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Detection of Avian Infectious Bronchitis Virus and Its Specific Antibody in Different Ages Layer Birds in Dinajpur District of Bangladesh.

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Abstract: ***Economical hazards due to drop in egg production by avian infectious bronchitis virus (IBV) is an alarming issue in Bangladesh. This study reports on the incidence of IBV infection by clinical manifestations, propagation of virus and viral specific antibody detection by serological test. Virological inocula prepared from the organ samples of dead birds according to the standard procedure were inoculated through allantoic sac (AS) route (@ 0.2 ml inoculum/embryo) of 10 days old embryonated indigenous chicken eggs. After 48 hours post infection, moderate mortality was found at day 4 and the highest mortality was at day 6. The growth of the IBV was determined by finding dwarfing and curling in the dead embryos which are path gnomic signs of IBV. The indirect ELISA (iELISA) test results revealed the level of antibody titre among 76.92% positive sera samples where the highest and the lowest antibody titre was 14849 and 204 respectively. The highest mean titre was found in 63-73 aged birds' group as 9206.53 indicating occurred frequently. Thus, the present study has established the IBV infection in layer birds by virus propagation and iELISA test where the test result motioned that the magnitude of this viral infection was stronger in 63-73 aged birds' group.***

Key words: IBV, iELISA, Antibody titre, Layer Birds.

I. INTRODUCTION

Avian Infectious bronchitis (IB) is an acute highly contagious viral infection of the upper respiratory tract of chicken caused by virus belonging to the member of the Coronaviridae family [1, 2]. This disease has been reported as endemic in all over the poultry producing countries of the world and was first reported by Bhattacharjee et al in Bangladesh [3, 4]. IB is common where chickens are raised commercially with the history of affecting chickens of all ages [5], although at the beginning, it was reported primarily as a disease of young chicks of 3 to 4 days [6]. In Bangladesh, the commercial poultry raisers did not know about IB disease in chickens, though the farmers of different corners faced, sometimes in layer chickens, a serious problem with drop in egg production and unabsorbed yolk. IBV causes decline in egg production up to 50% and produce soft and rough shell, misshaped; de-pigmentation of egg and also reduce hatchability in layer industry [7]. Moreover, some IBV strains can cause mortality [5, 8, 9] and also replicates in the oviduct and testes of infected birds resulting infertility problem. Among the etiological agents associated with drop in egg production, IBV might play a major role in Bangladesh. However, there are no published data of IBV infection available in different arena of Dinajpur district of Bangladesh. In this context, the aim of this present study was to isolate IBV and to detect antibody of IBV from non- vaccinated poultry farms in Dinajpur district of Bangladesh.

II. MATERIALS AND METHODS

A. Study area

The study was conducted in small scale layer farms in different areas of Dinajpur district of Bangladesh.

B. Sample collection

A total number of 42 suspected field samples comprising trachea, lungs, ovary and oviduct tissues were collected in 50% buffered glycerin aseptically from the dead birds after post mortem examination for the isolation of IBV and a total of 91 blood samples (without the history of IBV vaccine administration) were collected from the selected farms for the preparation of sera suspected to be

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infected with IBV aged ranging from 8 to 84 weeks. Samples were divided into seven groups from A1 to A7 according to age range of layer birds (Table. 1).

C. Isolation of the IBV in embryonated chicken egg

The preserved field samples were macerated separately in sterilized pestle and mortar to prepare a 10-20% (w/v) suspension in sterile PBS. After centrifugation at 3000 rpm for 30 minutes the supernatant thus obtained was treated with broad-spectrum antibiotic (Gentamycin) @ 50 µg /ml for 30 minutes at room temperature. Ten days old healthy embryonated eggs were selected by candling. A point for inoculation on a lateral side of the egg (at the site of embryo development but free from large blood vessel) and the air sac was marked with a pencil during candling and were disinfected with 70% alcohol for making a hole with the help of an electric egg driller without damaging the chorio-allantoic-membrane (CAM). 0.2ml of inoculums was inoculated into the allantoic cavity with tuberculin syringe. Both the holes were sealed with molten paraffin. The eggs were incubated at 37°C up to 7 days post inoculation (d.p.i.) and examined two times daily by candling. The embryos, which died after 24 hours as well as the live embryos at 7d.p.i. were chilled at 4°C for 1-2 hours to prevent the bleeding during samples collection. The embryos were open aseptically and examined for gross lesions.

