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Sero-Epidemiology of Avian Influenza Virus in Native Chicken in Bangladesh

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Abstract: Sero-epidemiological investigation was carried out to determine avian influenza virus in native chickens at Dagonbhuyian, Feni district in Bangladesh. A total of 224 sera samples were collected from the chickens of key beneficiaries during monsoon, winter and summer seasons. All sera samples were examined for the detection of antibodies of Avian influenza virus by indirect enzyme linked Immunosorbant assay using commercially available Kits. The seroprevalence was found 25% in Ram Nagar union, 14.28% Matubhuyian union, 0% in Rajapur union and 0% Jaylashker union. The overall seroprevalence of avian influenza was recorded 9.82%. Sero-epidemiology of virus in relation to seasonal variation was also carried out in the study areas and it was recorded 14.45% during monsoon, 3.70% during winter and 11.67% during summer season. The investigation exhibited higher prevalence of avian influenza in hens (10.83%) than cocks (8.65%). The investigation revealed the highest (12.80%) prevalence in birds > 34 weeks of age group and the lowest (3.13%) in birds of 8-16 weeks of age. However, Quick antigen detection kit for avian influenza virus identification did not show positive results of the samples collected for virological study.

Key words: Sero-epidemiology, avian influenza, prevalence, ELISA

Introduction

Avian influenza is a disease of viral etiology that ranges from a mild or even asymptotic infection to an acute, fatal disease of chickens, turkey, guinea fowls, and other avian species, specially migratory water fowl (Clavijo *et al*., 2003). The disease is caused by avian influenza virus A type, which is classified under the family Orthomyxoviridae. Influenza viruses vary widely in their ability to cause disease (Pathogenicity) and their ability to spread among birds. Wild birds usually act as carrier. Some strains of influenza viruses cause severe illness or death in chickens, turkeys and guinea fowl (Alexander, 2003; Katz, 2003). Among the 16 hemagglutinins and 9 neuraminidase antigens of type A influenza viruses (Akey, 2003) H5 N1 has been proved to be more virulent. It causes 100% morbidity and mortality to the poultry population (Cherbonnel *et al*., 2003).

Recent outbreak of Avian influenza in some Asian and European countries e.g., China, Hong-Kong, Thailand, Malaysia, Vietnam, Indonesia, Russia, Turkey, Japan, Romania and India drew attention to the world community (OIE, 2006). In addition to their effect on avian species the virus was found to be associated with death of human being. Thus it has created a serious public health concern (Alexander, 2003; Katz, 2003; Sins *et al*., 2003). There is no evidence of out break of Avian influenza in Bangladesh. It does not mean that Bangladesh is free from Avian influenza because wild birds and migratory waterfowls are available here which are considered to be the main source of infection in domestic poultry (Alexander, 2000). Pathogenic stains of Avian influenza may emerge from low virulent virus through antigenic drift and shift (Vander *et al*., 2003) that may cause havoc to national economy as well as to public health problem. Considering this a research project was undertaken to study sero-epidemiology of avian influenza in native chickens of selected poultry rearing areas in Bangladesh.

Materials and Methods

Study area: The present research work was conducted at Dagonbhuyian Upazilla under Feni district, in Bangladesh. In the area Small holder Livestock Development Project-2 (poultry rearing project) has been implemented by Grassroots Health and Rural Organization for Nutrition Initiative (GHARONI) a Non Government Organization and Directorate of Livestock Services. Laboratory work was conducted in the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh.

Samples: A total of 224 blood samples were randomly collected from the 'Native' chickens belonging to key

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rearers of Small Livestock Development Project (SLDP-2) areas from July 2005 to April 2006 to obtain sera conducting serological tests. At the same time 224 cloacal swabs were also collected from the same bird of the same area for the isolation and identification of avian influenza virus during the above period. The samples were preserved at –20°C until use.

Methods

Enzyme linked Immunosorbant Assay (ELISA): Commercial ELISA kits (BioChek, Crabethstraat 38-C 2801 AN Gouda, Holland) was used for the detection of antibodies against avian influenza virus.

