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Antimicrobial profile of resistant bacterial pathogens causing septicaemia in Japanese quails

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Abstract

In a private Japanese quail farm raising approximately 25,000 Japanese quail layers (*Coturnix coturnix japonica*) a history of long-term losses and gradual mortality rate of up to 6.3% was recorded. Suitable samples were collected at disease investigation and processed. Totally 5 types of different bacterial pathogens were isolated. Antimicrobial susceptibility testing (AST) was carried out using 12 different antibiotics for all the above pathogens. Multi drug resistant (MDR) serovars of *Salmonella* spp, Avian Pathogenic *Escherichia coli* (APEC) strains and *Mannheimia haemolytica* had caused mixed septicaemia infections and were multidrug resistant with a narrow window for treatment options.

Keywords: APEC, *Salmonella*, *M. haemolytica*, AST MDR, Japanese quail

1. Introduction

Japanese quail are generally considered more resistant to diseases. Quails are recent additions in the poultry market. Quail meat is believed to have medicinal properties and also is a delicacy in many cuisines (Chindo and Olowoniya, 2006) [5]. Despite their reputation for being resilient, they have specific health vulnerabilities that stem from intensive farming conditions. Unlike their wild counterparts who have vast spaces to roam, farmed quails live in confined spaces where diseases can spread rapidly (Van Hoorebeke *et al.*, 2011) [15]. So, the key to successful quail farming lies not in treating diseases after they occur, but in creating an environment where diseases will not occur easily.

Sepsis caused by Avian Pathogenic *Escherichia coli* (APEC), *Salmonella* serovars, *Mannheimia* etc., still pose significant health risk and an economic threat to farms, as it causes decreased egg production high mortality in young birds (up to 15-20%). Infections manifest as systemic diseases like colisepticemia, perihepatitis, and air sacculitis triggered by environmental stressors or concurrent viral infections. In a private Japanese quail farm raising approximately 25,000 Japanese quail layers (*Coturnix coturnix japonica*) a history of long-term losses and gradual mortality rate of up to 6.3% per day was recorded.

Methods: A disease investigation was undertaken after repeated approach of personnels from a private Japanese quail farm regarding considerable losses due to many health problems amongst the quails in spite of different approaches to contain the same recurrence of disease occurred within short duration.

History: As described by the farm personnel the disease manifestation in quails had various symptoms including difficult breathing, nasal discharge, white diarrhoea, and in severe cases, paralysis and twisted necks. Birds showed signs of depression, reduced egg production, and sudden death. Mortality rate was 6.3% and morbidity was 37% Interventions for addressing the problem was ineffective with a gradual raise in morbidity and sudden mortalities

The sign were suggestive of Ranikhet disease, but were ruled out by Spot haemagglutination test (WOAH) [14] unlike viral diseases, a bacterial infection like those caused by *E. coli* represents a different type of challenge in quail health as they are opportunistic in nature often indicating an underlying poor management. Typically, pathogens that cause sepsis, affect

when birds are already stressed due to environmental conditions, production stress, or other co-infections.

Clinical signs: Ailing birds showed symptoms like difficult breathing, reduced appetite, diarrhoea, and sudden death. As observed, sanitation was poor in the farm with moist litter which was stuck to the toes of quails. Birds huddled and were overcrowding near heat source. Inadequate ventilation produced an ammonia odour. This triggers opportunistic infections like those caused by *E. coli* which manifest in various forms, like respiratory, septicaemia, and digestive disorders.

Sample processing: All the samples received and collected from the affected J. quails (Table 1) were processed by bacteriological and serological methods. The routine procedures for isolation were done by inoculation in Tryptic soya broth along with positive cultures, sub-cultured on 5% sheep blood agar, BHI agar, MacConkey agar. Later incubated under aerobic conditions at 37 °C for 16-18 hours.

