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Original Article

Detection of Infectious Bronchitis Virus from Chicken and Efficacy trial of its commercially available Vaccine

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An attempt was made to detect and isolate Avian Infectious Bronchitis Virus (IBV) from chicken and efficacy trial of its commercially available vaccine with a view to diagnose and control of Infectious bronchitis infection in chickens of Bangladesh. This is a first preliminary report about the evidence of IBV detection. A total of 45 suspected infectious bronchitis virus field samples were collected from five different corners of Bangladesh. After receiving the samples, virus inocula were prepared aseptically maintaining standard procedure and inoculated into 10 days old embryonated chicken eggs through allantoic sac route. Characteristic curling and dwarfing of developing embryo of 2-7 days post inoculation manifested as the typical positive lesions of IBV. Moreover, embryo adherent amniotic sac and oedematous chorio-allantoic membranes were also considered for this purpose. Trachea and lungs were marked as a good source of IBV after cultivation in the laboratory. A group of layer and broiler chicks were challenged with the isolated virus after the administrtion of commercial vaccine of IBV. It was noted that, IBV vaccinated chicks could withstand the exposure where as 100% morbidity was observed in unvaccinated control group. The serum titre of antibody was detected by commercially available indirect ELISA in birds of prevaccinated, vaccinated and control groups. Thirteen samples were found to be positive to IBV. Samples collected from BRAC Diagnostic laboratory showed highest positive detection of IBV (53.8%). The prevailing local strain of IBV was preliminarily assessed as Massachusetts strain H120 as the chicks were vaccinated with the vaccine prepared from Massachusetts strain H120.

Keywords: Infectious bronchitis virus, vaccine

Introduction

Avian Infectious Bronchitis (IB) is a highly infectious and contagious viral infection of chickens. The virus being replicated primarily in the respiratory tract result in depression, coughing, sneezing, tracheal rales and the accumulation of excess mucus in the bronchi. The causal agent is infectious bronchitis virus (IBV) under the family *Coronaviridae*. IB was first observed in the United States in North Dakota in 1930s[1] . At present, IB is common throughout the world where chickens of all ages are raised commercially. IBV causes chick mortality, decline in egg production up to 50%, produce soft-rough shelled, misshaped and de-pigmented egg and takes 3-8 weeks to return to normal production level. IB can be nephrotropic causing acute nephritis, urolithiasis and mortality in adults. In the infected flocks, the losses from production inefficiencies are usually of greater concern than losses from mortality. The prevalence of IBV antibodies in chickens was first reported in Bangladesh[2]. Now commercial poultry raisers are using IBV vaccine without knowing the local isolates/serotypes of IBV upon concern that IB might play a major role for reduced egg production. Due to serotype variation, immunity following infection or vaccination with one serotype often is not protective against infection with unrelated serotypes[3]. IBV can be isolated in chicken embryo, tracheal organ culture and cell culture [4-6]. The identification and characterization was done by haemagglutination inhibition test using known sera, indirect immunofluorescence, virus neutralization, indirect immunoperoxidase (IP), enzyme-linked immunosorbent assay (ELISA), electron microscopy and polymerase chain reaction[7-11]. In the context of Bangladesh there is very little information available regarding local isolates of IB and its characters. So the present study was designed to detect and isolate the IBV from suspected field samples.

Materials and methods

Sample

A total number of 45 suspected field samples of IB were collected aseptically. Trachea, lungs, ovary and oviduct tissues of chicken were used for virus detection and isloation from the study areas (Anis Poultry Farm, Suzanagar, Pubna, Layer chicken farms of Society Development Committee [SDC], Faridpur, Layer chicken farms of Bangladesh Extension Education Services [BEES], Chunarughat, Hobigonj, Bangladesh Agricultural University [BAU] Poultry Farm, Mymensingh and samples reposited at BRAC Diagnostic Laboratory, Gazipur). The samples in 50% buffered glycerine were brought to the laboratory and kept at -70°c until used.

