

Salmonellosis in Japanese Quails – A Report From Central Kerala, India



MICROBIOLOGY

KEYWORDS : Salmonellosis, Japanese quails, Salmonella Gallinarum

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ABSTRACT

The present study deals with the isolation, biochemical characterization and antibiogram of Salmonella enterica subsps. Gallinarum from Japanese quails in a private farm in central Kerala. There were reports of heavy mortality among birds of 5 to 7 months of age in the farm. Necropsy of the birds revealed lesions suggestive of fowl typhoid. The organ samples were cultured on enrichment and selective media and the colonies obtained were subjected to further biochemical characterization and the organism was proved to be S. Gallinarum. The antibiotic sensitivity pattern of the isolate revealed that it was completely resistant to Streptomycin, Ampicillin, cotrimoxazole, oxytetracycline and Augmentin. They were sensitive to Ceftriaxone and Chloramphenicol and moderately sensitivity to Ciprofloxacin and Gentamicin.

Introduction

Salmonellosis is a fatal disease of poultry causing severe mortality resulting in massive economic loss. *Salmonella enterica* subsp. Gallinarum (*S. Gallinarum*) and *Salmonella enterica* subsp. Pullorum (*S. Pullorum*) are the species responsible for fowl typhoid and pullorum disease respectively. The disease affects a wide variety of avian species including chicken (Arora et al., 2013), duck (Cha et al., 2013), emu (Vanhoeser and Welsh, 1995) and turkey (Osman et al., 2010). The control of the disease mainly relies on the use of antimicrobial drugs. This leads to indiscriminate use of antimicrobial drugs in poultry industry that results antibiotic resistance and limits the therapeutic possibilities in the treatment of bacterial diseases. Despite its very high prevalence, salmonellosis in Japanese quails is rarely reported from Kerala. This report deals with an outbreak of salmonellosis in a private quail farm in the state of Kerala, India and the antibiogram profile of *S. Gallinarum* isolated from the cases.

Materials and methods

History and clinical signs

The outbreak occurred in a private farm in Perumbavoor, central Kerala. Three ailing birds of 7 months of age were presented to Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India during May 2011. The symptoms reported were drooping, reduced feed intake, ruffled feathers, whitish diarrhoea and death.

Isolation of the causative agent

Necropsy was performed aseptically and organ samples from liver and spleen as well as heart blood were collected. The isolation of the causative agent was done using standard bacteriological methods (OIE, 2008). The media and the antibiotic discs used were procured from M/s Himedia (Mumbai, India). The organ samples collected during post mortem examination were cultured onto brain heart infusion agar (BHIA) and to Mac Conkey agar (MCA) for primary isolation. A small portion of intestine (tied up on both ends with sterile cotton thread) was cut using sterile scissors and then immersed into 10 mL of buffered peptone water as pre-enrichment for *Salmonella*. After incubating for 12 h at 37 °C, 0.1 mL of the pre-enrichment broth was transferred to 10 mL of Rappaport-Vassiliadis broth which is selective for *Salmonella* and incubated at 42°C for 48 h. A loopful of inoculum was transferred to MCA; incubated for 24 h under aerobic condition in a bacteriological incubator.

Biochemical identification was done as described by OIE

(2008). Briefly, the tests employed were catalase, oxidase, Oxidation Fermentation, motility test using motility medium, triple sugar iron agar (TSI), urease, nitrate reduction, indole, methyl red, Voges Proskauer, citrate (IMVC), ornithine decarboxylase, lysine decarboxylase, growth on BGA, growth on MCA agar and various sugar fermentation tests.

Antibiogram

Antibiotic sensitivity testing was done using disc diffusion technique (Bauer et al., 1964). The following antibiotic discs are used: cotrimoxazole (25 µg), gentamicin (10 µg), ciprofloxacin (10 µg), oxytetracycline (30 µg), ampicillin (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), streptomycin (10 µg) and Augmentin (30 µg). The growth inhibition zones were measured and the degree of sensitivity was interpreted using National Committee for Clinical Laboratory Standards chart, provided along with the antibiotic discs.

Results

The post mortem examination revealed air sacculitis, peritonitis, perihepatitis, hepatomegaly with bronze discolouration and oophoritis (Figure 1). On BHIA, round translucent smooth convex colonies could be observed after 24 h from spleen and liver. Yellow colonies obtained on MCA 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. Gram staining of colony revealed Gram negative short rods arranged singly or in pairs.

Biotyping

The results are presented in Table1.

Table1. Biotyping of the isolate

TEST	RESULT
Gram's Staining	negative
Shape	Short rod
Motility	negative
Catalase	positive
Oxidase	negative
O/F Test	fermentative
Tsi	alkaline slant, acid butt with black coloration (H ₂ S production)
Indole	negative
Methyl Red	positive

Voges Proskauer	negative
Simmond's Citrate	positive
Urease	negative
Nitrate Reduction	positive
Lysine Decarboxylase	positive
Ornithine Decarboxylase	positive
Sugar Utilization	
Lactose	negative
Maltose	positive
Fructose	positive
Sucrose	negative
Dextrose	positive
Galactose	positive
Trehalose	positive
Adonitol	negative
Inositol	negative
Mannose	positive
Arabinose	negative
Raffinose	negative
Rhamnose	negative
Xylose	positive
Mannitol	positive
Dulcitol	positive

Antibiogram

The isolate showed complete resistance against the antibiotics Streptomycin, Ampicillin, cotrimoxazole, oxytetracycline and Augmentin. They were sensitive to Ceftriaxone and Chloramphenicol and moderately sensitivity to Ciprofloxacin and Gentamicin.