Staining: Gram's staining of the bacterial culture of clinical samples was done. Giemsa staining was carried out for heart blood smears while Leishman's stain for tissue impression smears. All stained smears were observed under oil immersion at 100X magnification. Biochemical characterization was carried out, by routine tests as, indole production, oxidase, catalase, and lysine decarboxylase utilization and to produce urease and ornithine decarboxylase (ODC) were performed. Cultures were subjected to IMViC test panel. Sugar fermentation tests, like dulcitol, sorbitol,

mannitol, trehalose, and maltose, sucrose, glucose, mannitol etc was carried out. Reactions were observed after inoculation of 1–2 colonies of pure culture in 2 ml of growth medium with appropriate indicator and incubation at 37 °C for 48 hr. Sorbitol reactions were recorded up to 14 days.

Antibiotic Sensitivity test: AST was performed as per Kirby–Bauer disc diffusion method (Bauer *et al.*, 1966) [3]. Different antibiotics were used such as FOS: Fosfomycin (300µg), NX: Norfloxacin (10 µg), DoHcl: Doxycycline hydrochloride (30µg), AMP: Ampicillin (10 µg), AMX: Amoxicillin (10 µg), CTX: Cefotaxime (30 µg), N- Neomycin (15µg) and CIP: Ciprofloxacin (5µg), COT: Co-trimoxazole (23.75 µg & 1.25µg), B: Bacitracin (10 IU), GEN: Gentamicin (10 µg), TE: Tetracycline (30 µg). The interpretation of the measured zone IN mm was done according to CLSI (2016). The ZOI was measured and interpreted as per guidelines of CLSI 2016 [7] (Table 2)

Results and Discussion

Confirmation of Bacterial pathogens: Strains of Avian Pathogenic *Escherichia coli* (APEC-16 Nos.) (Ewers Janssen and Wieler 2003) [10] and 5 isolates of *Mannheimia haemolytica*, *Pseudomonas* and *Staphylococcus* were isolated and characterized by cultural and biochemical tests. All suspected isolates for *Salmonella* spp., was also isolated from the samples by conventional methods and tested by biochemical test. All bacterial isolates were confirmed by identification of virulence and toxin genes by PCR. *Salmonella arizonae* serovar was the primary pathogen for the disease outbreak and long-term sepsis

Table 1: Bacterial isolates from different clinical samples collected at PM is given in table 1.

Post mortem done for 25 quails /batch at an interval of 3 months					
No	Sample type	Nos	Bacterial isolate	Total	%
1	Cloacal swabs	25	<i>Salmonella</i> serovar (n=43)	23	53
2	Intestinal ulcers	09		11	26
3	Liver	11		09	21
4	Spleen	07	<i>Mannheimia</i> spp	01	20
5	Heart blood	09		01	20
6	Intestine	15		03	60
7	Air sac (thick)	06	<i>E. coli</i>	06	38
8	Ileo caecal junction	11	<i>Enterobacter</i> spp	04	
9	Shell less Eggs	07	<i>E. coli</i>	02	13
10	Peritoneal fluid (3ml)	08	<i>E. coli</i>	05	31
11	Ovaries (Omphalitis)	09	<i>Clostridium perfringens</i>	03	100
12	Hydropericardial fluid (2ml)	07	<i>Pseudomonas</i> spp	03	43
		05	<i>Staphylococcus</i> spp	04	57
		05	<i>E. coli</i>	03	19
		07	<i>Pseudomonas</i> spp	02	29
		08	<i>Pseudomonas</i> spp	02	29
		08	<i>Staphylococcus</i> spp	03	43
		50	Total bacterial isolates	42	84

Table 2: AST pattern of resistant bacterial pathogens and percentage of resistance is given in table 2

No	Resistant bacterial isolates	Antibiotics (code) and Conc. in mcg (µg) or IU.											
		CIP (5)	GEN (10)	AMP (10)	AMX (10)	TE (30)	B (10IU)	N (15)	DoHcl (30)	CTX (30)	COT(23.7& 1.25)	FOS (200)	NX (10)
1	<i>Salmonella arizonae</i> (22)	09	11	17	09	13	06	-	-	-	02	-	-
	Resistance%	41	50	77	41	59	27	-	-	-	9	-	-
2	<i>Escherichia coli</i> (05)	03	03	03	01	03	-	01	-	-	2	-	-
	Resistance%	60	60	60	20	60	-	20	-	-	40	-	-
3	<i>M. haemolytica</i> (05)	-	02	-	-	03	02	-	-	03	02	-	02
	Resistance%	-	40	-	-	60	40	0	0	60	40	-	40
4	<i>Pseudomonas</i> spp (07)	-	05	03	01	03	04	-	-	02	03	-	-
	Resistance%	-	71	43	14	43	57	-	-	29	43	-	-

