

Case Study

CONCURRENT INFECTION OF INFECTIOUS BURSAL DISEASE AND PSEUDOMONIASIS IN POULTRY-A CASE STUDY

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Abstract: The present study investigates a case of concurrent infection by Infectious bursal disease (IBD) and pseudomoniasis in 20 day old chicks in a private farm in Koothattukulam, Kerala, which was presented to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Thrissur, with a history of anorexia, ruffled feathers, whitish diarrhoea and severe mortality. Tissue samples were collected for bacterial and fungal culture and nucleic acid extraction. The presence of *Pseudomonas aeruginosa* was confirmed by means of conventional cultural and biochemical methods and IBD outbreak was ascertained with the help of both gross lesions and Polymerase Chain Reaction (PCR) targeting the VP2 gene of Infectious Bursal Disease Virus (IBDV) with an amplicon size of 480 bp.

Introduction

Infectious Bursal Disease (IBD) is a contagious immunosuppressive disease of chicken caused by Infectious Bursal Disease Virus (IBDV). It is an economically important disease of poultry industry which causes severe mortality in the flock. The virus is a member of the family *Birnaviridae* and the genus *Avibirnavirus*. Two known serotypes of IBDV are present namely serotype 1 and 2. Serotype 1 is pathogenic to chicken while serotype 2 is non-pathogenic. The major economic impact is due to virus-induced immunosuppression or potential interactions between IBDV and other viruses, bacteria or parasites. (Giambrone *et al.*, 1977) The losses are due to secondary bacterial infections, growth retardation and condemnation of carcasses at the slaughterhouse. The first cases were observed in Delaware (United States of America [USA]), in the area of Gumboro, which is the origin of the name, although the terms 'IBD' or 'infectious bursitis' are more accurate descriptions. The first report of a specific disease affecting the bursa of Fabricius in chickens was made by Cosgrove (1962). The outbreaks have caused colossal losses to poultry farmers in India (Selvam *et al.*, 2006) and outbreak may be due to the occurrence of antigenic variant strains, interference by

maternal antibodies or other immunosuppressive afflictions such as aflatoxicosis/mycotoxicosis. Any flaw in vaccine preparation, its transport and administration can also lead to vaccination failure. In India, virulent types of variant strains of serotype 1 caused catastrophic losses to the poultry industry during the period 1993-95 (Mittal *et al.*, 2006)

Infection by *Pseudomonas* spp. in birds is of great significance because epidemics may spread rapidly through poultry flocks causing mortality in all ages (Shukla and Mishra, 2015). *Pseudomonas aeruginosa* (*P.aeruginosa*) is an opportunistic pathogen capable of infecting virtually all tissues. The disease (pseudomoniasis) may be localised in the infraorbital sinuses, air sacs or cause cellulitis or it can be a systemic septicaemic disease affecting many organs and tissues. Septicaemic infection in poultry has been reported by Nakamura *et al.* (1990). Morbidity and mortality rate may vary from 2 to 100%, but more commonly about 2-12% with greatest losses in very young birds. The most predominant *Pseudomonas* species causing mortality among birds especially chickens is *P.aeruginosa* which is Gram-negative, aerobic, motile, non-capsulated and non-spore forming bacterium. The organisms are ubiquitous, often associated with soil, water and humid environments. The present case study deals with the deadly outbreak of IBD in a flock of chicken, which were further complicated by pseudomoniasis.

Materials and Methods

Four 20 day old ailing birds with the history of anorexia, depression, and severe mortality were presented to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Thrissur, Kerala. The owner reported that among 500 birds in the flock, 60 died. Post-mortem examination was conducted and samples were collected aseptically. The samples (liver, lungs, spleen and bursa) were streaked on to blood agar (7% ox blood agar) and brain heart infusion agar (BHIA). The liver and intestinal contents were streaked onto MacConkey agar. The blood agar plates were incubated aerobically and with 5-10 per cent CO₂ tension at 37°C for 24 h and BHIA and MacConkey agar were incubated aerobically at 37°C for 24 h. The samples for fungal culture were streaked in duplicate on to Sabouraud's dextrose agar (SDA) and incubated at 37°C and room temperature, respectively.

