

MULTIDRUG RESISTANT *SALMONELLA TYPHIMURIUM* IN KUTTANADAN DUCKLINGS

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Summary

Salmonellosis is a destructive disease of ducklings caused by *Salmonella anatum* and *Salmonella typhimurium*. *Salmonella typhimurium* is a leading cause of human gastroenteritis. So this disease acquires zoonotic importance too. The disease is usually encountered in ducklings under two weeks of age and the morbidity and mortality varies considerably. The study deals with microbiological examination of pathogens of a *Salmonella* outbreak in Kuttanadan ducklings, the native ducks of Kerala. Antibiotic sensitivity test, pathogenicity test and plasmid isolation were performed. The organism was serotyped as *Salmonella typhimurium* (Antigenic structure 4, 12: i: 1, 2).

Keywords:

Salmonellosis, Kuttanadan ducklings, Antibiotic Sensitivity, Serotyping

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Introduction

Salmonellosis is a destructive disease of ducklings caused by *Salmonella anatum* and *Salmonella typhimurium*. Among this *S. typhimurium*, is a broad-host-range pathogen. Most cases of salmonellosis (~93%) in ducks were caused by *Salmonella typhimurium* (1). In poultry *Salmonella enterica* serovar typhimurium infection the so called paratyphoid may result in a high mortality rate in young birds, but affected adults typically have a nonlethal chronic or carrier status. In a carrier bird, *Salmonella* always exists in the alimentary tract and the reproductive system, and can thus be transmitted to humans through contaminated eggs and meat. Humans consuming the contaminated eggs or meat may contract salmonellosis (2) *Salmonella typhimurium* is a leading cause of human gastroenteritis. So this disease acquires zoonotic importance too. The disease is usually encountered in ducklings under two weeks of age and the morbidity and mortality varies considerably.

Methods

Disease outbreak was observed in a flock of Kuttanadan ducks which are the native ducks of Kerala. Out of 200 ducklings 75 died within a week period. The dead and affected ducklings were brought to the Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala for microbiological examination.

History revealed that the birds had clinical signs of depression, ruffled feathers, thirst and sudden death. The gross lesions observed were Pericarditis, thickening of pericardial sac, fluid in the pericardial sac and focal necrotic lesions in liver.

Blood smear prepared from the birds were stained using Leishman's stain. Heart blood, pieces of liver, spleen, brain and intestine were collected aseptically, processed and inoculated into the allantoic cavity of 9-day-old embryonated chicken eggs following standard procedure to identify viral etiology. The 20% tissue sample suspension in 0.2 mL amounts was inoculated onto chorioallantoic membrane (CAM) of an 11-day-old duck embryonating egg for the detection of Duck plague virus. Five eggs were inoculated while 5 eggs simultaneously received 0.2 mL of PBS and kept as control. Eggs were incubated at 37.5°C and embryo mortalities were monitored for 10 days by routine candling. The tissues were separately cultured on Brain Heart Infusion Agar and Rappaport-Vassiliadis broth. After eight hours one loop full of culture from Rappaport-Vassiliadis broth is inoculated into Brilliant Green Agar and both the plates were incubated at 37°C for bacterial growth. The pure cultures obtained were identified as per the method described by Quinn *et al.* (3)

Chicken embryo lethality test and mice pathogenicity test were done as recommended by Giovanardi *et al.* (2005) to prove the pathogenicity of the isolate (4). For chicken embryo lethality test, overnight incubated broth culture of the isolate was adjusted to a concentration of 1×10^4 cfu/ml and 0.1ml inoculum each was injected into the allantoic cavity of four 9-day-old embryonated chicken eggs. One egg was kept as control. Eggs were incubated at 37°C and were candled daily to identify dead embryos. For mice pathogenicity test, two mice were intraperitoneally inoculated with 0.1 - 0.2 ml of 24-hour nutrient broth culture containing 1×10^8 cfu/ml and monitored every 6 hrs interval for mortality. One mouse was kept as control.

A sensitivity test of eight antimicrobial agents frequently used in local poultry farms was carried out by standard disc diffusion method as per Bauer *et al.* (1966) (5). A 4- hours-old Brain Heart Infusion (BHI) broth culture of the organism was swabbed onto the surface of Muller- Hinton agar (Himedia). The antibiotics and amounts per discs used were given in table 1. The discs were placed on the medium and were incubated at 37°C for 24 hrs.

The inhibition zones were measured and the results were interpreted using the HiAntibiotic zone scale and Zone size interpretative chart. Serotyping was performed by sending the samples to National Salmonella and Escherichia Research Centre, Kasauli. Plasmid isolation was performed using Nucleospin plasmid isolation kit followed by agarose gel electrophoresis.

Table1: Antimicrobial agents and their disc concentration

Antimicrobial agent	Disc concentration	Antimicrobial agent	Disc concentration
Amoxicillin	10µg	Erythromycin	10µg
Cephalexin	30µg	Gentamycin	10µg
Chloramphenicol	30µg	Oxytetracyclin	30µg
Ciprofloxacin	5µg	Sulphadiazine	100µg

Results

On attempt for virus isolation, in chicken eggs, no death of embryos /haemagglutination of chicken red cells were noticed and the harvested allantoic fluid was passaged for a minimum of three times before declaring as negative for Newcastle disease. In duck embryos no death/thickening of chorioallantoic membrane/extensive petechial hemorrhages were noticed and the chorioallantoic membrane and the harvested allantoic fluid was passaged for a minimum of three times before declaring as negative for Duck plague.

Microscopical examination of blood smear did not reveal bipolar organisms but numerous short rods were seen. Next day, on the agar plate the colonies formed were mucoid, glistening and circular with entire edges. Red colonies were seen on Brilliant Green Agar since the bacterium does not ferment lactose or sucrose (6). On xylose lysine desoxycholate agar, the colonies formed were red colored with a black centre indicating fermentation of xylose and hydrogen sulphide production.

The isolate was Gram-negative short rods, motile, non sporing and non capsulated. It was catalase positive, oxidase negative, reduced nitrate and - + - + on IMViC test. It produced H₂S and lysine decarboxylase. It produced acid and gas from glucose, mannitol, maltose, dulcitol, sorbitol, arabinose, rhamnose, trehalose, xylose and failure to ferment lactose, sucrose, adonitol, cellobiose, inositol, raffinose and salicin.

Culture that gave characteristic biochemical reaction was serotyped as Salmonella typhimurium (Antigenic structure 4, 12 : i : 1, 2). On lethality test, all the inoculated embryos were found to be dead on 30-48 hrs post-inoculation (PI) and the organism was reisolated from the embryo. Also both the inoculated mice were dead on 30 hr PI. On post-mortem examination, hyperemia and congestion of viscera noticed and the organism was reisolated from the heart blood and liver.

The antibiotic sensitivity test showed the isolate was resistant to amoxicillin, cephalixin, chloramphenicol, ciprofloxacin, erythromycin, oxytetracycline, sulphadiazine but intermediate sensitive to gentamicin. Based on the results, rests of the ailing birds in the flock were treated with gentamycin and the mortality rate was decreased. On plasmid isolation it was found that the organism doesn't bear any plasmids.

Conclusions

The findings proved that the death could be due to salmonellosis. The organism shows complete resistance to seven antibiotics and upon plasmid isolation no plasmids were obtained. Since antibiotic resistance is a character assigned to the extra chromosomal DNA this has to be further studied for better understanding and control of the particular organism.

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