The protocol for ELISA was followed as per the instructions supplied with the kits. Briefly, the reagents were allowed to keep at room temperature and then mixed by inverting. The sample positions were recorded on a work sheet. Serum sample was diluted in 1:500 dilutions with diluent buffer. An amount 100 µl of pre-diluted positive and negative controls and diluted samples were added to appropriate wells of ELISA plate. Each sample and controls (positive and negative) was run in duplicate for optimum result. The plate was covered with an adhesive cover and incubated at room temperature for 30 minutes. Adhesive cover was removed and the plate was washed 4 times with wash buffer (300 µl per well).An amount of 100 µl of enzyme conjugate reagent was added to each well and the plate was covered with an adhesive cover and incubated at room temperature for 30 minutes. After washing @100 µl substrate was added to each well and incubated at room temperature for 15 minutes. An amount 100 µl of stop solution was added to each well and mixed by gently tapping at the side of the plate. The reading of the result was taken by using a microtitre plate reader at 405-nm wavelength The method of calculation for ELISA results was done as per protocol supplied.

Quick antigen detection test for avian influenza virus: Quick S-in flu A/B kit (Denka Seiken Co. Ltd. Tokyo, Japan) was used for detection of avian influenza virus. It is a colloidal gold immunoassay kit for the detection of nucleoprotein and differentiation of influenza virus type A and B. The test was performed according to manufacturer's instructions. Known positive and negative control samples supplied with the kit were used simultaneously.

Calculation of results: The presence or absence of antibody to avian influenza virus is determined by relating the A (650) value of the unknown to the positive control mean. The positive control has been standardized and represents significant antibody levels to AIV in chicken serum. The relative level of antibody in the unknown can be determined by calculating the sample to positive (S/P) ratio.

The equation of calculation provided in ELISA kit was used for the calculation of antibody titer.

a) Negative control mean ($\overline{NCX}$)

$$\frac{\text{Well A 1A (650) + Well A 2 A (650)}}{2} = \overline{NCX}$$

b) Positieco ntrol mean ($\overline{PCX}$)

$$\frac{\text{Well A 3A (650) + Well A 4 A (650)}}{2} = \overline{PCX}$$

c) S/ P Ratio

$$\frac{\text{Sample mean - } \overline{NCX}}{\text{PCX - } \overline{NCX}} = S / P$$

d) Titer- Relates S/P at a 1:500 dilution to an endpoint titer:

Log_{10} Titer=1.1 (Log_{10} S/P) + 3.156

Interpretation of Results: A serum samples with S/P ratios of less than or equal to 0.5 considered negative. S/P ratios greater than 0.5 (titters greater than 668) considered positive and indicates vaccination or other exposure to avian influenza virus.

Results

Seroprevalence: Results of the investigation revealed that the seroprevalence of Avian influenza was 25% in Ram Nagar union, 14.28% in Matubhuyian union, 0% in Rajapur and 0% in Jaylashker union (Fig. 1). However, the overall seroprevalence among the aforesaid four unions was found to be 9.82% (Table 1). The highest seroprevalence was found in Ram Nagar union (25%) and the lowest seroprevalence was found 0% in Rajapur and Jaylashker union (Fig. 1). Statistically a significant difference of prevalence between the areas was observed ($P<0.01$). The seasonal seroprevalence was found 14.45% in monsoon and 3.70% in winter season. In summer, the seroprevalence was found to be11.67%, next to the monsoon. The highest seroprevalence (14.45%) was found in monsoon and the lowest was found (3.70%) in winter. Significant difference ($P<0.01$) of prevalence rate was observed due to seasonal variation. The brief result about seasonal seroprevalence of avian influenza is presented in Fig. 2. The seroprevalence rate of avian influenza was found 10.83% in female birds (hens) and 8.65% in male birds(cocks). The results were analyzed statistically one-way ANOVA method and found significant ($p<0.01$) variation between male and female birds (Fig. 3). The possible explanation for the more prevalence rate in hen might be due to the stress during egg production and vulnerability of infection to the female.

