

Prevalence of Avian Influenza and Newcastle Disease Viruses in Poultry in Selected Areas of Bangladesh Using Rapid Antigen Detection Kit

M. S. Rahman^{1*}, M. G. Rabbani¹, M. J. Uddin¹,
A. Chakrabartty¹, Moon Her²

¹ Department of Medicine, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

² Animal, Plant and Fisheries Quarantine & Inspection Agency, Anyang, Gyeonggi-do, Korea

Correspondence:

✉ prithul02@yahoo.co.uk

Telephone: 88 01918181550

Abstracts

Background: Avian Influenza (AIV) and Newcastle Disease Viruses (NDV) are the two most important culprit in poultry diseases causing serious economic loss in the poultry industry in the world as well as in Bangladesh. However, there is no study for the simultaneously detection of AIV and NDV using rapid diagnostic kit in Bangladesh. Therefore, the present study was carried out to determine the prevalence of Avian Influenza and Newcastle Disease Viruses in poultry in the selected areas in Bangladesh.

Methods: Avian Influenza (AIV) and Newcastle Disease Viruses (NDV) were detected using rapid antigen detection kit from field samples of poultry in Bangladesh. The cloacal swabs were collected from randomly selected 10 birds (broiler, layer, and native chicken, and duck) to detect AIV and NDV in four different districts or areas, and a total of 160 field samples were successfully tested in this study.

Findings: Overall prevalence of AIV and NDV in different species of birds was 6.8% and 38.1%, respectively. The prevalence of AIV in layer and broiler bird was 15.0% and 12.5%, respectively. The prevalence of NDV in layer, broiler, native bird and duck was 37.5%, 32.5%, 55.0% and 27.5%, respectively. The prevalence of AIV in Dhaka, Gazipur, Mymensingh and Bogra districts were 12.5%, 12.5%, 0.0%, and 2.5%, respectively. The prevalence of NDV in Dhaka, Gazipur, Mymensingh and Bogra districts were 50.0%, 45.0%, 25.0%, and 32.5%, respectively. In galliformes, the prevalence of AIV and NDV was 9.16% and 41.6%, respectively. In anseriformes prevalence of AIV and NDV was 0.0% and 27.5%, respectively.

Conclusion: This is the first time that we are using these types of kit in Bangladesh and it's concluded that the test is very easy and rapid, less laborious, less time consuming and non expensive for the detection and differentiation of AIV and NDV in Bangladesh.

Key words: Avian Influenza virus, Newcastle Disease Virus, Rapid antigen detection kit, Poultry, Bangladesh



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Introduction

Of all the viral diseases, the highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) are considered as top ranking viral diseases of poultry by the Office International des Epizooties [1]. Influenza virus that infects birds is called Avian Influenza Virus (AIV). ND is a deadly endemic

disease caused by Newcastle Disease Virus (NDV), also called Avian Paramyxovirus type-1 (APMV-1). Both Avian Influenza (AI) and Newcastle Disease (ND) are zoonotic viral diseases of birds. Influenza also has caused severe losses throughout the century [2]. Both the viral diseases are responsible for serious economic losses every year to the poultry industry all over the world [1].

