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Isolation and identification of septicaemic pathogens in Japanese quails

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Abstract

Salmonellosis is a major zoonotic bacterial disease causing huge economic losses in the poultry industry globally. This study was carried out in response to long term production losses in a private commercial quail farm in Tamil Nadu. Septicaemia due to Mannheimiasis and virulent strains of Avian Pathogenic *E. coli* were recorded. After differential diagnosis, the major determinant of the disease outbreak was confirmed as *Salmonella arizonae* serovar. In all, *Salmonella* was isolated from 23 cloaca swabs (31%), 11 from intestines (15%) and 9 from the liver (12 %). From 9 different intestines *E. coli* was isolated (10%), 2 samples from bone marrow was positive for *M. haemolytica* (3%). As per AST *Salmonella arizonae* in Japanese quails was susceptible to Ciprofloxacin and Cefotaxime. The multiple infection was confirmed by detection of virulence genes (*invA*) and (*stx-1* and *eae A*) using specific primers by polymerase chain reaction for *Salmonella* and APEC strains respectively.

Keywords: *Salmonella arizonae*, *invA* *stx-1*; *stx-2*; *eaeA* genes, PCR, APEC, Japanese quails

Introduction

Poultry farming plays a major role in providing subsidiary income and nutritional security to the small-scale farmers of India. Presently in India quails have become the third largest avian species to be reared next to chicken and ducks Asha R.R. (2011) [2]. Japanese quail belongs to the order Galiformes, family Phasianidae, genus *Coturnix* and species *japonica*. In India two species are commonly noticed namely Black breasted quail found in jungle (*Coturnix Coromandelica*) and the Brown coloured Japanese quail (*Coturnix Coturnix japonica*), which is raised commercially. In Tamil Nadu, the climate is suitable for quail rearing as they adapt quickly to environmental fluctuations. Quail farming has always been farmer friendly due to its low capital investment, minimal labour cost, short lifespan, good returns from eggs and meat in minimal space requirement (Leonel Rabins and Sethupathi, 2022) [12]. Quail eggs are nutritive with increased amount of lysozyme-a natural antimicrobial enzyme which kills harmful bacteria. Also, quail meat is rich in protein with fewer calories (Chindo and Olowoniya, 2006) [4] and has medicinal qualities with a unique flavour (Jeke *et al.*, 2018) [9]. Quails have better immunity against diseases than chicken and are considered resilient (Khoshbakht *et al.* 2017) [10]. However, certain management lacunae, specific lethal infections, nutritional deficiencies, non-infectious and parasitic diseases etc., impede quail production. Intensive farming methods to commercially raise quails makes them vulnerable to severe septicaemic infections of concern like Mannheimiasis and Salmonellosis. Other common diseases include ulcerative enteritis, omphalitis, coccidiosis etc. (Shanaway, 1994) [16]. Salmonellosis is a major poultry disease worldwide. It is zoonotic and needs to be constantly surveyed (Shivaprasad and Barrow 2008) [17]. Salmonellosis is a systemic bacterial infection that results in high rates of mortality and morbidity with high productive influence (Barrow and Freitas Neto 2011) [3]. Mannheimiasis is yet another acute septicaemia to which quails are vulnerable as they are to virulent strains of avian pathogenic *Escherichia coli* strains which cause extra-intestinal infections in various organs.

The present study was conducted to isolate and characterize septicaemic pathogens from diseased quails reared in a private quail farm in Tamil Nadu.

Materials and Methods

Clinically ill and dead quails from a disease outbreak in a private quail farm were investigated. History, clinical signs, and post mortem lesions were suggestive of mixed infections due to septicaemia that had caused sudden mortality with few lesions. Hence it was given least attention by mere burial and disinfection.

