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Carrier State Studies of Infectious Bronchitis Virus in Asymptomatic Layer in Pakistan

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Abstract: Isolation of infectious bronchitis virus (IBV) was conducted in asymptomatic layer flocks with a history of IB or IB vaccination at least from 5 to 20 weeks before the day of sampling. A trypsin-induced hemagglutination (THA) assay to detect IBV in allantoic fluid of embryonated eggs was used. Detection of IBV through THA showed that group A [5 wks post-inoculation (PI)] was 100%, group B (10 wks PI) was 86%, group C (15 wks PI) was 74% and group D (20 wks PI) was 28% positive for IBV. Virus titre was found negatively correlated with the PI duration.

Key words: Infectious bronchitis virus, layer, allantoic fluid

Introduction

Infectious bronchitis (IB) is an acute, highly contagious viral disease of the respiratory and urogenital tracts of chickens, which is characterized by tracheal rales, coughing and sneezing. IBV causes poor weight gain and feed efficiency. IB may result in condemnation of broiler and causes egg quality decline.

The highly transmissible nature of the disease and confirmation that multiple serotypes of IBV have complicated and increased the cost of attempts to prevent the disease by immunization.

Serum from individuals who were in direct contact with chickens or poultry diagnostics was found positive for low titer of antibodies against IBV. (Jackwood *et al*., 1992).

Most strains of IBV are inactivated after 15 minutes at 56°C and after 90 minutes at 45°C (Johnson and Marquardt, 1975). Infected tissues stored in 50% glycerol are well preserved, which may be maintained without refrigeration (Fulton *et al*., 1993). Outdoor survival of IBV is reported upto 12 days in spring and 56 days in winter (Keeler *et al*., 1998). IBV in allantoic fluid (AF) lyophilized, sealed and stored in a refrigerator has remained viable for upto 30 years and is completely inactivated within 6 months when stored at 37°C (Fulton *et al*., 1993).

IBV spreads rapidly among chickens in a flock. Susceptible birds placed in a room/farm with infected chickens usually develop signs within 48 hours (Cook *et al*., 1991). IBV was isolated consistently from the trachea, lungs, kidneys and even from bursa of Fabricius of chicken (Gay, 2000). IBV can be isolated from 14 to 20 weeks post inoculation/exposure to the disease (Albassam *et al*., 1986). Re-excretion of IBV has been detected from the hens at the point of lay that had been virus-negative for several weeks after the exposure to IBV at the age of one-day old (Gelb *et al*., 1987). There are reports of IBV isolation from eggs upto 43 days after recovery (Cook *et al*., 1991). The nature of persistence of IBV infection is undefined but extended and intermittent shedding is evidence of potential risk of flock-to-flock transmission via contamination of personnel or equipment (Cook *et al*., 1987). Present study was aimed to estimate the prevalence of IBV in clinically normal layer farms.

Table 1: Percentage of positive samples from all the 4 groups

Groups	Total No. of samples	No positive sample	% age of positive samples
A	50	50	100%
B	50	43	86%
C	50	37	74%
D	50	14	28%

Materials and Methods

A total of 200 samples (trachea and kidneys mixed) were collected each from 20 different layer farms, which did not exhibit any symptoms of IB. These samples were divided into 4 groups i.e., 5, 10, 15 and 20 weeks post-exposure, which were designated as group A, B, C and D respectively. The samples were collected in screw capped test tubes containing sterilized phosphate-buffered saline (PBS) supplemented with penicillin (10,000 IU per ml), streptomycin (10,000 ug per ml) and nystatin sulphate (1000 IU per ml) to avoid bacterial and fungal contamination. The pH of this transportation media was adjusted at 7.2. After collection, the samples were stored in the refrigerator at -4°C. They were reconstituted in PBS, centrifuged at 5000 rpm for 20 minutes and the supernatant was stored at -4°C for further use.