Avian influenza (AI), bird flu, a highly infectious and emerging viral disease both for the poultry and human now a days, caused by any subtype (H1-16\N1-9) of type A influenza virus (AVI) [3]. This is a single stranded RNA, negative sense, enveloped, genome, segmented (8 segments) and encodes with ten different proteins, belongs to the family Orthomyxoviridae [4, 5]. Type A influenza virus infects a wide range of animals such as swine, horses, sea mammals, a large number of birds and human as well [6]. Avian flu can easily spread from domestic birds to other species of birds, but there are human cases also. World Health Organization (WHO: <http://nieman.harvard.edu/Microsites/NiemanGuideToCoveringPandemicFlu/TheScience/AvianFluBasics.aspx>) confirmed 442 cases of H5N1 in humans in Azerbaijan, Bangladesh, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Lao people's Democratic Republic, Myanmar, Nigeria, Pakistan, Thailand, Turkey and Vietnam, leading to 262 deaths, as up to 24 September, 2009 [3]. In Bangladesh in 2007-2008, the chicken population was 212.5 million and duck 39.8 million [7]. In Bangladesh aspect, poultry mainly used as source of income and employment, source of protein food, various industrial uses, use in research work [8]. Recently Bangladesh is facing a serious problem with a series of avian influenza outbreak and resulted massive culling of millions of poultry and poultry products lead to severe economic losses to the owner of more than 500 poultry farms. Until March 2010, AI has reported a total of 326 outbreaks in 49 districts out of 64 districts in Bangladesh (the Ministry of Fisheries and Livestock, Government of Bangladesh). Since first report of outbreak in 2003, the H5N1 in poultry reached epidemic proportion with reports of serious outbreaks in several Asian countries including Vietnam, Thailand, South Korea, Laos, Cambodia, Indonesia, Japan and Malaysia etc. [10, 11]. AI have the ability to affect different species of birds and mammal and the antigenic drift occur within the same strain of type A virus after a series of point mutation of amino acids at the distal region of HA. Existence of the H5N1 was first reported among poultry population of Bangladesh on 21, March 2007 after the catastrophic outbreak of HPAI at Biman poultry Complex, Savar, Dhaka, Bangladesh. The Armed Forces Medical Research Institute (AFRIM) of Thailand first confirmed presence of this subtype (H5N1) of the AIV type A in this country [9].

Newcastle disease (ND) is another highly contagious viral disease of many domestic and wild species of birds throughout the world [12] which is caused by an enveloped, single stranded, negative sense and non-segmented genomic ribonucleic acid (ssRNA) virus of Paramyxoviridae family. Newcastle disease viruses (NDV's) have three pathotypes: lentogenic, mesogenic, and velogenic. In the context of Bangladesh, ND of poultry is caused mostly by velogenic strains of NDV rather than mesogenic or lentogenic [8]. The rate of mortality in young and adult chickens due to very virulent (vvNDV) varies

from 80-100% respectively [13]. NDV affects the respiratory, nervous, and digestive systems. Symptoms are very variable depending on the strain of virus, species of bird, concurrent disease and preexisting immunity. The incubation period for the disease ranges from 2 to 15 days. An infected bird may exhibit the signs of respiratory problems including sneezing, gasping for air, nasal discharge, coughing, greenish, watery diarrhea, nervousness, depression, muscular tremors, drooping wings, twisting of head and neck, circling, complete paralysis and swelling of the tissues around the eyes and in the neck. NDV is so virulent that many birds die without showing any clinical signs. To date there is no known treatment for ND.

Both AI and ND of poultry often manifest similar clinical signs and post-mortem lesions and also appear either single or concurrent infection [14]. Until now, isolation and identification of the AIV and NDV from faeces, various internal organs, oro-nasal and cloacal swabs are considered as the standard method for the diagnosis of both the diseases. Although several diagnostic methods/tests have been designed for the confirmatory diagnosis of AI and ND but primary diagnosis is still preferably made by virus isolation through inoculation of field samples into avian embryos and cell culture, such cultures are followed by demonstration of viruses in the allantoic fluid (AF) and infectious cell culture fluid (ICF) by HA, Hemagglutination inhibition (HI), agar-gel immunodiffusion (AGID) or other antigen-detection methods [15]. The conventional methods such as clinical signs and post-mortem lesions of diagnosis are being practices in Bangladesh for the diagnosis of AI and ND both in the sick and dead birds. However, these techniques have been reported to be of less diagnostic value as such these diagnostic tests may have lots of limitation for confirmatory detection of both AIV and NDV either from field and laboratory samples as well [10]. Considering the above facts in the field of viral disease diagnosis particularly for AIV and NDV in the poultry population of Bangladesh, the present research work was aimed to determine the prevalence of both AIV and NDV in different species of poultry (Layer, Broiler, Native chicken and Duck) in the selected area using an immuno-chromatographic kit as rapid diagnostic tool for the detection and differentiation of AIV from NDV in poultry.

