J Vet Clin 29(4) : 309-314 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

Evaluation of Avian Influenza and Newcastle Disease Virus Detection Kit using Field Samples from Domestic and Semi-domestic Birds

Md. Siddiqur Rahman*, Md. Abdul Malek, Md. Alimul Islam**, Muhamad Jasim Uddin*, Md. Shamim Ahasan*, Amitavo Chakrabartty*, Md. N Sakib** and Joon-Seok Chae***[1]**

**Department of Medicine, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
***Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
****Laboratory of Veterinary Internal Medicine, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*

(Accepted: July 04, 2012)

Abstract : The study was undertaken to evaluate sensitivity and specificity of rapid Avian Influenza (AI) and Newcastle Disease virus (NDV) combo antigen kits from field samples of domestic (broiler and layer chicken, native chicken) and semi-domestic (duck, goose, pigeon and quail) birds of Bangladesh. Samples were collected from naturally infected AI suspected domestic and semi-domestic birds of five different outbreak areas in Bangladesh. From each area two birds were selected for sampling, and from each bird three types of samples (tracheal, cloacal and oro-nasal swabs) were collected. A total of 210 field samples from a total of 70 birds were collected and tested using AI and NDV combo antigen rapid diagnostic kits in the study. All three different samples from a bird showed similar pattern of reaction. Out of 210 samples, 15 samples (5 birds), 63 samples (21 birds) and 27 samples (9 birds) were positive for AIV, NDV and both for AIV and NDV, respectively; whereas the remaining birds were negative for either AIV or NDV in this screening test. Among the five AIV positive, a layer chicken from wet market in Mymensingh, Netrokona, Gibandha and Kurigram and a native chicken from wet market in Kurigram area was positive to AIV. The semi-domestic birds are either positive to NDV or free from both AIV and NDV. This study revealed that the AIV and NDV rapid diagnostic kits could be effectively use to diagnose the respective virus in trachea, oro-nasal and cloacal samples simultaneously. AIV-NDV combo Ag test result clearly indicates that the test kit designed for AIV and NDV could diagnose the disease rapidly with less effort and higher scientific know how which could be used for the detection of AIV and NDV using field samples in large scale.

Key words **:** avian influenza, New Castle disease, birds, field samples, Bangladesh.

Introduction

Newcastle disease (ND) and avian influenza (AI) are the two of the most important zoonotic viral diseases of birds throughout the world (1,21). Influenza viruses that infect birds are called avian influenza virus (AIV) that causes severe losses throughout the world (10). ND is a deadly endemic disease caused by Newcastle disease virus (NDV), also called avian paramyxovirus type-1 (APMV-1). Notably, both the viral diseases are responsible for serious economic losses every year to the poultry industry all over the world (20).

AI or bird flu is a "notifiable" highly infectious disease affecting many species of birds including chickens, duck, turkeys and geese (13). It can affect commercial as well as pet birds. There are various sub types of bird flu, but the sub type that is concerned at the moment is the deadly H5N1 strain. Avian flu can easily spread from domestic birds to other species of birds, but there are also some human cases. The humans cases are thought to have been due to contact with the virus through close or direct contact with infected birds. The matter of concern is that the H5N1 strain may undergo genetic changes, enabling it to spread easily from person to person. If these changed occur, there would be a pandemic threat. Since first report of outbreak in 2003, the H5N1 in poultry reached epidemic level with reports of serious out breaks in several Asian countries including Vietnam, Thailand, South Korea, Laos, Cambodia, Indonesia, Japan and Malaysia etc (17,14). Since near past, Bangladesh is also facing a series outbreak of AI following emergence of highly pathogenic avian influenza (HPAI) virus (H5N1) at different areas throughout the country. Existence of the H5N1 was first reported among poultry population of Bangladesh on 21, March 2007 after the catastrophic outbreak of highly pathogenic AI at Biman poultry Complex, Savar, Dhaka. The Armed Forces Research Institute of Medical Sciences (AFRIMS) of Thailand first confirmed presence of this sub-

[1]Corresponding author.
E-mail : jschae@snu.ac.kr

type (H5N1) of the AIV type A in Bangladesh.

