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Seroprevalence of Low Pathogenic Avian Influenza (H9) in Sonali Chickens of Joypurhat

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Abstract

The present study was designated to know the sero-prevalence status of H9 antibodies in Sonali chickens at Joypurhat district in Bangladesh by initial screening of AIV type A by ELISA followed by H9 LPAI detection by HI test. 180 sera samples from 18 flocks were collected and screened for Avian Influenza Virus type A antibodies by ELISA. All ELISA positive samples were tested by HI test, specific for H9 antibodies. On ELISA, sero-prevalence for AIV type A were 14.4% and 3.3% in older aged group (≥11weeks) and growing aged group (≤10 weeks) respectively, with an overall sero-prevalence of 8.89% in Sonali chickens at Joypurhat district. Among these AIV sero-positive samples, 92.31% 0f older aged group and 66.67% of growing aged group chickens found sero-positive to H9 antibodies by HI test with an overall 87.5%. Out of 180 sera samples, only 14 samples found positive to H9 antibodies, indicating 7.78% sero-prevalence in Sonali chickens of Joypurhat district. This study revealed higher sero-prevalence of AIV antibodies as well as H9 antibodies in older aged group which might be due long-time exposure to infections. Sero-prevalence of both AIV type A and H9 LPAI are higher in older aged Sonali chickens. The findings of the present study suggest that the overall seroprevalence of AIV type A and H9 LPAI in Sonali chickens are 8.89% and &.78% respectively, at Joypurhat district in Bangladesh and also suggest that H9LPAI virus in circulation in Sonali chickens at Joypurhat district in Bangladesh.

Keywords

Sero-prevalence, Avian Influenza, Sonali Chicken, ELISA, Sera

INTRODUCTION

Commercial poultry farming in Bangladesh is growing rapidly; in the nineties, the total investment in the poultry sector was only Tk 15 billion, but now it is more than Tk 150 billion. This industry has a

great potential for boosting the economic growth of the country as well as ensuring food security (Ali and Hossain, 2012).

Avian influenza is an important poultry disease caused by Type A influenza viruses under the family *Orthomyxoviridae*, that can cause mild to severe infection in different avian species resulting in severe damage to the poultry industry. Influenza A viruses infecting poultry are divided into two groups based on their pathogenicity: highly pathogenic avian influenza (HPAI) which cause generalized rather than respiratory disease with flock mortality as high as 100% and low pathogenic avian influenza (LPAI) which usually causes much milder respiratory disease with low mortality if there are no secondary viral and/or bacterial infection or poor environment condition (OIE Manual, 2009).

In 2003 to 2004, HPAI virus caused multiple and widespread poultry outbreaks in many Asian countries, including Cambodia, China, Indonesia, Japan, South Korea, Laos, Malaysia, Thailand and Vietnam. Stamping-out has been the national policy in Bangladesh in combating H5N1 HPAI. Recently vaccination against H5N1 has been introduced on trial basis. Non-H5 or non-H7 AI isolates that are not virulent for chickens are identified as LPAI (OIE, 2009). Low pathogenic avian influenza viruses H9N2 became panzootic in the mid-1980s among multiple avian species in Asia, the Middle East, Africa and Europe. The H9N2 viruses infection is generally characterized by mild respiratory infections, however nowadays these viruses produce significant disease problems associated with high mortality in poultry. Although H9N2 viruses are characterized as LPAI viruses, they may cause high morbidity and mortality that may increase the risk of infections of H5N1 HPAI (Park *et al.*, 2001).

Low pathogenic H9N2 viruses are circulating in poultry farms of Bangladesh (Negovetich *et al*., 2011; Jannat *et al*., 2013; Shanmuganatham *et al*., 2013; Parvin *et al*., 2014). Both HPAIV H5N1 and AIV H9N2 are co-circulating among poultry population in many Eurasian countries mostly reported from Bangladesh, China, India, Pakistan, Vietnam, Israel, Egypt and United Arab Emirates. Although the LPAI is circulating in Bangladesh, there are very limited studies on LPAI even not in Sonali Chickens. There are no activities for controlling LPAI in Bangladesh.

The research work is aimed to study the Prevalence of Avian influenza type-A as well as LPAI in Sonali Chickens at Joypurhat District for the prevalence study of LPAI in Sonali Chickens and detection of antibodies to avian influenza type-A in Sonali Chickens in Joypurhat district.

MATERIALS AND METHODS

Research Area

Samples were collected from Sonali chicken raring farms at Joypurhat and most of the laboratory research work was conducted in the Central Disease investigation Laboratory (CDIL), Department of Livestock services, Dhaka.

