull all the positive reactors, make the sheds rodent proof, ensure thorough disinfection of incubators and to periodically screen the birds for *Salmonella*.

*Salmonella* outbreaks can seriously affect the functioning

of poultry farms and hatcheries. Disinfection and eradication measures are extremely tedious and chances of further infections are many fold after an initial attack. Futher studies of these strains like PCR identification, plasmid analysis, virulence studies, outer membrane proteomics and serotyping are to be done.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

*Background*

This is an interesting manuscript reporting the prevalence of *Salmonella* species in three different farms in Southern part of India. The study is very significant because understanding the biotype of *Salmonella* species is critical to implement prophylactic and therapeutic measures.

*Research frontiers*

Molecular identification and characterization using PCR, ribotyping, plasmid profiling, proteomics *etc*.

*Related reports*

Limited reports are available regarding the species specific characterization of *Salmonella* during outbreak from India particularly from Kerala.

*Innovations and breakthroughs*

Although current case report didn't use innovative techniques, the report has practical application in disease diagnosis and designing treatment strategies.

*Applications*

The isolation and characterization of the causative agent is very important for the effective control and prevention of any disease. The present study gives an idea about the prevalence of Salmonella in poultry farms, techniques for isolation, identification of the causative agent and the control measures which should be implemented in case of an outbreak. The study also provides information about the antibiotic sensitivity and resistance pattern of the bacteria which will help in the selection of a suitable antibiotic in case of a future outbreak.

*Peer review*

This is a good study of three outbreaks of Avian salmonellosis in which the authors isolated/characterized the causative agent and compared their antibiogram profile. The results are significant in terms of antibiotic resistance profile which will prove useful in future outbreak cases.

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