D. Collection and preparation of antiserum

Blood samples were collected aseptically from the wing vein using 3 ml disposable sterile syringes from seven groups (Table 1). The sera were separated from blood samples using cooling centrifugation. Then the sera were kept in clean sterilized micro tubes and stored at -20°C for iELISA test.

E. Detection of infectious bronchitis virus-specific antibody by iELISA

The indirect enzyme linked immunosorbent assay (iELISA) was performed according to Das et al. [10]. Antibody titres were calculated from a single dilution described by Subramanyam et al. [11] and Ching et al. [12]. In iELISA, the titre was predicted from the absorbance value of 1:500 dilution of a serum at 405 nm. The IBV positive control has been carefully standardized to represent significant amounts of antibody to IBV in Chicken serum.

III. RESULT

A. History and clinical manifestation

The size of the suspected flocks was less than 1000 layer birds in each farm and the chicks were not vaccinated against IBV. Serous and catarrhal exudates in trachea, caseous materials that blocked the nasal passage, cloudy air sacs and swollen kidneys were found in postmortem examination of IBV affected sick and dead layer birds. In laying birds, fluidly yolk materials were found in abdominal cavity. Occasionally swollen lungs were found in 52-62 weeks aged birds.

Table I
Propagation of IBV suspected field samples in 10-days–old embryonated chicken eggs

SL. No.	Group	Age range	Organ Samples	samples propagated	samples	
					Positive	Negative
01.	A1	08-18 weeks	Trachea	2	0	2
			Lungs	2	1	1
			Ovary and oviduct	2	1	1
02.	A2	19-29 weeks	Trachea	2	1	1
			Lungs	2	0	2
			Ovary and oviduct	2	1	1
03.	A3	30-40 weeks	Trachea	2	2	0
			Lungs	2	1	1
			Ovary and oviduct	2	1	1
04.	A4	41-51	Trachea	2	2	0

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		weeks	Lungs	2	1	1
			Ovary and oviduct	2	2	0
05.	A5	52-62 weeks	Trachea	2	2	0
			Lungs	2	2	0
			Ovary and oviduct	2	2	0
06.	A6	63-73 weeks	Trachea	2	2	0
			Lungs	2	2	0
			Ovary and oviduct	2	2	0
07.	A7	74-84 weeks	Trachea	2	2	0
			Lungs	2	1	1
			Ovary and oviduct	2	2	0
Total				42	30	12

B. Isolation of IBV suspected field samples

A total of 42 IBV suspected field samples were propagated in embryonated eggs and out of them 30 samples were detected as positive to IBV infection (Table 1). Positive to IBV infection was determined through embryo mortality after inoculation of IBV suspected field samples and also considering different ages of layer birds as well as the organs of susceptibility (Table. 1). The overall incidence was recorded as 71.42% where the highest rate was 100% in case of 52-62 and 63-73 weeks aged layer birds in Dinajpur, Bangladesh. The lowest incidence rate was estimated as 33.33% in case of 08-18 and 19-29 weeks aged layer birds (Fig. 1). The highest mortality was found in A5 and A6 groups and most of them are died between 4^{th} and 6^{th} days post infection (Table 2). The gross pathological lesions were observed that stunting, curling and dwarfing of the embryos in comparison to uninoculated control group. In addition, there were found internal lesions consisting of cutaneous hemorrhages after four or more days of post inoculation. Congested lungs and swollen kidneys were also found. The yolk sac appeared shrunken with an increased volume of allantoic fluid. The allantoic and chorioallantoic membranes (CAM) were edematous.