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Antisera

A commercial combined freeze-dried live virus vaccine with IB virus H-120 strain manufactured by Biochek B.V., Holland was used to raise antibody against IBV. A total number of thirty 1-day-old broiler chicks (Cobb 500) of Kazi Farms Ltd. Dhaka and another 30 indigenous layer chicks both without the history of vaccination in parents were collected to raise the antibody. A total of 70 sera samples encompassing sera from pre-vaccinated, vaccinated and unvaccinated control chicks, were collected. Broiler and layer chicks were vaccinated following the manufacturer schedule and a no. of birds kept as control.

Virus isolation

Ten days old embryonated eggs were inoculated with 0.2 ml of suspected sample following allantoic sac route. The inoculam was prepared from preserved field samples as 10-20% (w/v) suspension in sterile PBS. The suspension was centrifuged at 3000 rpm for 30 minute, supernatant treated with antibiotics, monitored for bacterial contamination by culturing on blood agar plates and kept at -70ºC for further use. The eggs were incubated at 37ºC up to 6 days post inoculation and examined twice daily by candling. Each suspected sample was inoculated into three embryonated chicken eggs. Five embryonated eggs were kept as uninoculated control.

The dead embryos within 24 hours were discarded and embryos died after 24 hours were chilled at 4ºC for 18 hours and examined for gross lesions. The whole embryos and the allantoic fluid were collected aseptically into sterile test tubes and stored at -70ºC for further study. Each sample was propagated maximum upto 2nd passage in the embryonated eggs. The ELD_{50} of the test viruses were calculated following the method of Reed and Muench[12].

Infectious bronchitis virus-specific antibody detection

The indirect enzyme linked immunosorbent assay (iELISA) was performed according to the manufacturer's instruction using IBV pre-coated plates and pre-diluted, ready to use reagents and buffer (BioChek B.V., Holland). In case of iELISA, the titre was predicted from the absorbance value of 1:500 dilution of a serum at 405 nm. The following equation relates the S/P (color absorbance value of a sample to positive ratio) of samples to an end point titre; $\log_{10}$titre=1.09 ($\log_{10}$ S/P) + 3.36. Samples with S/P value of ≥0.2 (titre ≥834) contain anti-IBV antibodies and were considered positive. S/P value ≤0.149 (titre ≤624) and 0.150-0.199 (titre 625-833) were considered as negative and suspected respectively.

Haemagglutination (HA) test

Haemagglutination test was performed with 1% chicken RBC to see the HA activity of suspected field isolates.

Challenge

The IBV vaccinated chicks including control groups were challenged with 0.5 ml of 10^2 ELD_{50} virulent suspected field IBV isolate through intramuscular route at day 26 in case of broiler and in case of layer at day 28. The survivability and the mortality of chicks were recorded to further proof of IBV suspected local field virus.

Results and Discussion

Propagation of IBV suspected field samples

A total of 13 samples were detected as positive to IBV infection. Positive or negative to IBV infection was determined through embryo survivability or mortality and/ gross lesions after inoculation of IBV suspected field samples. The highest positive rate was recorded as 53.8% in samples of both layer and broiler chicken came to BRAC Diagnostic Laboratory, Gazipur and the lowest positive rate was recorded as 10% in case of BAU Poultry Farm, Mymensingh (Table 1). The incidence of IBV was found more in layer chickens.

Rather than the embryo mortality, gross pathological lesions of the inoculated embryonated chicken eggs were considered as a supportive determinant for the identification of IBV infection. The embryos those died after 24 hours of post inoculation (PI) were examined for the gross pathological lesions. The typical gross pathological lesions were visually observed as curling, dwarfing of the embryo with cutaneous haemorrhages. There was also found an increased volume of allantoic fluid; chorioallantoic membrane (CAM) and the amniotic membrane were oedematous. The gross lesions observed in the embryos inoculated with the isolated field strains of IBV at passage number two is shown (Figure 1).

After challenge exposure all the exposed chicks either broiler or layer were kept up to 3 weeks under close observation for the development of any clinical signs and symptoms of IBV infection. All the chicks of IBV vaccinated broiler group resisted against the virulent challenge of isolated IBV from field samples, only one layer chick shows mild respiratory infection. But the respective unvaccinated control group of broiler and layer showed 100% morbidity. The mortality was 23.07% and 30.8% respectively for broiler and layer control group with IBV virulent challenge exposure (Table 2). The infected chicks showed sneezing, coughing, tracheal rales and nasal exudates which were typical clinical signs and symptoms of IBV. Isolated virus samples didn't show any haemagglutination activity.