Table 2: Passage wise percentage of positive allantoic fluid and maximum virus titres respective to each passage

Group	First Passage		Second passage		Third passage		Fourth passage	
	% age of +ve samples	Virus titre	% age of +ve samples	Virus titre	% age of +ve samples	Virus titre	% age of +ve samples	Virus titre
A	96	10^7	4	10^5	-	-	-	-
B	64	10^6	14	10^5	8	10^5	-	-
C	20	10^6	30	10^4	18	10^3	8	10^3
D	0	-	4	10^6	18	10^4	6	10^3

Embryonated chicken eggs inoculation: Nine-day-old embryonated chicken eggs (ECEs) were obtained from Experimental Poultry Farm Department of Poultry Husbandry, University of Agriculture Faisalabad. Each sample purified from the specimens following the method of Doherty (1967) was inoculated into a set of four nine-day-old ECEs via the chorioallantoic membrane (CAM).

Trypsinization: Working solutions of reagent grade trypsin (Sigma Chemical Company St. Louis, MO) were prepared containing 2.0 percent trypsin in PBS with the adjusted pH 7.2. Allantoic fluid from inoculated dead and live embryos was collected 72 hours post-inoculation (PI) and treated directly by mixing 0.25 ml of AF from each set of inoculated embryos with 50 ul from each of the working solutions. After the addition of trypsin, all samples were held at 37°C for 30 minutes. After incubation at 37°C, all the samples were placed at 4°C for 5 minutes.

Trypsin-induced Hemagglutination Assay: Trypsin-induced Hemagglutination assay (THA) was performed for all the samples separately by gently mixing 50 ul of trypsin-treated AF with 50 ul of a 2% solution of CRBCs in a microtiteration plate. Direct agglutination of the CRBCs was read within 5 minutes (Corbo and Cunningham, 1959; Mahmood *et al.*, 2004).

Results

HA activity was recorded with all IBV positive samples. Complete, clear and consistent HA reactions were observed with in 5 minutes of incubation at 37°C and remained stable for more than 2 hours at room temperature.

Table 1 shows the percentage of prevalence of IBV in 4 different groups of layer farms. Detection of IBV through THA showed that group A was 100%, group B was 86%, group C was 74% and group D was 28% positive for IBV.

Table 2 shows the passage wise results of IBV adapted and grown in AF. Out of four groups, the samples from group A gave 96% (48) THA positive reaction on first passage, which gave 1024 virus titre. The rest of 4% (2) samples gave positive THA reaction on second passage with a virus titre 512. 64% samples from group B were found positive after first passage with virus titre 512, 14% after second passage with virus titre 256 and 8% after third passage with the virus titre 256. Twenty percent samples from group C were found positive after first passage with virus titre 512, 30% after second passage with virus titre 256, 18% after third passage with the virus titre 64 and 8% of the samples were positive after fourth passage with the virus titre 64. Zero samples from group D gave positive THA, 4% samples were found positive after second passage with the virus titre 512, 18% were positive after third passage with virus titre 128 and 6% of the samples were positive after fourth passage with virus titre 64.

Discussion

The objective of this study was to study the presence of IBV in asymptomatic layer in Pakistan. Detection of IBV through THA showed that group A was 100%, group B was 86%, group C was 74% and group D was 28% positive for IBV. The results agree Keeler *et al.*, 1998 who reported the outdoor survival of IBV upto 12 days in spring and 56 days in winter. The results also confirm the findings of Albassam *et al.*, 1986 that are; IBV can be isolated from 14 to 20 weeks post inoculation/exposure to the disease, Gelb *et al.*, 1987; re-excretion of IBV has been detected from the hens at the point of lay that had been virus-negative for several weeks after the exposure to IBV at the age of one-day old and Cook *et al.*, 1991, there are reports of IBV isolation from eggs upto 43 days after recovery.

These carrier layers can transmit IBV to a young and immunologically nude poultry flocks. The nature of persistence of IBV infection is undefined but extended and intermittent shedding is evidence of potential risk of flock-to-flock transmission via contamination of personnel or equipment (Cook *et al.*, 1987).

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