Materials and methods

Collection of AIV/NDV suspected sample

The cloacal swabs samples were collected from randomly selected ten birds from each of the four different species (layer, broiler, native birds and duck) (Table 1) from four selected areas or districts namely Dhaka (Savar), Mymensingh (Sadar), Gazipur (Sadar) and Bogra (Sadar) in Bangladesh (**Figure 1**)

during the period from September 2010 to March 2011. From four selected districts, a total of 160 cloacal swabs were collected (Table 1). These four districts were selected since major poultry industries are located in these areas. All the samples were collected aseptically in separate sterile plastic vial and kept in ice carrier and then tested in the lab immediately.

Table 1. Total number of samples collected from the different species of four districts.

Name of the District	Name of birds	Source of sample	No. of birds (from each district)	No. of sample (from each district)
Dhaka	Layer, Broiler and Native chicken and Duck	Colocal swabs	10	40
Mymensingh			10	40
Gazipur			10	40
Bogra			10	40
Total	4		40	160

Test of AIV-NDV Rapid Ag kit

After collection of samples the test were performed with the help of AIV-NDV rapid Ag test kit (NDV Antigen Rapid Test, AIV Antigen Rapid Test, AIV+NDV Antigen Combo Rapid Test, RapiGEN, South Korea). The test was performed according to the manufacturer's instructions. Results of the test were observed within 3-5 minutes, recorded naked eye detection of single band for negative control, double band for AIV (H5N1) or NDV positive and triple band both for AIV (H5N1) and NDV positive (Figure 2). Since these kits were tested by the manufacturing company, the efficiency of these kits was not tested but positive and negative control was included in this study.

Statistical analysis

The results were statistically analyzed for interpretation by using Chi-square tests (χ^2). A probability associated with the observed values of chi-square was determined from relevant tables. Significance was determined at 1% and 5% level.