The seroprevalence of avian influenza was studied based on age and presented on Fig. 4. It was observed

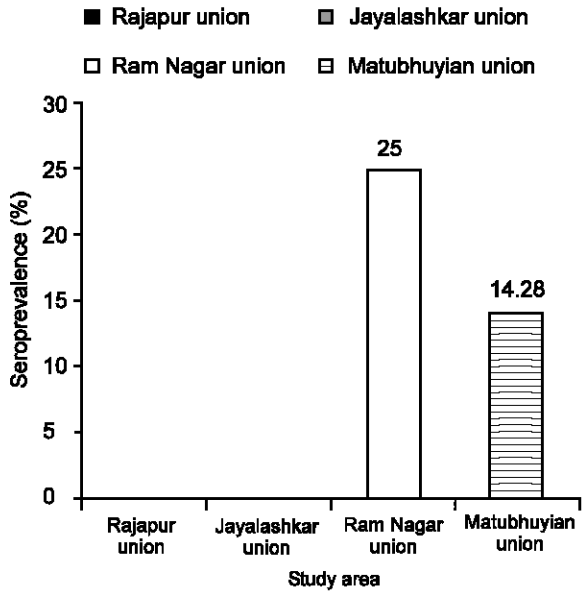

Fig. 1: Seroprevalence of avian influenza in native chickens of Dagonbhuyian upazilla under Feni district

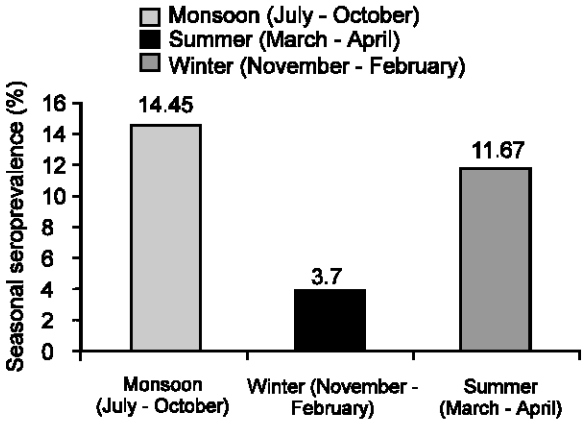

Fig. 2: Seasonal seroprevalence of avian influenza in native chickens of Dagonbhuyian upazilla under Feni district

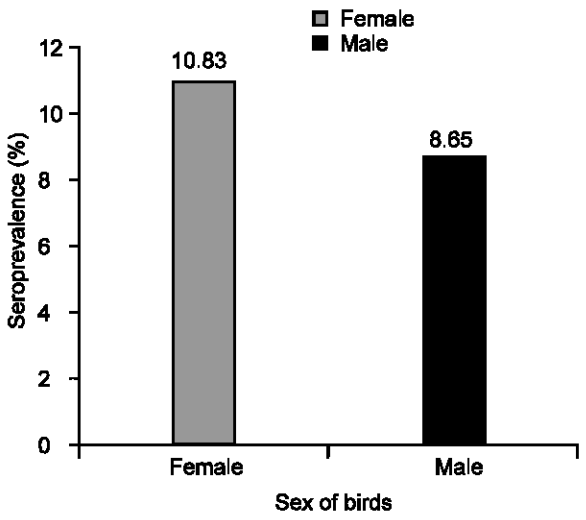

Fig. 3: Seroprevalence of avian influenza on the basis of sex in native chickens of Dagonbhuyian upazilla under Feni district

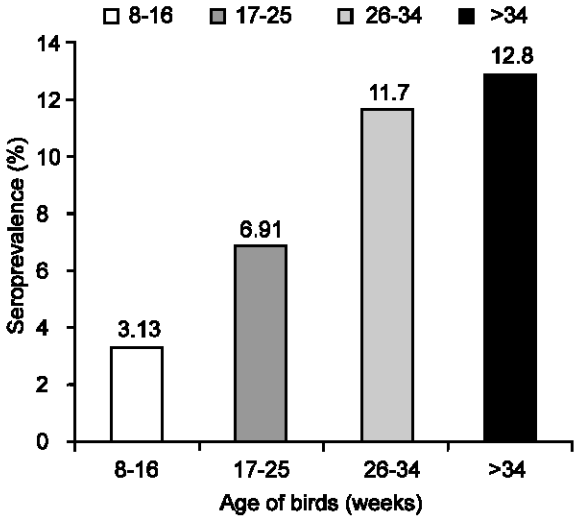

Fig. 4: Seroprevalence of avian influenza on the basis of age in native chickens of Dagonbhuyian upazilla under Feni district