- **History:** Problem was said to be present persistently over a period of 3 months and had escalated with increased mortality and severe morbidity by 3 months. Young birds exhibited mortality in the first 3 weeks of life and were stunted.
- **Clinical signs:** Diseased quails showed laboured respiration. Typical signs included somnolence, closed eyes, drooped wings, ruffled feathers and huddling near heat sources. They were anorectic and emaciated. Convalescent birds exhibited acute illness and ruffled feathers. Diarrhoea was distinct sulfur, white or green in

colour or bloody in nature. Severely infected quail chicks, (3 weeks) had swollen eyes and joints. (Kumar *et al.*, 2001)^[11].

- **Post mortem examination:** At necropsy a systemic dissection was made and gross changes in different tissues were recorded and suitably collected. There was generalized tissue congestion and hemorrhages in the lungs. The dark friable and bronze coloured enlarged liver was suggestive of fowl typhoid. Spleen was enlarged, in most birds. There was necrotic foci on entire liver parenchyma suggestive of Mannheimiosis. There was catarrhal inflammation of the intestine with petechial hemorrhages. Cheesy caeca cores clogged the ileo-caecal junction. Many birds had hemorrhagic ovarian follicles and unabsorbed yolks. (Erdogru *et al.*, 2002)^[6].
- **Sample collection:** A total of 73 samples were collected from quails suffering from septic mortalities. During post mortem examination, heart blood was collected aseptically from 10 birds. Deep swab of cloaca, intestinal contents and pieces of ovary, follicles, liver and long bone marrow (femur) were collected for bacteriological examination (Figure 1).

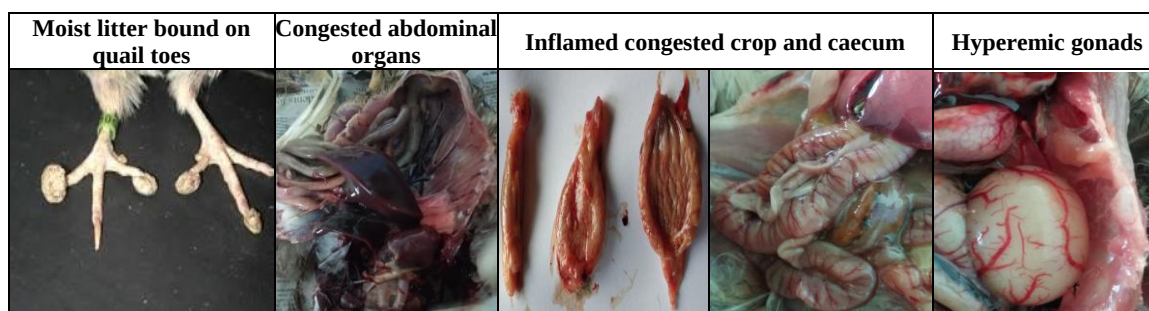


Fig 1: Postmortem examination of Japanese quails

- **Bacterial isolation:** Samples were inoculated immediately into 5ml of Selenite Broth (e.g., Selenite Cysteine or Selenite F) as the primary selective agent to inhibit growth of faecal coliforms and for selective enrichment of Salmonella organisms for 6-8 h. After selective enrichment, one loopful of each sample's culture was streaked onto on to MacConkey agar (MacC), Brilliant Green Agar (BGA), Xylose-Lysine-Deoxycholate (XLD) agar, Eosin Methylene Blue agar and 5% sheep Blood agar. (Quinn *et al.*, 1994)^[14] Mixed type colonies were re-inoculated in 5ml of Rappaport-Vassiliadis (RV) Medium which has a low pH to select for Salmonella and incubated at elevated temperature of 42 °C. Samples were also inoculated in Nutrient and Brain Heart Infusion broth and incubated at 37°C for 16-18 h at 70rpm/min.
- The non-lactose fermenting pale colonies and pink colonies on MacConkey and dewdrop like translucent colonies on BHI agar were stained by Gram's method. All bacterial isolates were phenotypically characterized by biochemical tests viz. IMViC pattern, TSI and urease slants and by sugar fermentation.
- **Blood examination:** Blood was directly collected from the heart and jugular veins of ailing quails and a thin blood smear was prepared. Impression smears were made with cut surfaces of liver and spleen on glass slides, air dried and stained by Leishman's method.
- **Antibiotic Sensitivity Test:** For this, 10 different antibiotics were used against all the isolates obtained viz.,