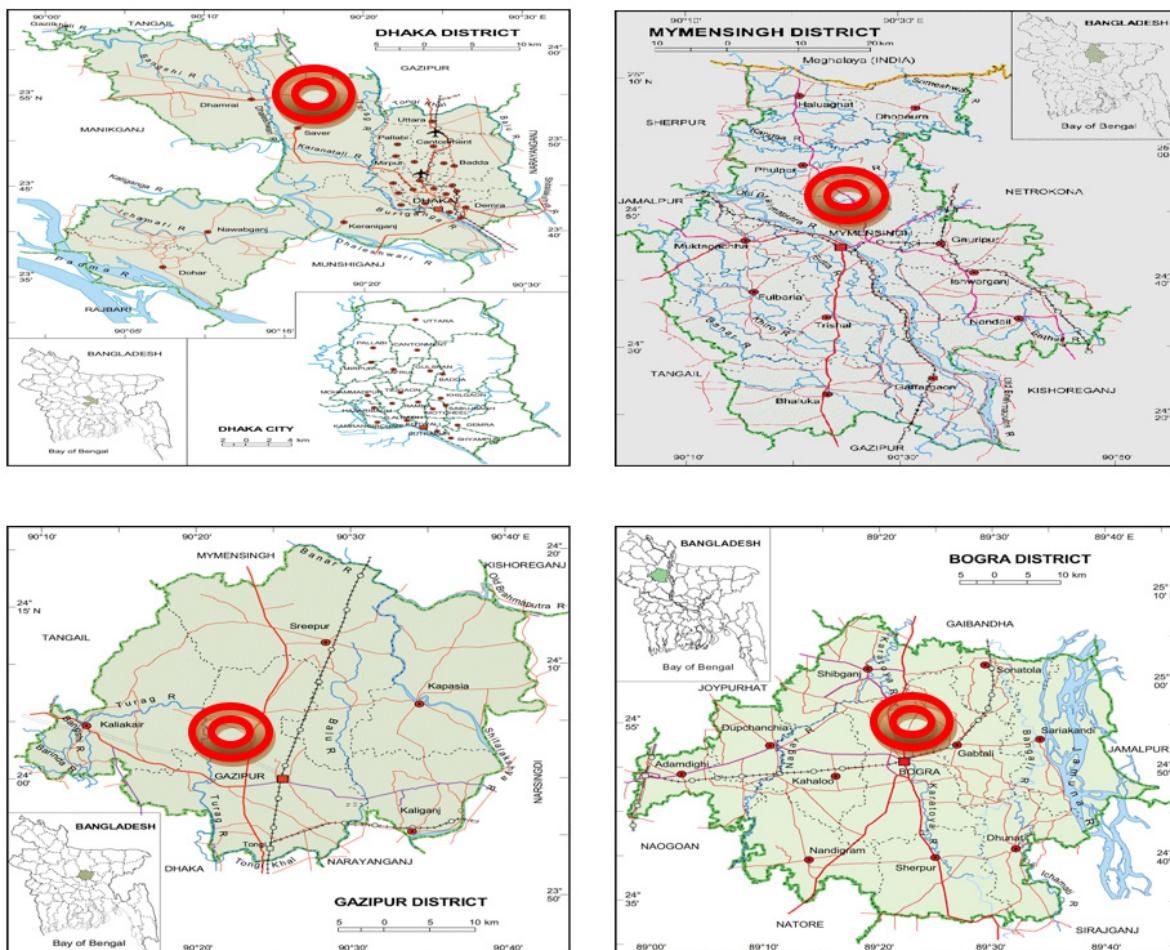
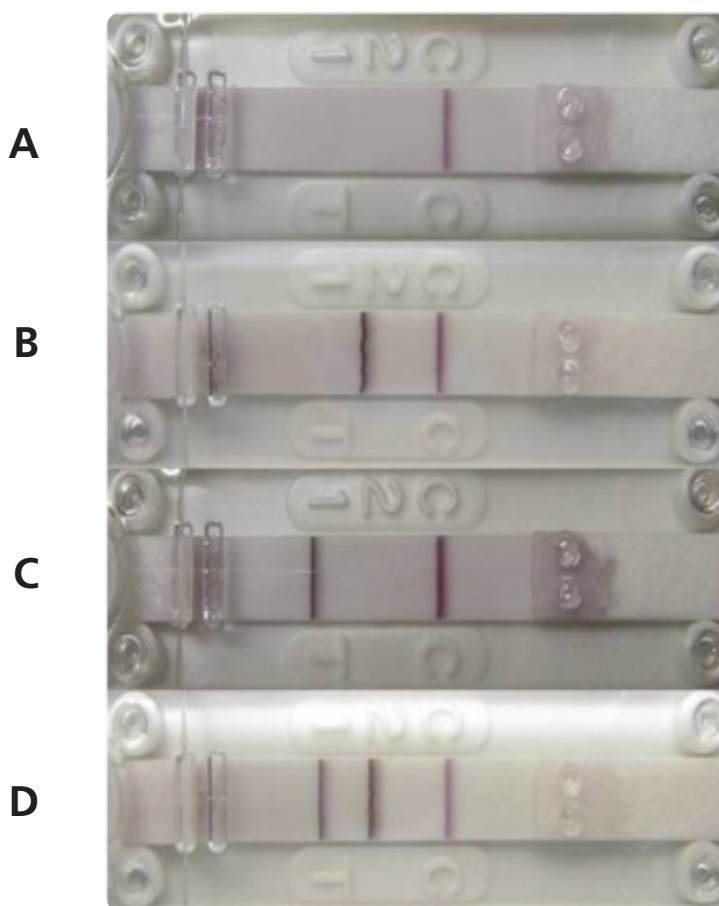


Figure 1. Map of study area-Savar, Dhaka; Gazipur sadar, Gazipur; Mymensingh Sadar, Mymensingh; Bogra sadar, Bogra.