Newcastle disease is another highly contagious viral disease of many domestic and wild species of birds throughout the world (2) caused by NDV belonging to the genus *Rubulavirus* of subfamily *Paramyxovirinae* and family *Paramyxoviridiae* (11). Based on the pathogenic and virulence properties, NDV is categorized into three major pathotypic strains i.e. lentogenic, mesogenic and velogenic strains (3). In the context of Bangladesh, ND of poultry is caused mostly by velogenic strains of NDV rather than mesogenic or lentogenic strains (9). Chickens are highly susceptible, while ducks and geese can be infected and show a few or no clinical signs to the same strain. NDV affects the respiratory, nervous, and digestive systems. Symptoms are variable depending on the strain of virus, species of bird, concurrent disease and preexisting immunity. The rate of mortality in young and adult chickens due to very virulent NDV (vvNDV) varies from 80-100% respectively (6).

Both AI and ND disease of poultry often manifest similar clinical signs and post-mortem lesions and also appear either single or concurrent infection. 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 and demonstration of hemagglutinating (HA) agents or 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 have low specificity and sensitivity and as such these diagnostic tests may have lots of limitation for random use as a reliable detection method for both AIV and NDV either from field and laboratory samples as well (18).

Considering the above facts in the field of viral disease diagnosis particularly for AI and ND in the poultry population of Bangladesh, it is necessary to assess the sensitivity and specificity of rapid AI and NDV diagnostic kits. Therefore, the aim of this present research to assess an immunochromatographic kit as rapid diagnostic tool for the differentiation of AI from ND in domestic and semi-domestic birds and to evaluate the sensitivity and specificity of the immunechromatographic kit for the detection of AIV and NDV from the field and laboratory samples.

Materials and Methods

Experimental design

On the basis of the birds considered for this study, the samples were divided into two subgroups namely domestic birds and semi-domestic birds. Where, domestic birds were consists of chicken and water fowl, and semi- domestic birds were consists of pigeon and quail. Specifically, commercial layer, broiler and native bird were grouped in chicken, whereas duck and goose were considered as water fowl in this study. Three types of samples (tracheal, then oro-nasal and later cloacal swab) were collected from each birds from both the commercial farms and wet markets in five different areas or districts named Dhaka, Mymensingh, Netrokona, Gaibandha and Kurigram in Bangladesh during the outbreak period from January 2008 to May 2009 since influenza outbreak were more common in these foresaid areas. From each districts or areas, two suspected chickens were selected for collecting these swab samples. From a total of 70 birds, a total of 210 field samples were collected for this study (Table 1). In the case of broiler and layer chicken, a broiler and a layer chicken was selected from both the commercial farm (CF) and from wet market (WM) in all five areas (Table 2); whereas in the case of native chicken and semi-domestic birds, two birds were selected from WM in all five areas (Table 3). After collection, the samples were transferred to the laboratory in the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh with aseptic condition and the tests were performed using the AIV-NDV combo Ag test kit (RapiGEN®, South Korea) following the manufacturer's protocol. Results of the test were observed within 3-5 minutes by naked eye and recorded: single band for negative control, double band for the AIV positive and triple band for both the AIV and NDV positive.

Principle of the AIV-NDV combo Ag test

The RapiGEN Avian Influenza virus and Newcastle disease virus Ag test kits are rapid one step test kits based on the

Table 1. Samples collected from the different species of birds (n = 70) in five different districts

Name of the districts	Number of selected birds	Species of birds	Number of samples*
Dhaka	2	layer, broiler, native chicken, duck, goose, pigeon and quail	42
Mymensingh	2	layer, broiler, native chicken, duck, goose, pigeon and quail	42
Netrokona	2	layer, broiler, native chicken, duck, goose, pigeon and quail	42
Gaibandha	2	layer, broiler, native chicken, duck, goose, pigeon and quail	42
Kurigram	2	layer, broiler, native chicken, duck, goose, pigeon and quail	42
Total	10	35	210

*From each of the birds three different samples (tracheal, oro-nasal and cloacal swabs) were collected.