Table II
Mortality pattern of the chicken embryos with field strains of IBV

Group	Embryos No.	Route	Dose	Embryos mortality at day (Post inoculation)							
				Dl	D2	D3	D4	D5	D6	D7	D8
A1	5	AS	0.2ml	-	-	-	-	-	2	-	-
A2	5	AS	0.2ml	-	1	-	-	1	-	-	-
A3	5	AS	0.2ml	-	-	-	1	2	-	1	-
A4	5	AS	0.2ml	-	-	1	-	2	1	1	-
A5	5	AS	0.2ml	-	-	1	2	-	2	1	-
A6	5	AS	0.2ml	-	1	-	1	1	3	-	-
A7	5	AS	0.2ml	-	-	-	2	1	2	-	-
E	5	-		-	-	-	-	-	-	-	-
Total	40			0	2	2	6	7	10	3	0

E = Uninoculated control group, D = Day, AS= Allantoic sac

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C. ielisa test for IBV specific serum antibody titre

The result of antibody titres from iELISA test of the suspected layer birds' sera samples revealed the highest antibody titre at 63-73 weeks aged birds and lowest antibody titre at 08-18 and 19-29 weeks aged layer birds (Table. 3).

Table III
Infectious bronchitis virus-specific antibody titre of different ages layer birds.

Sample No.	Age of the chicken (weeks)													
	08-18		19-29		30-40		41-51		52-62		63-73		74-84	
	Ab Titre	+/-	Ab Titre	+/-	Ab Titre	+/-	Ab Titre	+/-	Ab Titre	+/-	Ab Titre	+/-	Ab Titre	+/-
01	3210	+	450	+	2197	+	1893	+	2539	+	14849	+	11843	+
02	204	-	254	-	308	-	1196	+	13331	+	13898	+	10159	+
03	271	-	1726	+	277	-	1117	+	13582	+	2676	+	11526	+
04	342	-	300	-	1184	+	1292	+	5027	+	11476	+	3994	+
05	4898	+	1709	+	300	-	1251	+	4473	+	7212	+	4615	+
06	254	-	254	-	950	+	850	+	3026	+	4006	+	1726	+
07	4536	+	2847	+	438	-	855	+	3452	+	10864	+	1913	+
08	304	-	304	-	550	-	755	-	5586	+	7762	+	3227	+
09	475	-	1913	+	4348	+	400	-	10626	+	14843	+	2435	+
10	855	+	855	-	1301	+	450	-	8554	+	12740	+	2847	+
11	217	-	1196	+	2376	+	663	-	7737	+	4064	+	1709	+
12	276	-	2197	+	2801	+	713	-	8258	+	10684	+	2659	+
13	475	-	445	-	304	-	2639	+	14311	+	4611	+	1913	+

Legends	Titre Range	Antibody status (According to manufacturer instruction)
+	624 or less	Negative
_	834 or greater	Positive

IV. DISCUSSION

The present research work was conducted in order to detect the IBV infection and the IBV specific serum antibodies in context of Bangladesh in Dinajpur district using the propagation technique of IBV and serological detection of viral antibodies. IBV is responsible for sudden drop in egg production and causes economic losses in layer farms [7]. The natural infection of IBV was characterized by depression, coughing, sneezing; tracheal rales and accumulation of excess mucous exudates in the bronchi. These observations are in conformity with the previous report of Buxton et al [6]. In the present study virus isolates caused embryo

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mortality. The mortality of embryo after 24 hours of post infection was considered to be IBV positive. Moderate mortality was observed at day 4 whereas the highest mortality was at day 6 which is similar to David et al [13] where the authors declared that embryo mortality in between 2 to 7 days p.i. were IBV specific. The dead infected embryo showed dwarfing and curling signs. These gross lesions of the present findings are consistent with Calnek et al [14]. However, in this experiment the mortality pattern and the magnitude of lesions found in the embryo were not consistent. This might be due to the indigenous embryonated eggs used for virus isolation instead of using SPF (specific pathogen free) eggs. Because it is believed that the indigenous chickens are relatively more resistant to IBV infection than commercial chickens. IBV suspected field sera samples were collected from layer birds and specific serum antibody titres were measured by using indirect ELISA test. 76.92 % sera sample were positive to IBV and the highest and lowest antibody titre was recorded as 14849 and 204 respectively in 63-73 weeks aged layer birds (Table. 3). In this age group, the highest mean titre was found as 9206.53 (Fig. 2). This significant increase of antibody titre at 63-73 aged layer birds among the seven age groups of different small scale layer farms in Dinajpur district might be due to infection with IBV. Emikpe et al. [15] reported that IBV prevalence in indigenous chickens at the age of over 56 weeks was higher i.e. 78.32% in Nigeria which is quite similar to present study. Whereas, in Bangladesh, the highest incidence was recorded in birds of peak-production age group (›20 to 45 weeks) by Bhattacharjee et al. [3] which is a little bit incompatible with the present study. Concerning, the iELISA test for virus-specific antibody titres and IBV propagation through allantoic sac in 10-days-old embryonated indigenous chicken eggs, it may be hypothesized that the affecting virus was absolutely IBV.

