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Identification and Characterization of Hemagglutinating Viruses in Native Chickens in Bangladesh

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Abstract: A total number of 224 cloacal swabs were examined for hemagglutinating activity from 4 different poultry farms of Noakhali district in Bangladesh. Out of the total samples 15 exhibited positive reactions with chicken erythrocytes. Among the 15 positive samples, 11 were found to be positive for Newcastle disease virus. The highest (100%) prevalence of Newcastle disease virus was found in Ram Nagar union and the lowest (66.66%) were found in Rajapur union. Seventy five percent prevalence was found in Jaylashker and Matubhuyian union. The overall prevalence of Newcastle disease virus was found to be 79.17% in the four unions. No egg drop syndrome virus and avian influenza viruses were identified in the samples those gave positive result in hemagglutination test.

Key words: Newcastle disease virus, hemagglutination test, prevalence

Introduction

Most of the fatal avian viruses are known to have hemagglutinating activity with chicken erythrocytes (Sarker, 2006). As such this property is being used for the preliminary screening of the poultry farm for the presence of any such devastating viruses such as Avian influenza, Newcastle, Egg drop syndrome etc.

Recent outbreak of Avian influenza in some Asian and European countries e.g., China, Hong-Kong, Thailand, 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). On the other hand, Newcastle disease virus is known as highly contagious and the most fatal virus of avian species. It can destroy the poultry population within a few days if proper vaccination has not been done in a farm. In Bangladesh poultry industry is a rapidly growing segment at present. Therefore, continuous monitoring of poultry farms is prerequisite to prevent and control the malady. Keeping this in view, the present study was undertaken for the preliminary detection of hemagglutinating viruses and subsequent identification of their specificity.

Materials and Methods

Collection of virological samples: 224 cloacal swabs samples were collected from live 'Native' chickens of a poultry farm in Noakhali district, Bangladesh. After collection, the samples were transported to the Laboratory of the Department Microbiology and Hygiene, BAU maintaining proper cool chain. All the samples were kept at -20°C in the Virology laboratory until use.

Antisera: Antisera of Newcastle Disease Virus (Newcastle disease, Charles River SPAFAS) and Egg Drop Syndrome-76 virus (Egg drop syndrome virus-76, Charles River SPAFAS) were used to differentiate Newcastle Disease Virus and Egg Drop Syndrome-76 virus from the other hemagglutinating viruses.

Inoculum preparation from collected cloacal swabs:

Collected each swab sample was placed in individual test tube containing phosphate buffer solution (pH 7.2). Then the cotton bud was removed and the solution was treated with antibiotic (gentamicin @ 50 µg/mL) for one h. Then few drops of the inoculum was streaked on nutrient agar media for sterility test and incubated at 37°C for 24 h. Bacteriologically sterile inoculum was selected for inoculation and stored at -20°C until use.

Inoculation of prepared inoculum in embryonated chicken egg:

The protocol for embryo inoculation was as follows-

- (i) An amount of 0.2 mL inoculum was inoculated through allantoic cavity route in 5 embryonated chicken eggs of 9-11 days old for each sample.
- (ii) After inoculation, eggs were incubated at 37°C for 5 days.
- (iii) The embryos died within 24 h were discarded.
- (iv) The eggs containing dead embryos were chilled and AF(s) were collected. Similarly, the eggs containing live embryos after five days also selected and allantoic fluid was collected and preserved as a source of viruses.
- (v) The collected fluids were kept in sterile eppendorf tubes with marking and stored at -20°C until use.

Preparation of 2% and 0.5% chicken red blood corpuscles (chickenRBC) suspension: Blood was collected from wing vein with sterile syringe and needle

Nooruddin *et al.*: Identification and Characterization of hemagglutinating viruses

Table 1: Detection of hemagglutinating viruses in the allantoic fluid

Area	No. of sample tested	Positive cases (%)	Overall Prevalence (%)	Mean±SD	P-value
Rajapur	56	6 (10.71)	6.69	3.75 ± 2.06	0.00**
Jaylaskher	56	4 (7.14)			
Ram Nagar	56	1 (1.78)			
Matubhuyian	56	4 (7.14)			

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

Table 2: Detection of Newcastle disease virus in the allantoic fluid positive for hemagglutinating viruses

Area	No. of sample tested	Positive cases (%)	Overall Prevalence (%)	Mean±SD	P-value
Rajapur	6	4 (66.66)	79.17	2.75±1.26	0.00**
Jaylaskher	4	3 (75)			
Ram Nagar	1	1 (100)			
Matubhuyian	4	3 (75)			

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

containing anticoagulant (4% trisodium citrate) at the rate of 1 mL for 10 mL of blood. Following collection, blood sample was mixed with phosphate buffered solution (PBS) and centrifuged at 1000 rpm for 10 min. The supernatant was discarded and resuspended with PBS and centrifuged at 1000 rpm for 10 min. This process was followed for three times and finally the supernatant was discarded. One mL of chicken RBC was mixed with 49 mL of PBS to prepare 2% RBC suspension. For plate hemagglutination (HA) and plate hemagglutination inhibition (HI) test 10 mL of 2% RBC suspension was mixed with 30 mL of PBS to make 0.5% chicken RBC and stored at 4-8°C for 3 days.