Table 2. Results of screening for AIV and/or NDV in field samples from layer and broiler chicken of commercial farm and wet market using AIV-NDV combo Ag test

Birds	Clinical Samples	Districts/areas									
		Dhaka		Mymensingh		Netrokona		Gaibandha		Kurigram	
		CF	WM	CF	WM	CF	WM	CF	WM	CF	WM
Layer chicken	Tracheal swab	+++	+++	-	+	-	+	++	+	++	+
	Oro-nasal swab	+++	+++	-	+	-	+	++	+	++	+
	Cloacal swab	+++	+++	-	+	-	+	++	+	++	+
Broiler chicken	Tracheal swab	++	+++	++	++	-	++	-	++	-	++
	Oro-nasal swab	++	+++	++	++	-	++	-	++	-	++
	Cloacal swab	++	+++	++	++	-	++	-	++	-	++

CF, commercial farm; WM, wet market; +, AI positive; ++, ND positive; +++, AI and ND positive; -, AI and ND negative.

Table 3. Results of screening for AIV and/or NDV in field samples from native chicken, water fowl and semi-domestic birds of wet market using AIV-NDV combo Ag test

Birds	Clinical samples	District / areas									
		Dhaka		Mymensingh		Netrokona		Gaibandha		Kurigram	
		Wet Market									
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Native chicken	Tracheal swab	+++	+++	++	++	+++	+++	+++	+++	+	++
	Oro-nasal swab	+++	+++	++	++	+++	+++	+++	+++	+	++
	Cloacal swab	+++	+++	++	++	+++	+++	+++	+++	+	++
Duck*	Tracheal swab	-	-	++	++	++	++	-	-	-	-
	Oro-nasal swab	-	-	++	++	++	++	-	-	-	-
	Cloacal swab	-	-	++	++	++	++	-	-	-	-
Goose	Tracheal swab	-	-	-	-	-	-	-	-	-	-
	Oro-nasal swab	-	-	-	-	-	-	-	-	-	-
	Cloacal swab	-	-	-	-	-	-	-	-	-	-
Pigeon*	Tracheal swab	++	++	++	++	-	-	-	-	-	-
	Oro-nasal swab	++	++	++	++	-	-	-	-	-	-
	Cloacal swab	++	++	++	++	-	-	-	-	-	-
Quail*	Tracheal swab	-	-	++	++	-	-	-	-	-	-
	Oro-nasal swab	-	-	++	++	-	-	-	-	-	-
	Cloacal swab	-	-	++	++	-	-	-	-	-	-

*In case of duck, pigeon and quail area Mymensingh means BAU campus poultry farm. S1, Sample One; S2, Sample Two; +, AI positive; ++, ND positive; +++, AI and ND positive.

immuno-chromatographic assay. A specific antibody to conserved nucleocapsid protein of AIV-NDV is conjugated with 'gold' particles and another specific antibody is immobilized as a band on a nitrocellulose membrane (RapiGEN®, South Korea). If AIV and / or NDV is present, the labeled antibody gold conjugates bind to it, forming an antibody-antigen (Ab-Ag) complex. As the mixture flows along the membrane, the complex is captured by the antibody immobilized in the test line of the membrane, producing a visible red / purple color band. Another gold-conjugated reagent is captured by the antibody immobilized in the control line of the membrane (RapiGEN®, South Korea).