E. coli (20%); *Mannheimia haemolytica* (04%) and Salmonella serovar (59%), as per (Finegold SM and Martin WG, 1982)^[7]. AST was performed in Muller Hinton agar by disc diffusion method as per Kirby and Bauer (1982) with FOS: Fosfomycin (300µg), NX: Norfloxacin (10 µg), DoHcl: Doxycycline hydrochloride (30µg), AMP: Ampicillin (10 µg), AMX: Amoxicillin (10 µg), CTX: Cefotaxime (30 µg), E:Erythromycin (15µg), CTR: Ceftriaxone (30mcg) GEN: Gentamicin (30mcg) and CIP: Ciprofloxacin (5µg). Interpretation of the zone of inhibition was done according to (CLSI M02, 2024)^[5].

- **Genotypic characterization:** Detection and confirmation of virulence genes of *Salmonella arizonae* serovars, *Mannheimia* spp and APEC strains was also done by PCR. (Zhang *et al.*, 2017)^[19] For all samples DNA was extracted from bacterial cultures using the QIAamp DNA Mini kit (Qiagen, Germany) with some modifications. PCR was done as 20µl per reaction using Promega Green amplicon master mix with gene specific primers.
- **Analysis of the PCR Products:** After the completion of reaction cycles, the amplified products were electrophoresed on 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml). The images of stained DNA bands were analyzed under ultraviolet trans-illuminator and digitized using a gel documentation system (Bio-rad, USA).

Results

In all, 23 *Salmonella* was isolated from deep cloaca swabs (32%), 11 was from intestinal ulcers (15%) and 9 from liver (12%) as reported from Kerala in India (Ambily and Mini, 2014) [1]. From 9 different intestines 07 *E. coli* was isolated (10%), from 03 spleen and 05 ovaries 2 (3%) and 3

(4%) *E. coli* isolates were obtained. From 05 caecum samples 02 was *E. coli* (3%). From 02 bone marrow 2 isolates (3%) and from 05 heart blood samples 01 isolate (1%) was positive for *M. haemolytica* respectively (Table 1). The same is depicted in Figure 2.

Table 1: Details of bacterial isolates that caused mixed septicaemia infections in J. quails

No	Sample type	NOS	Bacterial isolate	Total (+ve)	+ve (%)	Overall (+ve) & (%)
1	Cloacal swabs	25	<i>Salmonella arizonae</i> serovars (N=43)	23	32	43 Nos (59%)
2	Intestinal ulcers	09		11	15	
3	Liver	11		09	12	
4	Spleen	03	<i>Escherichia coli</i>	02	03	07 Nos (10%)
5	Ovaries	05		03	04	
6	Caecum	05		02	03	
7	Heart blood	05	<i>Mannhiemia haemolytica</i>	01	01	03 Nos (4%)
8	Bone marrow	02		02	03	
9	Intestinal content	08	<i>Escherichia coli</i>	07	10	07 Nos
	Total sample	73	3 types	60	-	(10%)