Figure 2. Results of Avian Influenza Virus (AIV, H5N1) and Newcastle Disease Virus (NDV) Test kits (A = Negative, B = NDV positive, C = AIV positive, D = NDV and AIV positive).



Results

Screening of AIV/NDV in different avian species

A total of 40 samples were collected from the cloacal swab of layer birds from the wet market of four districts. The samples were tested for the presence of AIV or NDV. Among the 40 samples, 10 samples were collected from each of four districts. The samples were tested from Dhaka, Gazipur and Bogra district, AIV was found positive 30.0%, 20.0% and 10.0%, respectively. On the other hand in Mymensingh district, AIV was found negative. In Dhaka, Gazipur, Mymensingh and Bogra district, NDV was found positive 50.0%, 40.0%, 30.0% and 30.0%, respectively (**Table 2, Figure 2**). In this study, there were a significant relationship of AIV and NDV in layer birds of different districts ($p < 0.01$).

Table 2. Results of rapid antigen test for screening of AIV and NDV using cloacal swab in layer birds.

District	AIV*		NDV†	
	No. of sample tested	No. of positive (%)	No. of sample tested	No. of positive (%)
Dhaka	10	3 (30.0)	10	5 (50.0)
Gazipur	10	2 (20.0)	10	4 (40.0)
Mymensingh	10	0 (0.0)	10	3 (30.0)
Bogra	10	1 (10.0)	10	3 (30.0)
Total	40	6 (15.0)	40	15 (37.5)

* Avian Influenza Virus,
† Newcastle Disease Virus.

A total of 40 samples were collected from the cloacal swab of broiler birds from the wet market of four districts. The samples were tested for the presence of AIV or NDV. The samples were tested from Dhaka and Gazipur district, and AIV was found positive 20.0% and 30.0%, respectively. On the other hand in Mymensingh and Bogra district, AIV was found negative. In Dhaka, Gazipur, Mymensingh and Bogra district NDV was found positive in 40.0%, 50.0%, 20.0% and 20.0% cases, respectively (Table 3).

Table 3. Results of rapid antigen test for screening of AIV and NDV using cloacal swab in broiler birds.

District	AIV*		NDV†	
	No. of sample tested	No. of positive (%)	No. of sample tested	No. of positive (%)
Dhaka	10	2 (20.0)	10	4 (40.0)
Gazipur	10	3 (30.0)	10	5 (50.0)
Mymensingh	10	0(0.0)	10	2 (20.0)
Bogra	10	0 (0.0)	10	2 (20.0)
Total	40	5 (12.5)	40	13 (32.5)

* Avian Influenza Virus,
† Newcastle Disease Virus.

A total of 40 samples were collected from the cloacal swab of native birds from the wet market of four districts. The samples were tested for the presence of AIV or NDV. The samples were tested from Dhaka, Gazipur, Mymensingh and Bogra district, and AIV was found negative. In Dhaka, Gazipur, Mymensingh and Bogra district, NDV was found positive 60.0%, 50.0%, 50.0% and 60.0%, respectively (Table 4).

Table 4. Results of rapid antigen test for screening of AIV/NDV using cloacal swab in native birds.

District	AIV*		NDV†	
	No. of sample tested	No. of positive (%)	No. of sample tested	No. of positive (%)
Dhaka	10	0 (0.0)	10	6 (60.0)
Gazipur	10	0 (0.0)	10	5 (50.0)
Mymensingh	10	0 (0.0)	10	5 (50.0)
Bogra	10	0 (0.0)	10	6 (60.0)
Total	40	0 (0.0)	40	12(55.0)

* Avian Influenza Virus,
† Newcastle Disease Virus

A total of 40 samples were collected from the cloacal swab of ducks from the wet market of four districts. The samples were tested from Dhaka, Gazipur and Bogra district, and IV was found negative. In Dhaka, Gazipur and Bogra district, NDV was found positive 50.0%, 40.0% and 20.0%, respectively. On the other hand, samples from Mymensingh district were negative for both AIV and NDV (Table 5).