4	<i>Enterobacter</i> spp (03)	-	02	-	03	02	03	03	01	02	03	-	-
	Resistance%	-	67	-	100	67	100	100	33	67	100	-	-
5	<i>Cl. perfringens</i> (01)	-	01	01	-	01	01	-	01	-	01	-	-
	Resistance%	-	100	100	-	100	100	-	100	-	100		
6	<i>Staphylococcus</i> spp (04)	02	02	04	02	02	02	01	04	02	03	-	-
	Resistance%	50	50	100	50	50	50	25	100	50	75		

Multidrug resistance patterns to at least two antimicrobials has been reported in *Salmonella* spp. by (Christian *et al.* 2016) [6]. MDR *Salmonella* is resistant to Chloramphenicol and Ampicillin with necessitates the need to use new classes of antibiotics (Crump *et al.*, 2015) [8]. Similarly, in this study also, *Salmonella arizonae* was resistant to 7 out of the 12 antibiotics used. It was most resistant to Ampicillin (77%) followed in order by Tetracycline (59%); Gentamicin (50%) Ciprofloxacin and Amoxicillin (41%) each and to Co-trimoxazole (9%).

The sepsis and colibacillosis caused multidrug resistant APEC strains was resistance to 60% antibiotics like Ciprofloxacin, Gentamicin, Ampicillin and Tetracycline followed by a 40% resistance to Co-trimoxazole and 20% resistance to Amoxicillin and Neomycin (Bhattarai *et al.*, 2024; Aljuhaishi and Albawi, 2024) [4, 14]. *Mannheimia haemolytica* considered a primary pathogen to J. quails was resistant by 60% to Tetracycline and Cefotaxime and 40% resistant to each of Gentamicin, Bacitracin, Norfloxacin and Co-trimoxazole.

Japanese quails might be small, but when it comes to health management, they require just as much attention as any other poultry. These compact birds are generally hardy, but like other birds, they succumb to various diseases and can devastate economy of the farm.

Salmonella species were listed as critical antibiotic-resistant bacteria since 2017 by WHO. In this study, *Salmonella arizonae* was the main etiological agent of sepsis which caused immunosuppression and hence subsequent invasion by opportunistic APEC (Kathayat *et al.*, 2021) [12] and Mannheimiosis in the quail flock. The resistance to numerous antibiotics also made the pathogens refractory to regular treatment (EFSA, 2013) [16]. Similarly, the other bacterial isolates were also resistant to numerous antibiotics (This makes research in drug susceptibility profile of zoonotic pathogen a top priority).

Antibiotic misapplication and misuse in food animal production and the steady development of antibiotic-resistant bacteria has become a serious public health concern (Antunes *et al.*, 2016) [2]. Salmonellosis is a common bacterial infection that results in decreased egg production and mortality with significant economic losses in poultry farms worldwide (Rothrock *et al.*, 2015) [13]. Antibiotic resistance is considered a threat to the health of humans and animals, and to food safety. It is associated with transfer of genetic elements of bacteria to humans and other bacteria via poultry products. Ciprofloxacin and Ceftriaxone were used to treat *Salmonella* infections as per (Andi *et al.*, 2017) [1], which was similar to the results of our study.

For an entire six months clinical samples like cloacal swabs, intestinal contents, liver, eggs etc. were collected from convalescent and ill birds and from pens marked earlier as contaminated and routinely processed and tested (Jahan *et al.*, 2018) [11]. Over duration of 3 months the mortality stopped completely. Further elaborate testing for water and litter quality along with effective management measures were put into practice to contain the mixed sepsis infections in the quail farm. Non-therapeutic alternatives to antibiotics need to be considered for alternate use.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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