Since the clinical signs and gross lesions were suggestive of IBD, the tissue samples were processed and subjected to RNA extraction using Trizol method. The RNA was reverse transcribed to cDNA using commercial cDNA synthesis kit according to the manufacturer's instructions and subsequently subjected to PCR standardised using specific primers as mentioned by Singh *et al.*(2014) and the Reverse transcriptase-PCR was carried out as per the

following conditions (Table 1). The PCR products were detected by electrophoresis in one per cent agarose gel in Tris acetate EDTA buffer (1X) and the gel was visualised and results were documented in a gel documentation system.

Table 1. Polymerase chain reaction conditions for amplification of IBD Virus

Steps		Temperature	Time
Initial denaturation		95°C	5 min.
34 cycles	Denaturation	95°C	30 sec.
	Annealing	59.5°C	60 sec.
	Extension	72°C	30 sec.
Final extension		72°C	6 min.
Hold		4°C	10 min.

Results and Discussion

Infectious bursal disease is a highly contagious viral disease affecting young chickens, and is characterised by the destruction of lymphoid organs, and in particular, the bursa of Fabricius, where B lymphocytes mature and differentiate. The target cell of the virus is B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine. *Pseudomonas aeruginosa* is an opportunistic pathogen that can produce a localised or systemic disease in newly hatched chicks and growing poultry. *Pseudomonas* infections in birds are of great importance because epidemics may spread rapidly through poultry flocks causing mortality in all ages (Shukla and Mishra, 2015).

In the present study, postmortem examination of the chicks revealed hepatomegaly, splenomegaly with mild congestion, haemorrhages on thigh muscle, enlarged bursa and congestion in small intestine. Bacteriological examination revealed the presence of large, irregular, translucent colonies which produced a greenish diffusible pigment on BHIA and is characterised by a fruity smell. On blood agar, the colony produced beta haemolysis. On Gram's staining, Gram-negative bacilli could be isolated. On MacConkey agar, colonies were found to be pale and non lactose fermenting. On SDA, no growth could be obtained at 37°C and at room temperature even after seven days of incubation. Biochemical reactions revealed

that isolates were oxidase, catalase and citrate positive, while methyl red, Voges-Proskauer and indole tests were negative. The isolates were found to be negative for sucrose, lactose, dextrose, maltose, mannitol and xylose fermentation. As per Quinn *et al.* (1994) these characters were in conformity with those of *P. aeruginosa*.

On PCR, the tissue samples were found to be positive for IBD virus using specific primers targeting the VP2 gene of infectious bursal disease virus with an amplicon size of 480 bp (Fig 1) which is in accordance with Singh *et al.* (2014). The gold standard method for diagnosis of virus is isolation and identification which is tedious and time consuming. Molecular detection methods like RT-PCR are found to be highly sensitive and will give rapid and accurate results. This in conjunction with conventional bacterial and fungal culture followed by antibiogram will enable the clinician to follow effective strategies to combat the infection, thereby preventing further mortality in the flock.

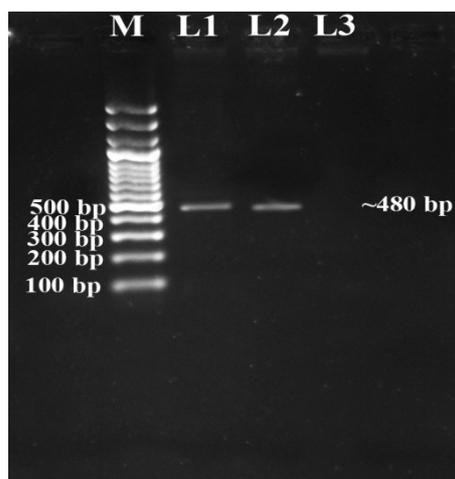


Fig:1 M – ladder, L1 – positive control, L2 – sample, L3 – negative control.

Conclusion

In the present study, the concurrent infection of IBD and pseudomoniasis was detected in a flock of birds. The molecular diagnostic methods like RT-PCR along with conventional bacteriological culture and have made timely diagnosis possible, which helped in advocating adequate measures to combat the infection and it prevented further economic loss in the flock.

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