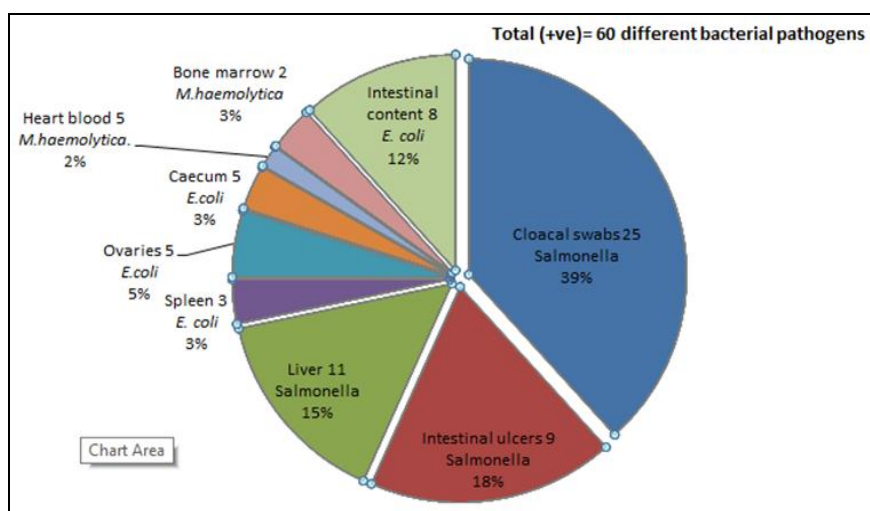


Fig 2: Bacterial pathogens isolated from different clinical samples of diseased J. quails

The results of blood smears stained by Leishman's method, depicted presence of bipolar organisms under oil immersion microscopically. As per cultural characteristics, on Brain Heart Infusion agar *Mannheimia haemolytica* strains were observed typically as translucent dew drop like colonies and also it grew on MacConkey agar as pink, minute, moist colonies and when streaked on Blood agar produced beta haemolysis suggestive of *M. haemolytica*. The culture was Gram negative cocco-bacilli.

Colonies from BGA when stained was long slender Gram-negative rods and was *Salmonella* spp. It appeared as translucent colourless colonies with or without black centres on XLD agar also grew on MacConkey agar as colourless non-lactose fermenting colonies. The same was tested by IMViC and on TSI slants for biochemical differentiation (Quinn *et al.*, 1994) [14]. Lactose fermenting colonies on MacConkey agar produced pink colonies and stained as Gram negative rods. All *E. coli* strains produced metallic sheen on Eosin Methylene Blue agar.

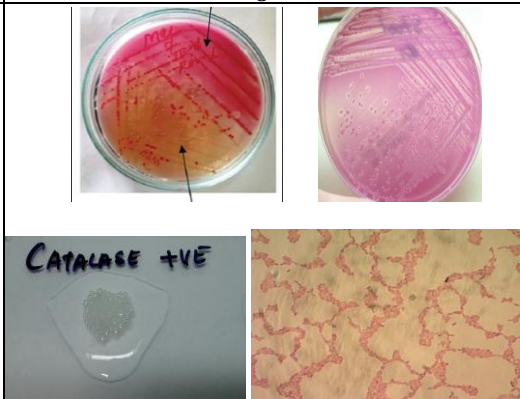
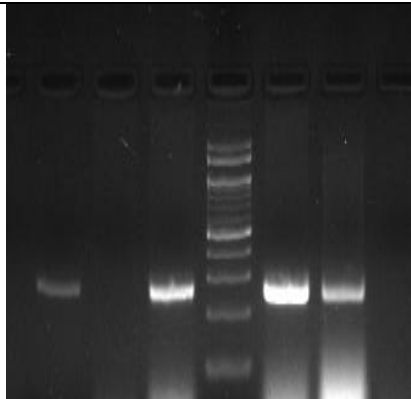
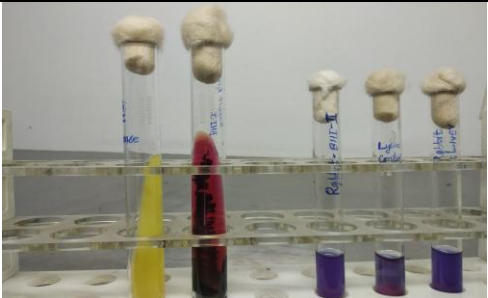
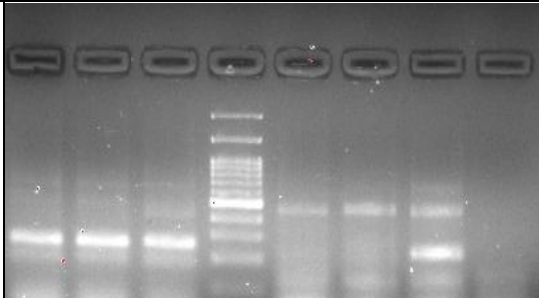
Salmonella enterica subsp. *arizonae* is a Gram-negative, motile, facultative anaerobic, rod-shaped bacterium. It was motile. Biochemical tests gave the following results. Isolates were negative for Oxidase and did not give blue colour on discs. It was positive for catalase with effervescence. Fermentation tests revealed a rapid lactose fermentation