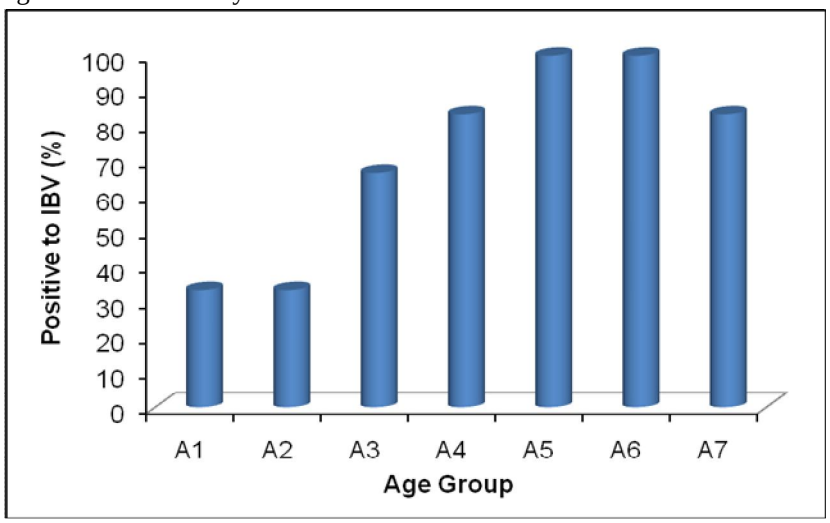

Figure 1: Incidence of Infectious bronchitis virus in chickens of different ages in small scale commercial layer farms in Dinajpur.

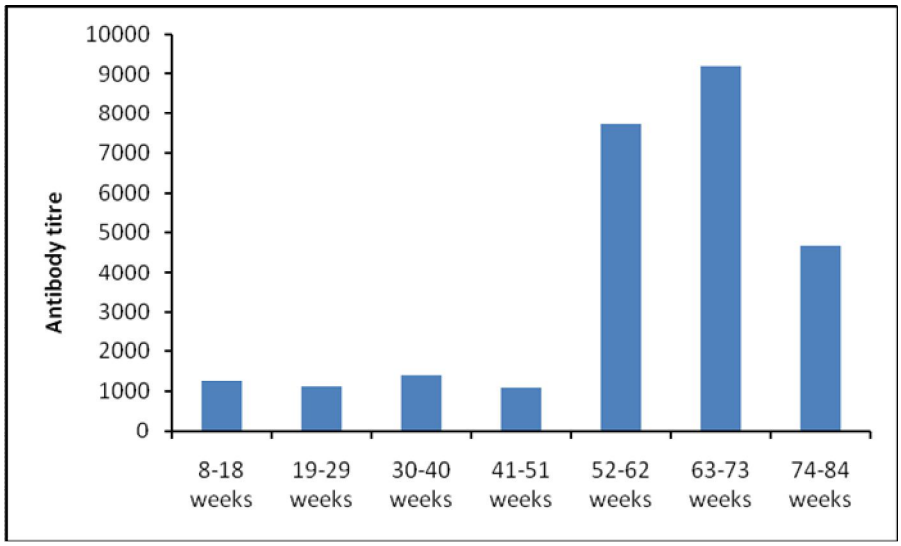

Figure 2: Mean antibody titre of chickens of different ages in small scale commercial layer farms in Dinajpur.

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V. CONCLUSIONS

In this work the avian infectious bronchitis virus-specific antibodies were successfully detected from the field outbreaks through antibody test kit (iELISA kit) and the virus induced a significant ELISA antibody titre which indicates that the affecting virus was absolutely IBV. In layer birds of 63-73 weeks ages at the small scale commercial poultry farm in Dinajpur was occurred frequently than other ages. The propagation of IBV in chicken eggs through AS route cause death of embryo with showing of specific symptoms that is specific for IBV infection. This work has future value with suitable vaccination program using common field serotype through the isolation and serotyping of IBV from all over the Bangladesh.