Table 5. Results of rapid antigen test for screening of AIV/NDV using cloacal swab in duck.

District	AIV*		NDV†	
	No. of tested sample	No. of positive (%)	No. of tested sample	No. of positive (%)
Dhaka	10	0 (0.0)	10	5 (50.0)
Gazipur	10	0 (0.0)	10	4 (40.0)
Mymensingh	10	0 (0.0)	10	0 (0.0)
Bogra	10	0 (0.0)	10	2 (20.0)
Total	40	0 (0.0)	40	11 (27.5)

* Avian Influenza Virus,
† Newcastle Disease Virus.

Prevalence of concurrent infection of AIV and NDV in same birds of different species

The prevalence of concurrent infection of AIV and NDV in same birds of different species is shown in Table 6. Considering AIV disease, the result shown that the higher prevalence was in layer (15.0%) than that of other species of birds. On the other hand for NDV, native bird had higher experience of NDV (55.0%) and that was higher in case of other species of birds. In this study there are significant association of prevalence of AIV and NDV in same birds of different species ($p < 0.01$).

Table 6. Prevalence of concurrent infection of AIV and NDV in same birds of different species.

Species	Total number of sample tested	Positive reaction for AIV* No. (prevalence)	Positive reaction for NDV† No. (prevalence)
Layer	40	6 (15.0)	15 (37.5)
Broiler	40	5 (12.5)	13 (32.5)
Native birds	40	0 (0.0)	22 (55.0)
Duck	40	0 (0.0)	11 (27.5)
Total	160	11 (6.8)	61 (38.1)

* Avian Influenza Virus,
† Newcastle Disease Virus.

Prevalence of concurrent infection of AIV and NDV in different districts

The comparison of prevalence rate of AIV and NDV in different district is shown in Table 7. For AIV, the higher prevalence was (12.5%) in Dhaka and Gazipur district and lowest was in Mymensingh (0.0%). In this study there are significant association of prevalence of AIV and NDV in different districts of Bangladesh ($p < 0.01$).

Table 7. Prevalence of concurrent infection of AIV and NDV in different district of Bangladesh.

Districts	Total number of sample tested	Positive reaction for AIV* No. (prevalence)	Positive reaction for NDV† No. (prevalence)
Dhaka	40	5 (12.5)	20 (50.0)
Gazipur	40	5 (12.5)	18 (45.0)
Mymensingh	40	0 (0.0)	10 (25.0)
Bogra	40	1(2.5)	13 (32.5)
Total	160	11 (6.8)	61 (38.1)

* Avian Influenza Virus,
† Newcastle Disease Virus.

Overall prevalence of AIV and NDV

The overall prevalence of AIV and NDV showed that out of 160 cloacal samples, positive cases were 11 (6.8%) and 61 (38.1%) for AIV and NDV, respectively. In this study there are no significant association was detected between overall prevalence of AIV and NDV in different species of birds. The result of prevalence of AIV and NDV according to taxonomic order is shown in Table 8. In case of AIV, higher prevalence was observed in galliformes species (9.2%) than that of anseriformes species (0.0%). In the case of NDV, prevalence was 41.7% and 27.5% in galliformes and anseriformes, respectively. In this study there was a significant association between prevalence of AIV and NDV in galliformes ($p < 0.05$) but no significant difference among different species of birds could be detected.

Table 8. Order wise prevalence of AIV and NDV in birds.

Name of order	Total number of sample tested	Positive reaction for AIV* No. (prevalence)	Positive reaction for NDV† No. (prevalence)
Galliformes	120	11 (9.2)	50 (41.7)
Anseriformes	40	0 (0.0)	11 (27.5)

* Avian Influenza Virus,
† Newcastle Disease Virus.