Plate Hemagglutination (HA) test: This test was done to detect the hemagglutinating viruses in the collected samples. The procedure of plate hemagglutination test was as follows:

- (i) 25 µL of PBS was dispensed in each well of a plastic U-bottomed microtitre plate.
- (ii) 25 µL of AF was placed in the first well and mixed well.
- (iii) From first well 25 µL of mixture was transferred into second well to make two fold dilutions. This process was continued up to the last well (11th) and from there 25 µL of mixture was discarded. Well 12 was used as control.
- (iv) 25 µL of 0.5% (v/v) chicken RBC was dispensed into each well.
- (v) The plate was tapped gently and was allowed to keep at room temperature for about 15 min.
- (vi) HA was determined by tilting the plate and observing the hemagglutination of the RBC.
- (vii) A uniform layer of hemagglutination covering the bottom of well of the plate was considered as positive HA and a sharp buttoning of RBC at the bottom of well of the plate was considered as negative.

Plate hemagglutination inhibition (HI) test for detection of Newcastle disease virus:

- (I) 25 µL of PBS was dispensed into each well of a plastic U-bottomed microtitre plate, except well 1 and 7 of each raw.

- (ii) 25 µL of known positive antiserum (Newcastle Disease, Charles River SPAFAS) was placed into first and second wells of each raw of the plate.
- (iii) From well two and eight of each raw two-fold dilution was made across the plate.
- (iv) 25 µL of HA positive virus was added into each well and left for a minimum of 30 min at room temperature.
- (v) 25 µL of 0.5% (v/v) chicken RBC was added to each well. After gentle mixing the plate was allowed to keep for about 40 min at room temperature.
- (vi) In each raw two wells (six and twelve) were kept as control.
- (vii) The hemagglutination inhibition activity was observed after 40 min and compared with control one.

Hemagglutination inhibition (HI) test for detection of Egg drop syndrome-76 virus: The test was performed following procedure of detecting Newcastle virus in plastic U-bottomed microtitre plate using @25 µL of known reference positive antiserum (Egg dropsyndrome-76, Charles River SPAFA).

Quick antigen detection test for avian influenza virus: Quick S-influ A/B kit (Denka Seiken Co. Ltd. Tokyo, Japan) was used for detection of AIV. 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.

Results

Hemagglutination inhibition (HI) test with ND specific antiserum and EDS-76 virus specific antiserum: Hemagglutination inhibition test was done to differentiate Newcastle disease virus and EDS-76 virus from other hemagglutinating viruses. Out of 15 plate HA positive samples, 11 samples were found to be positive

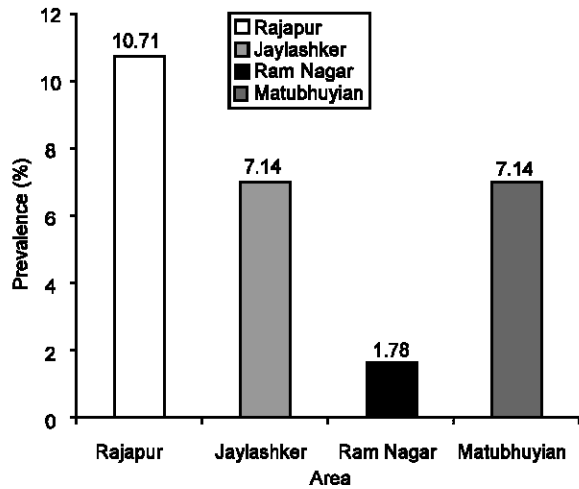


Fig 1: Allantoic fluid positive for hemagglutinating viruses

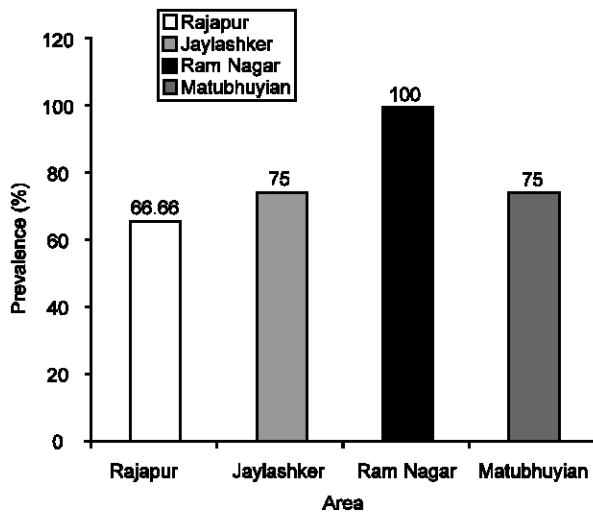


Fig 2: Identification of Newcastle viruses from hemagglutinating allantoic fluid

for Newcastle disease virus. The highest (100%) prevalence of Newcastle disease virus was found in Ram Nagar union and the lowest (66.66%) were found in Rajapur union. 75% prevalence was found in Jaylashker and Matubhuyian union. The overall prevalence of ND virus was found to be 79.17% in the four unions. There was significant difference observed at <0.01 levels. The results of prevalence of Newcastle disease virus are given in the Table 2. The four other samples were negative for both Newcastle disease virus and EDS-76 virus and were subjected to quick avian influenza virus detection test.