observed in 18-20 hours. Glucose fermentation was normal and produced bubbles due to gas production with yellow slant/butt with and typically red (unfermented) or yellow (non-sucrose-fermenting) butt. There was black discoloration on TSI slant due to production of hydrogen sulphide (H₂S) which had a foul rotten egg odour as given in the image. For the IMViC test, isolates were negative for Indole production. They were MP positive and VP negative (Figure 3).

They were able to utilize citrate as a carbon source and were positive on green citrate slants changed to indigo blue. They were Lysine decarboxylase positive and negative on Urease slants. These biochemical tests aid to differentiate *S. arizonae* from other bacteria within the *Enterobacteriaceae* family.

S. enterica subsp. *arizonae* is defined by its ability to ferment lactose, differentiating it from other *Salmonella*, but its sugar profile can vary slightly, with sucrose fermentation being rare. The isolates fermented maltose and mannitol, L-rhamnose, L-arabinose as well as trehalose which helped to differentiate from suspected *Mannheimia* spp. which can ferment arabinose or trehalose as per the biovar though they produce pink colonies on MacConkey agar. All isolates amplified positively for the *invA* gene which is specific for *Salmonella* spp., infection (Jahan *et al.*, 2018) [8]. Strains of APEC amplified for *stx-1*; *stx-2* and *eaeA* genes.

Fig 3: Phenotypic and Genotypic characterization of the isolated bacterial pathogens

No	Phenotypic characterization	No	Genotypic confirmation
1	Colorless NLF's on MacConkey agar, Catalase +ve and Gram negative bacilli	1	Amplification of <i>inv A</i> gene (284bp) of <i>Salmonella</i> spp
			
2	Black coloration and H ₂ S production in TSI slant and Citrate utilisation +ve	2	Amplification of toxin genes <i>stx-1</i> ; (282bp); <i>stx-2</i> (198 bp) and <i>eaeA</i> (420 bp) genes of APEC
			

Discussion

In quails, incidence of infection with *Salmonella enterica subspecies arizonae* is on the rise. Infected adult birds may become asymptomatic carriers, and shed the organism intermittently in their feces. In this study, Salmonellosis was caused by *Salmonella arizonae* serovar in Japanese quails (Yahi *et al.*, 2024) ^[18]. It produced mortality rates (up to 68%) in birds of all ages, causing substantial economic loss. Overall screening was done systematically for a period of 6 months. No relation between age and rate of isolation of *Salmonella* from birds was observed and these results agree with (Palanisamy and Bamaïyi, 2015) ^[13] wherein it is reported that there is no significant difference between the prevalence of *Salmonella* spp. in different age groups of quails.

In the study area poor hygiene, farming mismanagement and rainy season augmented mixed infections (Rahman, 2004) ^[15]. High rate of moisture and humidity aids growth and invasion by pathogens. Virulence genes such as *invA* for *Salmonella* and *stx-1*; *stx-2* and *eaeA* genes for APEC were detected in all examined isolates (Figure 3). *Salmonella* infections can be prevented with proper sanitary management techniques. Use of pre and probiotics as an immune supplement has an effective role in enhancement of immunity in healthy and diseased birds.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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