that 3.13% seroprevalence was found in 8-16 weeks of age of birds, 6.91% in 17-25 weeks age 11.70% in 26-34 weeks age and 12.80% >34 weeks age of birds. The highest (12.80%) prevalence was found in > 34 weeks age of birds and the lowest (3.13%) was found in 8-16 weeks age of birds. Seroprevalence of different age groups was also found significantly different ($P < 0.01$). This may be due difference of immune status at various age groups of birds.

Quick avian influenza virus detection test: Cloacal samples were collected besides sera samples for virological study to detect avian influenza virus. It was observed that quick antigen detection kit of avian influenza did not give any positive results of the samples tested for the avian influenza virus.

Discussion

Study in relation to the determination of avian influenza virus by sera analysis was also carried out by Alam *et al.* (2003) in another area in Bangladesh who reported that the seroprevalence in the native chickens was 38.6% in Cox's Bazar, 32.30% in Barisal and 14% in Bogra districts. Their findings showed higher seroprevalence rate than the present study. On the other hand, a study

Table 1: Seroprevalence of avian influenza in native chickens of Dagonbhuyian upazilla under Feni district in Bangladesh

Study area	Total sera tested	Positive cases (%)	Overall Prevalence (%)	Mean ± SD	Level of ignificance (P-value)
Rajapur union	56	0			
Jaylashker union	56	0	9.82	5.5 ± 6.81	0.003**
Ram Nagar union	56	14 (25)			
Matubhuyian union	56	8 (14.28)			

SD= Standard deviation, ** = P < 0.01 level of significance, %= Percentage

conducted by Biswas *et al.* (2004) based on serological study. The results of their investigation have the close agreement with present study. Seasonal variation and seroprevalence was also studied by Biswas *et al.* (2004) and found variable results in different seasons. In our study the highest (14.45%) prevalence was detected in monsoon and the lowest (3.70%) in winter. It might be due to the influence of hot weather and rain that might reduce the immune status of the birds, thus made them vulnerable to infection (Alexander, 1990). The result of the present study have the similarity with the result of Biswas *et al.* (2004) with minor variation in winter season.

The seroprevalence was higher (10.83%) in female than (8.65%) in male (Fig. 3) indicated that female birds were found to be more susceptible to Avian influenza than male birds (Halvorson *et al.*, 1983).

Antibody against Avian influenza may be found at any age (OIE, 2003) of birds (Fig. 4). The highest (12.80%) prevalence was found in >34 weeks age of birds and the lowest (3.13%) was found in 8-16 weeks age of birds. The result showed similarity with the study conducted by Brug *et al.* (1987).

In this present experiment, Avian influenza virus could not be detected from cloacal swabs of the native chickens of the study areas though it is known that avian influenza multiplies in the respiratory tract and gastro-intestinal tract and virus sheds through respiratory secretions and faeces. This finding suggested that avian influenza viruses were not sheded in the environment during the experimental period.

In this experiment, no influenza virus could be detected by antigen detection although antibodies of avian influenza were detected in the sera samples of native chickens of the study areas by Indirect ELISA test. This might be that the chickens were exposed with previous natural infection with low virulent avian influenza virus, as wild and domestic ducks are potent carriers of avian influenza virus. In the study areas, the native chickens were reared under semi-scavenging system and were allowed to scavenge with ducks in the yard, in the crop fields near to water reservoirs where domestic ducks, wild ducks and migratory birds used to scavenge over there. This factor may contribute in natural infection to the native chickens (Alexander, 2003; De Marco *et al.*, 2003; Senne *et al.*, 2003; Vander *et al.*, 2003; Capua and Alexander, 2004).

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