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Isolation of Infectious Bronchitis Virus from an Outbreak in Parent Layer Stock

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Abstract: Trachea and lung tissue samples collected from a disease outbreak at Regional Poultry Farm, Kerala, India were submitted for virological investigation at Institute of Animal Health and Veterinary Biologicals (IAH & VB), Bangalore. The outbreak had taken a toll of 385 layer parent stock birds. The main symptoms and lesions were air saculitis, tracheal rales, oopheritis and salphingitis. The samples were processed and inoculated to 10th day old chicken embryos. Lesions of dwarfing and curling characteristic of Infectious bronchitis were noticed in inoculated embryos. The virus was confirmed by a modified Haemagglutination (HA) test, wherein the allontoic fluid was pretreated with crude filtrate of *Clostridium perfringens* before carrying out HA.

Key words: Infectious bronchitis, corona virus, haemagglutination test

Introduction

Infectious bronchitis (IB) is an acute and highly contagious viral respiratory disease of chickens characterized by respiratory rales, coughing and sneezing. IBV is a pathotype species in the family *Coronaviridae* and is placed in Group 3 of Genus Corona Virus belonging to the Order Nidovirales and the disease is considered as one of the major causes of economic loss to the poultry industry world wide (King and Cavanagh, 1991). The ability of the virus to undergo continuous genomic shift and drift has lead to emergence of several new serotypes especially in the areas of intensive poultry farming (Zanella *et al*., 2003). Pathogenecity of the virus is greatly enhanced by secondary bacterial infection of respiratory tract like *E. coli* and *Mycoplasma spp*. (Arthur Sylvester *et al*., 2003). The widespread use of live and inactivated IB vaccines complicate the disease diagnosis by serological tests. Hence isolation and demonstration of virus appears to be confirmative of the disease (OIE Manual, 2003). Present communication describes isolation of IB virus from an outbreak at Regional Poultry Farm, Kerala, India and its confirmation by a modified Haemagglutination test.

Materials and Methods

History of the disease: An outbreak of disease in parent layer stock of Regional Poultry Farm, Chatamangalam, Kerala with the symptoms and lesions of air saculitis, catarrhal exudates in tracheal lumen, hydropericardium, fragile enlarged liver, corrugation of proventriculus mucosa, narrowing of small intestinal lumen, oopheritis and salphingitis, congested kidney and gelatinization of sub cutaneous fat, was recorded. The outbreak which was unabated by usage of higher antibiotics had taken a toll of 385 birds. Trachea and lung tissue were collected and submitted to Diagnostic Virology, Southern Regional Laboratory, Institute of Animal Health and Veterinary Biologicals (IAH and VB), Bangalore for virological investigation.

Virus isolation: Trachea and Lung tissue were homogenized in phosphate buffered saline (20%) and the suspension were clarified by low speed centrifugation. The supernatant was filtered in 0.45 u syringe filter and was inoculated in 10 day old chicken embryos (Veterinary college, Bangalore) by intra allontoic route and were incubated at 37^{o}C (Gelb, 1989). Inoculated eggs were checked twice a day. Those that died within 24 hrs of inoculation were discarded. The eggs were harvested after 8 days of incubation. Allontoic fluid and chorioallontoic membranes were collected for subsequent passages. During the present study a total of ten passages were given to the suspected samples.

Haemagglutination test: The IB virus has an unique property of agglutinating chicken red blood cells(RBC's) after the virus is enzyme treated (Schultze *et al*., 1992). The allontoic fluid collected from embryos showing specific IBV lesions (Zanella *et al*., 2003) were centrifuged at 30000 g for 3 hrs and the pellet resuspended at 100 fold concentration in 0.01 M Tris HCl buffer (pH 6.5). The allontoic fluid suspension was added with an equal volume of the crude filtrate of *Clostridium perfringens* culture which is believed to have the neuraminidase enzyme which induces the HA activity to IBV. The mixture was incubated at 37^{o}C for 2 hrs. Using this mixture of allantoic fluid and enzyme the HA test were carried out as per the protocol described by Alexander (1983). The test was carried out with three

control wells wherein the first control well had allontoic fluid without enzyme treatment, while the second had RBC's with prior enzyme treatment, whereas the third control well had RBC control without enzyme treatment.

Results and Discussion

IBV grows well in the developing chicken embryos compared to chicken organ cultures like chicken kidney and trachea (Cook *et al*., 1976). Upon inoculation by intra allontoic route, no visible changes were observed in first and second passages. The changes of dwarfing and embryo mortality were noticed from third passage onwards. Survival in third passage was 90% and with mild stunting of embryos. Embryo mortality, curling, dwarfing and stunting of embryos increased as the number of passage increased. The isolated virus was probably very virulent as evidenced by mortality of 90% of embryos from sixth passage onwards.

Upon opening the egg, the embryos appeared curled with deformed feet and compressed over the head. The amnion had thickened, shrunkened yolk sac, fragile membranes, increased volume of clear allontoic fluid were the other characteristic lesions noticed in the inoculated chicken embryos. The lesions noticed in the embryo were in argument with previous works carried out on IBV in chicken embryo. (Wang, 1996., Arthur Sylvester, 2003 and Zanella, 2003)

The harvested embryos did not show the lesions of urolithiasis, which could be due to the fact that, all IB virus isolates are not nephropathogenic and all nephropathogenic IB isolates need not induce urolithic disease which is attributed to strain differences (Wang *et al*., 1996)

The history and lesions in the birds indicated respiratory tract infection which was uncontrolled by antibiotics. The allontoic fluid collected after fourth passage failed to agglutinate the chicken RBC's ruling out the possibility of Newcastle Disease virus which readily agglutinates chicken RBC's. The lesions in the embryo supported towards the suspicion of IB. IB virus contains Alpha 2, 3 linked N-acetyl neuraminic acid which hinder the viral HA activity. When the virus is treated with crude filtrate of *Clostridium perfringens* culture which is believed to contain neuraminidase enzyme, this enzyme removes the neuraminic acid from the virus surface and induces HA activity (Schultze *et al*., 1992). The induction of viral HA activity by neuraminidase enzyme is the unique property of Corona viruses. HA activity was determined by observing the presence of tear shaped streaming of RBC's. The allontoic fluid collected after 10^{th} passage yielded HA titre of 1:16. All the three controls failed to give the HA activity. Further the virus was confirmed by performing Haemagglutinatin inhibition (HI) test using the isolated virus as the antigen and known IBV positive serum (Alexander, 1983).

In conclusion, it is necessary to verify, by a continuous epidemiologic surveillance and by a wide application of improved virologic and serologic methods, if new variants or IBV serotypes are diffused in the same and in different areas or countries and to determine, thereafter, the risk factors and the means of viral transmission.

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