Results

A total of 210 samples collected from a total of 70 different species of birds from five different AI affected areas of Bangladesh and were tested for AI and NDV (Table 1) in this study. The pattern of AI and NDV distribution was not same in all the areas selected in this study (Table 2 and 3). In the case of Dhaka region, the layer and broiler chicken in wet market (WM) as well as layer in commercial farm (CF) were

detected to be positive for both the AI and NDV (Table 2), but the broiler in CF were negative to AI. In the case of Mymensingh and Netrokona areas, the layer chicken showed similar results, where CF chicken were negative to both AI and NDV but WM chicken were positive to NDV. The broiler chicken in both the CF and WM were found to be positive to only NDV (Table 2). The layer chicken from Giabandha and Kurigram areas showed similar pattern of infection; where CF and WM chickens were positive only to the NDV and AI, respectively. Broiler chicken from WM in Netrokona, Giabandha and Kurigram showed positive reaction NDV, whereas CF chickens were from any of these viral diseases (Table 2).

Native chicken at WM in Dhaka, Netrokona and Gibandha were found to be positive to both the AI and NDV (Table 3). Both the samples in Mymensingh and a sample in Kurigram were positive to NDV, whereas a sample in Kurigram was positive to AI. The samples collected from duck in Dhaka, Gibandha and Kurigram were found to be negative to any of these two viral diseases in this study (Table 3), whereas the duck in Mymensingh and Netrokona were positive to only the NDV. All the samples collected from geese in any areas or districts in Bangladesh were free from both the AI and NDV diseases (Table 3). Only the pigeons at wet market in Dhaka and Mymensingh districts were positive to only the NDV, whereas pigeons from other districts were free from both the NDV and AI (Table 3). All the tracheal, oro-nasal and cloacal swab samples collected from quails at WM in this study were found to be negative to both AI and NDV, except the quails in Mymensingh district that were positive to only the NDV (Table 3).

Discussion

This study was devoted for the detection of AIV A type and NDV in different species of birds from five different areas or districts in Bangladesh where AI outbreak was occurred. The sensitivity and specificity of the immuno-chromatographic kit was carried out for simultaneous detection of AIV and NDV using the field and laboratory samples.

Chicken of commercial farm and wet market at Dhaka region was found to be positive to both the AIV and NDV (Table 2) which may due to the mass gathering of different type of birds during transportation to this region. The commercial farm in Gaibandha and Kurigram are affected with NDV only which could be explained by the insufficient management, improper vaccination, and lack of awareness about biosecurity in the commercial farm. Similar results in the commercial layer farms were also observed by Capua *et al*. (4). AIV was also found positive in the wet market of the districts of Mymensingh, Netrokona, Gaibandha and Kurigram. The detection of AIV and NDV in the wet market poultry was also recorded by Chang *et al*. (5). Notably, the patterns of positive reaction for layer chickens in Mymensingh and Netrokona, and for both the broiler and layer in Gibandha and Kurigram were similar (Table 2) which might be due to the geographic location of these areas since Mymensingh and Netrokuna district was neighbour to each other, and Gibandha and Kurigram were close to each other. The study revealed that the broiler chicken at commercial farm in Dhaka and Mymensingh region was NDV positive (Table 2) which could be due to the lack of biosecurity measures, insufficient management, improper vaccination, vaccination failure or incorrect vaccine strains. NDV was absent in the chicken at other farms may be due to proper uses of appropriate vaccine and maintenance of proper biosecurity measures. Presence of NDV in the commercial farms was also reported by Gohm *et al*. (7) and Roy *et al*. (16), whereas broiler chicken at wet market was found to be positive to the both AIV and NDV in Dhaka district and only NDV was present at wet market in the selected districts. This could be due to the mixing of different species of birds at the wet market that pick up the infection and spread viruses among the broiler birds. These results were coincided with the previous results reported by Seal *et al*. (19).