Research Period

The duration of experiment was 6 months from June, 2015 to December, 2015.

Equipment

Balance (AND, USA); Microcentrifuge (Eppendorf, Germany) Bench centrifuge; ClassII Biosafety cabinet (ESCO, Singapore); Micropipettes (Eppendorf, Germany); pH meter; Vortexer; Benchtop autoclave; Refrigerators, Freezers, ELISA Reader (Multiskan-EX Lab system, Thermo-scintific) etc.

Plastic Ware and Other consumables

Pipette tips (Eppendorf, Germany); Falcon tubes: 15 ml (Becton Dickinson Labware, USA and Eppendorf, Germany); Sterile filter tips (Eppendorf, Germany); Syringe and Needle (Opsonin, Bangladesh), etc.

Sera samples

A total of 180 blood Samples of Sonali Chickens of different age groups were collected from farms of

Joypurhat District in this study and subjected to ELISA and HI test.

Test Kits and Reagents

Test kit	Use	Source
Avian Influenza Antibody test ELISA kit	Type-A AIV antibody detection based on Matrix protein	IDEXX(U S A)
H9 antigen	HI test to detect H9N2 antibody in serum	Newcastle and Avian influenza Reference Laboratory, Italy

Table-1: Test kits and Antigen used in the present study

Solutions, Buffers and Chemicals

1X PBS, Alsever's solution, 1% Chicken RBC were used in the present study.

METHODS

Blood Collection and Sera Separation

- 1ml of each of 180 blood samples from Sonali chickens were collected in 3ml disposable syringe and lebelled with separate numbering. Samples were collected from those Sonali chicken rearing farms which have previous history of respiratory illness at Joypurhat.
- The syringe containing blood samples were then kept in horizontal position at room temperature overnight to separate serum from the collected blood.
- Sera samples were then transferred to separate eppendorf tube (1.5 ml) and subjected to low speed centrifuge at 1000 rpm for 5 minutes and supernatant were collected as serum samples in separate labeled eppendorf tube.
- Separeted serum samples were then stored at -20^{0}C until use.

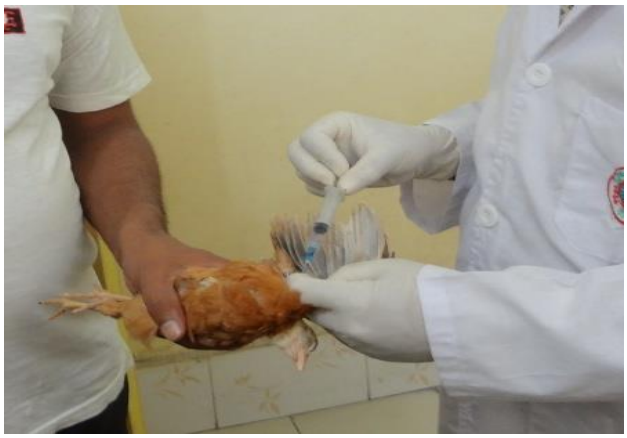

Figure 1: Collection of blood from Sonali chicken

Figure 2: Sera sample collected from chicken blood

Elisa Test for AIV Type – A Antibody

The Elisa Kit was obtained from Biocheck Company. ELISA test was done following manufacturer's instruction.

Calculation of Result

The relative levels of antibodies in the unknown samples were determined by calculating sample-positive (S/P) ratio. The equation of calculation provided in ELISA kit was used for the calculation of antibody titer.

Hemagglutination (Ha) Test for Preparing Of 4hau Antigen

- 25 µL of PBS was added to each of the well in 96 well V-bottomed plastic microtiter plate.
- Then 25µl of antigen (H9N2) was added at first row and serial two-fold dilution was done up to 11th well and 12 th well was kept as control.
- Again, 25 µL of PBS was added to each of the well in 96 well V-bottomed plastic microtiter plate.
- 25 µL freshly prepared 1%(V/V) Chicken RBC was then added into each well and mixed by tilting and kept at room temperature for 30 minutes.
- The higest dilution of antigens that completely agglutinate the chicken RBC was recorded as 1 HAU.
- 4HAU was prepared with PBS for the use on Hemagglutination Inhibition test.