VI. ACKNOWLEDGEMENT

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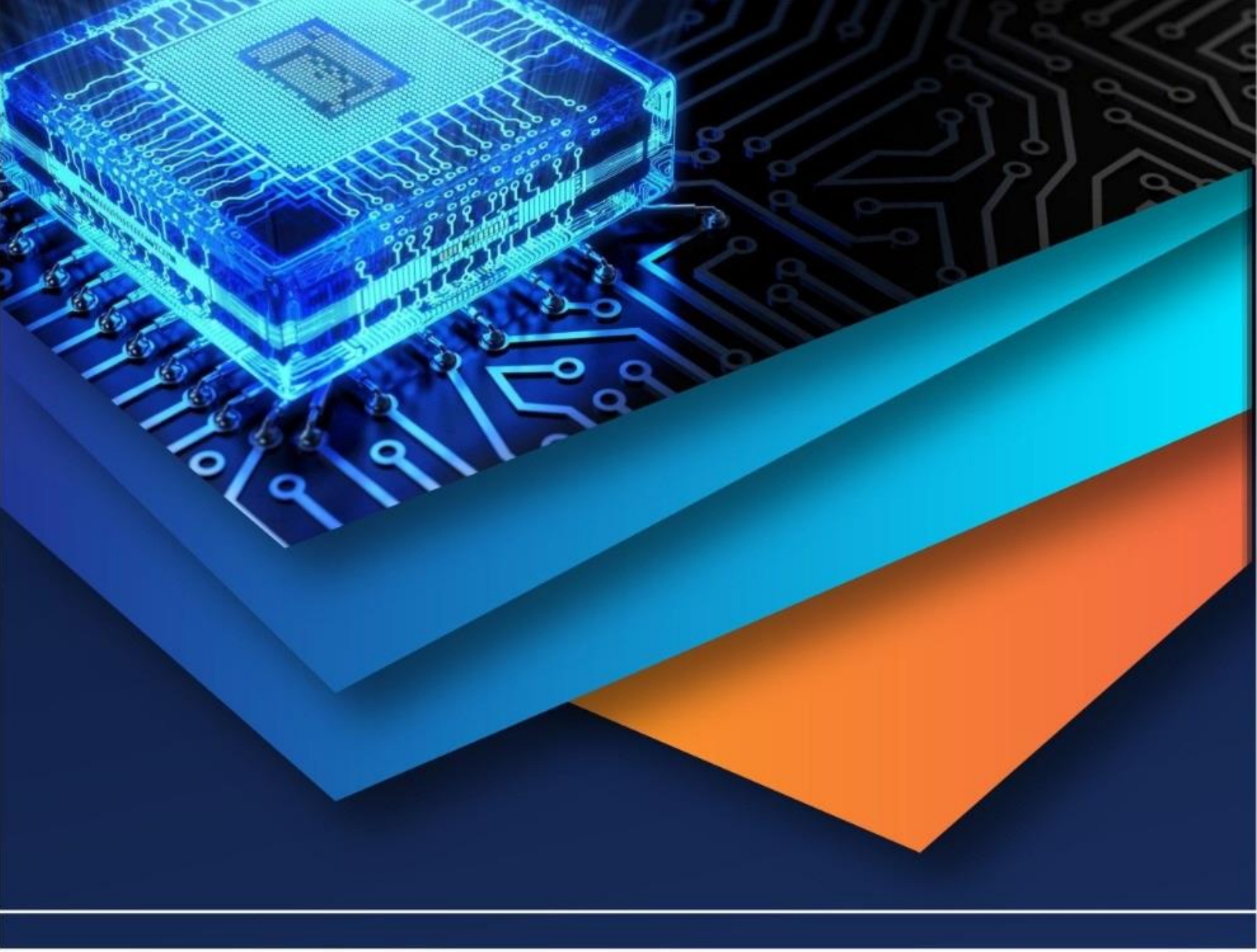

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