With the regards of backyard poultry, both AIV and NDV was found in the wet market in Dhaka, Netrokona and Gaibandha districts (Table 3) which might be due to the lack of vaccination program, weak biosecurity measures and mixing birds with different species as well as mixing of domestic and semi-domestic birds during selling. This finding is in good agreement with the finding of Horimoto & Kawaoka (8). On the other hand, only NDV was present in wet market in Mymensingh and Kurigram district which may be due to the lack of biosecurity and absence of vaccination in these areas. Only AIV was present in one sample in wet market of Kurigram district which may be due to border area of Bangladesh and infection can easily pick up from India.

Duck of wet market of Netrokona district and Bangladesh Agricultural University (BAU) campus poultry farm was found positive to NDV among the five districts (Table 3) which might be due to the rearing of different species of birds together and selling together in wet market in BAU campus poultry farm results the spread of virus among the ducks. Presence of NDV in duck of wet market was also reported by Seal *et al*. (18). It was observed that the goose of all areas were negative to both AIV and NDV in wet market, although presence of these infections are reported to be occurred in goose (14). Munster *et al*. (14) detected both AIV and NDV in the cloacal samples of goose.

With regards to the pigeon at wet market in the areas of Dhaka, Netrokona, Gaibandha and Kurigram and Bangladesh Agricultural University (BAU) campus poultry farm (Mymensingh) samples, NDV was only found in the samples from the wet market in Dhaka and BAU campus poultry farm. This might be due to the higher density of different species of birds in Dhaka wet market which could get the infection as well as due to the collective rearing system in BAU campus poultry farm. Similar results were previously reported by several studies (12,22). Only the NDV was detected in the quails only in BAU campus poultry farm (Table 3) which

might be due to the lack of biosecurity measures, rearing of different species together and easy movement of wild animals in the farm. The finding of this study is in good agreement with the previous results in the BAU campus poultry farm (9). However, except AI and NDV, other infections such as bacteria, protozoa and parasitic infections might present in these individuals that was not detected in the present study.

This immuno-chromatographic test kit is very easy to use and rapid, less laborious, less time consuming and non expensive; therefore, this kit could be used in large scale for the detection and differentiation of AIV and NDV in filed level as well as in the Lab. The limitation of the use of the comb AIV-NDV Ag test kits that detect only antigen against ND and AI. The present study also had some limitations such as birds used for this study were not taken from the specific pathogen free (SPF) flocks, which might have hampered to get the exact result in the detection of AIV and NDV. Considering the findings of the present study, further studies are needed to be conducted on complete subtyping and serotyping of AIV and NDV by sero-diagnosis (HI and NI) and phylogenetic analysis with the nucleotide and amino acid sequencing of the HA and NA genes of AIV and F gene of NDV isolates from Bangladesh to discover the origin of these viruses in the poultry population in Bangladesh.

AIV-NDV combo Ag test result clearly indicated that the test kit designed for AIV and NDV are characterized of rapid, economic and easy, and could be used in large scale for the detection of AIV and NDV quickly using different kinds of field samples.

Acknowledgements

The authors are grateful to RapiGEN®, Republic of Korea for kind supplying the kits to the first author in Bangladesh as free samples.