Discussion

Fowl salmonellosis instigates enormous impact on the economy in the form of epidemics and difficulty posed in the control of the disease. The humid climatic conditions and paucity of efficient control measures favour the environmental spread of these microorganisms and thus *Salmonella* remain as a serious economic problem (Barrow & Frietas Neto, 2011). Quail farming is in expansion in the state of Kerala and the report of an outbreak of salmonellosis in a private quail farm needs immediate attention. Several outbreaks of salmonellosis in different avian species have been reported from India over the years and *S. Gallinarum* constitutes 6.5% of the poultry isolates (Kumar et al., 2010). *Salmonella enteritidis* had been isolated from quail eggs previously (Erdogrul et al., 2002). Recently *Salmonella Gallinarum* was proved to be pathogenic in Japanese quails (Rocha-e-Silva et al., 2013). The present study deals with the isolation of *Salmonella Gallinarum* from Japanese quails in a private quail farm in central Kerala.

The organism was isolated at a rate of 2.3% and 0.8% from the yolk sacs of eggs of orally infected and egg-infected adult Japanese quails birds respectively (Awaad et al., 2010). This result confirmed that *Salmonella Gallinarum* infection could be transmitted vertically in Japanese quail in a similar way to that in chicken. However, the organism is transmitted usually through faecal oral route and pass through the crop prior to entering the proventriculus and gizzard. The acidic pH of crop induces acid adaptation mechanisms that help in passing through the proventriculus and gizzard (Chappell et al.,

2009). The main site of *Salmonella* colonization is the ceca. The organisms are taken up by macrophages or dendritic cells following intestinal invasion and transported to the spleen and liver. Replication occurs in the spleen and liver leading to lesions and can shed back into the gastrointestinal tract. This will result in death of the bird within 6 to 10 days following infection (Shivaprasad, 2000). The lesions produced by certain strains of *S. Gallinarum* are indistinguishable from those produced by *S. Pullorum* and the differentiation of pullorum disease and fowl typhoid cannot be made obviously after necropsy, necessitating biochemical characterization of the organism. In the present study, the gross lesions observed were similar to previous reports (Rocha-e-Silva et al., 2013; Shivaprasad, 2000). From liver, heart and spleen, pure cultures could be obtained which were characterized as *S. Gallinarum*. But mixed cultures were obtained from intestinal contents even after pre-enrichment.

Various antimicrobial agents have been used for restricting the mortality. Avian *Salmonella* was reported to have resistance to many antimicrobials like tetracycline, oxytetracycline, penicillin, aminoglycosides, sulphadiazine and fluoroquinolones (Taddele et al., 2012, Sjolund-Karlsson et al., 2010). According to some other reports, the isolates were sensitive to ciprofloxacin, nitrofurantoin, Sulphamethoxazole-trimethoprim and amoxicillin, tetracycline and resistant to penicillin-G and erythromycin (Akter et al. 2007). In another study, the isolates demonstrated poor susceptibility to oral antibiotics including Nalidixic acid, Ciprofloxacin, Ofloxacin, Azithromycin, Amoxicillin, Tetracycline, Ceftriaxone and Trimethoprim-sulfamethoxazole whereas 100% susceptibility to Chloramphenicol. Substantial variations in the resistance pattern of different isolates of *Salmonella* were observed by Singh and Gupta (1999) and the isolates showed 100% sensitivity to chloramphenicol, ciprofloxacin, cephazoline, gentamicin and 100% resistance to penicillin G. In the present study, the isolate showed complete resistance to the antibiotics Streptomycin, Ampicillin, cotrimoxazole, oxytetracycline and Augmentin. They were sensitive to Ceftriaxone and Chloramphenicol and moderately sensitivity to Ciprofloxacin and Gentamicin which may become resistant in near future. The antibiotic resistance of *Salmonella* strains of avian origin is attributed to chromosomal mutation or genetic recombination. The possibility for treatment is getting restricted to presently sensitive antibiotics, for which the bacteria will ultimately attain resistance as they will be used indiscriminately. The prophylactic use of many antimicrobials in poultry feed can also lead to acquired antibiotic resistance (Iovine & Blaser, 2004). In the present case, the birds were treated with ceftriaxone and it contained the infection in the farm.

Salmonellosis is having complex epidemiology. Majority of bacteria are cleared by the immune response. Carrier state infections frequently occur in birds more than a few days old due to the presence of bacteria persisting within intracellular niches (Chappell et al., 2009). Hatcheries play a vital role in spreading the *Salmonella* infection (Kumar et al., 2010). The control of infection is difficult as the organisms can remain in the environment. The role of rodents in persistence of salmonella in poultry farms cannot be underestimated and the infections can seriously affect the functioning of poultry farms. Since the disinfection and eradication measures are very tiresome, there is every chance of further infections. In the present case, the farm owners were advised to disinfect the entire farm by formaldehyde spraying and fumigation, keep the sheds rodent proof, confirm thorough disinfection and to periodically screen the birds for *Salmonella*.

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Figure 1. Necropsy showing hepatomegaly, perihepatitis and airsacculitis



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