Hemagglutination Inhibition (Hi) Test for H9 Subtype Detection

HI test was done as per instruction outlined in OIE manual. Briefly,

- 25 µL of PBS was added to each of the well in 96 well V-bottomed plastic microtiter plates.
- Then 25µl of ELISA positive sera was added into the first well of each row separately and recorded properly.
- Two-fold serial dilutions of sera were made across the row up to 11th well and 12th well kept as control.
- 25 µL of 4HAU avian influenza virus subtype H9N2 was added into each well up to 11th well in each row and incubated at room temperature for 30 minutes.
- 25 µL freshly prepared 1%(V/V) Chicken RBC was then added into each well and mixed by tilting and kept at room temperature for 40 minutes.
- The highest dilution of the test sera that completely inhibited the RBC agglutination was recorded as antibody titre against H9N2.
- Titer of H9 antibody in the test sera $\geq \log_2^4$ was considered as positive (Ghaniei et al., 2013).
- The titer was analyzed statistically by measuring arithmetic mean titer (AMT).

RESULTS

The present study revealed that 3 out of 90 samples of ≤10 weeks aged group were positive and 13 out of 90 samples of aged group ≥11 weeks were positive. The sero-prevalance of avian influenza type A was 3.3% and 14.4% in ≤10 weeks aged group and ≥11 weeks aged group respectively. The overall sero- prevalence of AIV antibodies in sonali chicken at Joypurhat district was 8.89%.

Age group	No of samples tested	No of ELISA positive samples	No of ELISA negative samples	Sero-prevalence (%)	Over all sero- prevalence (%)
≤10 weeks	90	3	87	3.3	8.89
≥11 weeks	90	13	77	14.4	
Total	180	16	164		

Table-2: Sero-prevalence of avian influenza type A in sonali chickens of Joypurhat district

Figure 3: ELISA Plate

Hemaglutination Inhibition Test

All 16 AIV type A positive sera on ELISA test were subjected to HI test. 14 samples out of 16 samples were positive on HI test with HI titer > $\log_2^4$. Results are presented in Table-3.

Age group	ELISA positive Sample	HI titer (Log_2)	AMT	%CV
	1	3	4.33	35.2
	2	4		
≤10 weeks	3	6		
≥11 weeks	1	6	5.62	26.7
	2	4		
	3	8		
	4	5		
	5	6		
	6	7		
	7	4		
	8	7		
	9	6		
	10	7		
	11	6		
	12	4		
	13	3		

Table-3: Results of ELISA test and HI test

DISCUSSION

AIV causes frequent epidemics and occasional pandemics in various animals and thus present a significant public health problem associated with considerable economic consequences. AIVs of various subtypes are circulating in poultry (Abbas *et al*., 2010; Jeong *et al*., 2010; Kim *et al*., 2010). In particular, H5N1 and H9N2 AIVs are predominant among poultry flocks causing severe disease outbreaks with high morbidity and mortality (Nagarajan *et al*., 2009; Xu *et al*., 2007; Cameron *et al*., 2000) in many countries including Bangladesh.

180 sera samples from 18 flocks were collected and screened for AIV type A antibodies by ELISA. ELISA positive samples were subjected to HI test, specific for H9 antibodies. On ELISA, sero-prevalence for AIV type A were 3.3% and 14.4% in growing aged group (≤10 weeks) and older aged group (≥11 weeks) respectively, with an overall sero-prevalence of 8.89% in sonali chickens at Joypurhat district. Among, these AIV sero-positive samples, 66.67 % of growing aged group and 92.31% of older aged group chickens found sero-positive to H9 antibodies by HI test with an overall of 87.5%. Out of 180 sera samples, only 14 samples found positive to H9 antibodies, indicating 7.78% sero-prevalence in sonali chickens of joypurhat district. This study revealed that sero-prevalence of AIV antibodies as well as H9 antibodies is higher in older aged group which might be due long-time exposure to infections as the virus is in circulation since 2006 (Parvin *et al*. 2013). Similar studies were done by Nooruddin *et al*. (2006) on native chicken of Bangladesh and found over all 8.92% sero-prevalence of AIV antibodies. In another studies, conducted by Alam *et al*. (2003) found 14% sero-positive native birds in Bogra district. Findings of both studies are nearly close to the present study, although, Alam et al. (2010) recorder higher sero-prevalence in other district of Bangladesh (Cox's Bazar 38.60% and Barishal 32.30%).Cheng *et al*. (2002) found 7% H9 sero-positive chicken and Li et al. (2004) found 12.8% H9 sero-positive chickens in their study areas. Antibody against Avian influenza may be found at any age (OIE, 2013) of birds.

CONCLUSION

So the present study may be concluded as;

a) Sero-prevalence of both AIV type A and H9 LPAI are higher in older aged Sonali Chickens.
b) H9 LPAI is in circulation in Sonali chickens at Joypurhat District in Bangladesh.

c) The overall seroprevalence of AIV type A and H9 LPAI in Sonali Chickens are 8.89% and 7.78% respectively, at Joypurhat district in Bangladesh.
d) Further studies can be taken to find out the LPAI scenario in Bangladesh.

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