Table 1. *Detection of Infectious bronchitis virus from suspected field samples of different farms*

Farms/organization with location	Types of Chicken	Number of samples propagated	Number and % of samples positive to IBV
BRAC Diagnostic Laboratory, Gazipur	Layer	8	6 (75.0%)
	Broiler	5	1(20.0%)
Anis Poultry Farm, Suzanagar, Pubna	Layer	8	2 (25.0%)
Layer chickens reared with help of SDC, Sadar Branch, Faridpur	Layer	7	1 (14.29%)
Layer chickens reared with the help of BEES, Chunarughat, Hobigonj	Layer	7	2 (28.57%)
	Layer	5	1 (20.0%)
BAU Poultry Farm, Mymensingh	Broiler	5	0 (0%)
Total		45	13 (28.89%)

Table 2. *Protection capacity of IBV vaccine in broiler and layer chicks*

Type of chicks	Age of vaccination (day)	Age of challenge (day)	Total number of chicks	Number of chicks died	Number of chick infected	Number of chicks survived	Morbidity (%)	Mortality (%)
Broiler	4, 14	26	13	0	0	13	0	0
Layer	2, 12, 20	28	13	0	1	13	7.7	0
Broiler (Control)	-	26	13	3	13	10	100	23.07
Layer (Control)	-	28	13	4	13	9	100	30.8

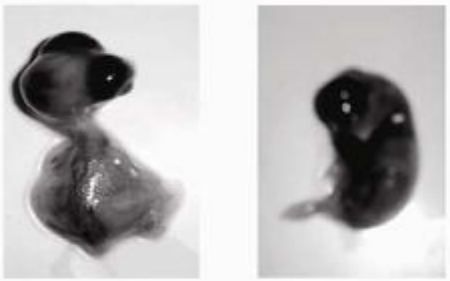

Figure 1. *Comparison of normal 16 day-old embryo (left) and curled, dwarfed, haemorrhaged infected embryo of the same age (right)*

Infectious bronchitis virus-specific antibody

Infectious bronchitis virus-specific antibodies from commercially available IBV vaccinated sera were detected by iELISA test kit against infectious bronchitis. The sera of vaccinated chicks revealed positive titre level while checking in ELISA plate reader and negative titre as expected from the pre-vaccinated and control unvaccinated group (data not shown).

Dwarfing and curling of infected embryo is considered as a phathognomonic sign found in the positive sample to be IBV. Thickened amniotic membrane adhered with the embryo and oedematous chorio-allantoic membrane was also observed[13-14]. Allantoic fluid was subjected for haemagglutination test and gave a negative HA reaction with chicken red blood cells (cRBCs). Embryo mortality and dwarfing increases as the number of serial passages increase, so that by the 10th passage most of the embryos are stunted, and up to 80% may die by the 20th day of incubation[1]. Due to limitation second passages were maintained for the propagation in this study. The embryo lesions might be clearly evident (such as stunting) and more no of positive sample would be found with increase no of blind passages. Trachea and lungs were found to be the good source for getting infectious bronchitis virus. All IBV strains can be isolated from the respiratory tract, with the highest concentration of IBV in the trachea during the first 3 to 5 days post-infection[15]. According to Cavanagh[16] the oviduct is also susceptible to IBV which may contribute to diminished egg production. So we in this study attempted to isolate virus using ovary and oviduct tissues and found two samples positive. All breed and age groups are observed equally susceptible. The challenge experiment identified that the chicks of vaccinated group (both broiler and layer) could resist 100% against the isolated IBV infection while morbidity reached up to 100% in unvaccinated groups. The protection capability of the IBV vaccine in broiler and layer chicks further proved that the suspected IBV field sample was actually IBV[17]. In scenario to commercial poultry farms, IB vaccination is carrying out routinely, so it is difficult to speculate whether the dead birds had the infection or just exposed to IBV along with other concomitant infection. It is a preliminary assessment that the local infectious bronchitis virus might be Massachusetts strain H120. As cross protection is not possible with unrelated serotypes, so vaccination with the local circulating virus is essential for effective protection against IB.

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