Discussion

The research work was carried out to determine the prevalence of Avian Influenza Virus (AIV) type A and Newcastle Disease Virus (NDV) in different species of birds from four districts of Bangladesh. In this study the cloacal swabs were collected from wet market and the test was performed using AIV and NDV rapid Ag test Kit. Results of the test were recorded by observing the single band for negative control, double band for AIV or NDV positive, triple band positive for both AIV and NDV cases within 3-5 minutes. It was observed that the layer birds of wet market of Dhaka, Gazipur and Bogra were positive for both AIV and NDV which might be due to mass gathering of different type of birds since these the major poultry industries are located in these areas in Bangladesh. Additionally, the transportation might be an important cause of spreading of both types of viruses in these areas. In the study it was also observed that the prevalence of AIV in layer birds from the wet market sample was 15.0%. It is comparable with the finding of Negovetich *et al* [16], who reported 23% prevalence of AIV in live bird market. It was also observed that the broiler birds of wet market of Dhaka and Gazipur district were positive for both AIV and NDV which might also be due to the accumulation of birds, transportation of poultry materials and vehicles that pick up the infection and spread the viruses among the broiler birds in wet market. It was further observed that the birds of wet market, AIV was present in Dhaka and Gazipur district and only NDV was present in wet market of the some districts. It may be due to mixing of different species of birds in the wet market that pick up the infection and spread viruses among the birds. In case of AIV, the higher prevalence was (12.5%) in Dhaka and Gazipur district and in Mymensingh the preva-

lence rate was 0.0%. In this study there were a significant association of prevalence of AIV and NDV in different districts of Bangladesh ($p < 0.01$).

In this study it was observed that the duck wet markets of three districts were found NDV positive out of four districts. This may be due to rearing of different species of birds together and selling together in wet market resulting spreading of viruses among the duck. The prevalence rate of AIV was higher in layer 15.0% than that of other group (broiler 12.5% and 0.0% for both native bird and duck) (Table 6). This finding was in good agreement with Capua *et al* [19]. However, prevalence rate of NDV was higher in native birds 55.0% followed by 37.5%, 32.5%, and 27.5% in layer, broiler and duck respectively. There was almost similar finding (60.8%, 57.2%, 37.8% and 25% in native bird, layer, broiler and duck, respectively) reported by Sadiq *et al* [18]. In the present study it was observed that the prevalence of NDV in native birds was 55.0%. The finding was lower than the findings of Chowdhury *et al* [17] and Sadiq *et al* [18], who reported prevalence of NDV in native birds as 67.4% and 60.8%, respectively.

Out of 160 cloacal samples, positive cases were 11 (6.8%) and 61 (38.1%) of AIV and NDV, respectively. There is no significant relation between the overall prevalence of AIV and NDV in different species of birds. In case of AIV, higher prevalence observed in galliformes species (9.2%) than that of anseriformes species (0.0%). In case of NDV, galliformes had higher prevalence of 41.7% than that of anseriformes of 27.5%. Similar result was reported by Lawal *et al* [20], who found 35.7% in galliformes and 28.6% in anseriformes. There is a significant relation between the prevalence of AIV

and NDV in galliformes, but no significant difference among different species of birds.

This is the first time that we are using these types of kit in Bangladesh and it is concluded that the test is very easy and rapid, less laborious, less time consuming and less expensive that is why, immuno-chromatographic kit would be considered as a rapid screening method for the detection and differentiation of AIV and NDV in Bangladesh in large scale. Considering the findings of the present study, further studies are needed to be conducted to determine the sensitivity and specificity of the kit for wide use in field condition in addition to virus isolation, serotype determination and molecular detection of the field isolates as well as phylogenetic analysis with the nucleotide and amino acid sequencing of the HA and NA genes of AIV, and F gene of NDV isolates in Bangladesh to know the origin of these viruses in the poultry population of Bangladesh.

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