Quick avian influenza virus detection test: The 4 sample those were plate HI negative for both NDV and EDS-76 virus were subjected to perform quick antigen

detection test for avian influenza virus. No positive sample was found for avian influenza virus.

Discussion

In the present study, plate HA test was performed using allantoic fluid to detect the hemagglutinating viruses as AIV hemagglutinates chicken RBC (Palmer *et al.*, 1975; Beard, 1980). In this study, 15 samples were found to be positive to hemagglutinating viruses (Table 1). However, it is important to determine whether the hemagglutinating activity detected in the allantoic fluid is due to Avian influenza viruses or other hemagglutinating viruses, such as paramyxovirus like NDV, egg drop Syndrome-76 and other paramyxoviruses. Considering that those allantoic fluid containing hemagglutinating viruses were tested for NDV and EDS-76 virus by plate HI test (Easterday *et al.*, 1997). It was found that those allantoic fluid exhibited positive results were due to the NDV (Fig. 2) and rest of the samples were found negative for both NDV and EDS-76 virus. The samples (4) those did not give the positive result for the above were subjected to quick antigen detection test for avian influenza. However, these were not proved to be avian influenza virus.

In this experiment, no influenza virus could be detected by antigen detection although antibodies of avian influenza virus were detected in the sera samples of native chickens of the areas by indirect ELISA test. This might be that the chickens were exposed with previous natural infection with low pathogenic 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). The sera samples, which showed positive reaction to ELISA, those might be due to antigenic similarity with other hemagglutinating viruses (Kodihalli *et al.*, 1993).

References

- Alexander, D.J., 2003. Report on Avian Influenza in the Eastern Hemisphere during 1997-2002. *Avian Dis.*, 47: 792-797.
- Beard, C.W., 1980. Isolation and Identification of Avian Pathogens by S.B. Hitchner, C.H. Domermuth, H.G. Purchase and J.E. Williams. *Am. Assoc. Avian Pathol.*, Kennett Square, PA., pp: 67-69.
- Capua, I. and D.J. Alexander, 2004. Avian influenza: recent developments. *Avian Pathol.*, 33: 393-404.

Nooruddin *et al.*: Identification and Characterization of hemagglutinating viruses

- De Marco, M.A., G.E. Foni, L. Campitelli, E.L. Roffini, D.I. Trani, M. Delogu, V. Guberti, G. Barigazzi and I. Donatelli, 2003. Circulation of influenza viruses in waterfowl wintering in Italy during the 1993-1999 periods: Evidence of virus shedding and seroconversion in wild ducks. *Avian Dis.*, 47: 861-866.
- Easterday, B.C., V.S. Hinshaw and D.A. Halvorson, 1997. Influenza. In *Disease of Poultry*, 10th edn. by Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, Iowa State University Press, Ames, Iowa., pp: 583-605.
- Kodihalli, S., V. Sivanandan, K.V. Nagaraya, S.M. Goyal and D.A. Halvorson, 1993. Antigen capture enzyme immunoassay for detection of avian influenza virus in turkeys. *Am. J. Vet. Res.*, 54: 1385-1390.
- OIE, 2006. Avian influenza, an update. The Office International des Epizooties. www.oie.int.
- Palmer, D.F., M.T. Coleman, W.R. Dowdle and G.C. Schild, 1975. *Advance Laboratory techniques for Influenza Diagnosis*. United States Department of Health, Education and welfare Immunology series No.6, Procedure Guide. Center for Disease control, Atlanta, GA.
- Sarker, A.J., 2006. Personal communication. Professor, Microbiology and Hygiene, BAU, Mymensingh, Bangladesh.
- Senne, D.A., D.L. Suarez, J.C. Pedersen and B. Panigrahy, 2003. Molecular and Biological Characteristics of H5 and H7 Avian Influenza Viruses in Live-Bird Markets of the Northeastern United States, 1994-2001. *Avian Dis.*, 47: 898-904.
- Vander, G.V.A., G. Kach, M.C.M. Gong and M.V. Boven, 2003. Transmission dynamics of low and high pathogenicity A/chicken/Pennsylvania/83 avian influenza viruses. *Avian Dis.*, 47: 939-941.