References

1. Aldous EW, Alexander DJ. Detection and differentiation of Newcastle disease virus (Avian paramyxovirus type 1). Avian Pathol 2001; 30: 117-128.
2. Aldous EW, Alexander DJ. Newcastle disease in pheasants (Phasianus colchicus): A review. Vet J 2008; 175: 181-185.
3. Beard CW, Hanson RP. Newcastle disease, In: M. S. Hofstad, Disease of poultry, 8th ed. Ames: Iowa State University Press. 1984: 452-470.
4. Capua I, Dalla PM, Mutinelli F, Marangon S, Terregino C. Newcastle disease outbreaks in Italy during 2000. Vet Rec 2002; 150: 565-568.
5. Chang WL, David ES, Jose AL, Dennis A S, David LS. H5N2 avian influenza outbreak in Texas in 2004: the first highly pathogenic strain in the United States in 20 years. J Virol 2005; 79: 11412-11421.
6. Eisa M, Omer EA. A natural outbreak of Newcastle disease in pigeons in Sudan. Vet Rec 1984; 114: 297.
7. Gohm D, Schelling E, Audigé L, Thür B. Newcastle disease seroepidemiologic study of a highly contagious epizootic in poultry and in wild birds in Switzerland. Schweizer Archiv Tierheilkunde 1999; 141: 549-558
8. Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. J Clin Microbiol 2001; 14: 129-149.
9. Islam MA, Ito T, Takakuwa H, Itakura C, Kida H. Acquisition of pathogenicity of a Newcastle disease virus isolated from a Japanese quail by intracerebral passage in chickens. Japanese J Vet Res 1994; 42: 147-156.
10. Kuszewski K, Brydak L. The epidemiology and history of Influenza. Biomed Pharmacol 2000; 54: 188-195.
11. Mayo MA. A summary of the changes recently approved by ICTV. Arch Virol 2002; 147: 1655-1656.
12. Mishra S, Kataria JM, Verma KC, Mishra JP. Strain differentiation of Newcastle disease viruses by laboratory tests. Indian J Ani Sci 2000; 70: 346-348.
13. Munster V, Anders W, Bestebroer TM, Herfst S, Derek S, Rimmelzwaan G, Björn O, Osterhaus M. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol 2005; 79: 2814-2822.
14. Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003; 362: 1733-1745.
15. Noroozian H, Vasfi MM, Razazian M. Detection of avian influenza virus of H9 subtype in the faeces of experimentally and naturally infected chickens by Reversetranscription-Polymerase Chain Reaction. Acta Vet Brunensis 2007; 76: 405-413.
16. Roy P, Venugopalan AT, Manvell R. Characterization of Newcastle disease viruses isolated from chickens and ducks in Tamilnadu. Indian Vet Res Commun 2000; 24: 135-142.
17. Ryan-Poirier KA, Katz JM, Webster RG, Kawaoka Y. Application of Directigen FLU-A for the detection of influenza A virus in human and nonhuman specimens. J Clin Microbiol 1992; 30: 1072-1075.
18. Seal BS, King DJ, Sellers HS. The avian response to Newcastle disease virus. Develop and Comp Immunol 2000; 24: 257-268.
19. Seal BS, Wise MG, Pedersen JC, Senne DA, Alvarez R, Scott MS, King DJ, Yu Q, Kapczynski DR. Genomic sequences of low-virulence avian paramyxovirus-1 (Newcastle disease virus) isolates obtained from live-bird markets in North America not related to commonly utilized commercial vaccine strains. Vet Microbiol 2005; 106: 7-16.
20. Swayne DE. Pathobiology of H5N2 Mexican avian Influenza virus infections of chickens. Vet Pathol 1997; 34: 557-567.
21. Wang LC, Pan CH, Severinghaus LL, Liu LY, Chen CT, Pu CE, Huang D, Lir JT, Chin SC, Cheng MC, Lee SH, Wang CH. Simultaneous detection and differentiation of Newcastle disease and avian influenza viruses using oligonucleotide microarrays. Vet Microbiol 2008; 18: 217-226.
22. Yu L, Wang Z, Jiang Y, Chang L, Kwang J. Characterization of newly emerging Newcastle disease virus isolates from the People's Republic of China and Taiwan. J Clin Microbiol 2001; 39: 3512 -3519.

닭과 야생사육조류로부터 야외샘플을 사용한 조류인플루엔자와 뉴캣슬병 바이러스 검출 키트의 평가

라만 씨딕*・마렉 압둘・이슬람 알리물**・어딘 무하마드 자심*・아산 샤민***
챠크라바티 아미타보*・사키브・채준석*****[1]

*방글라데쉬 농업대학교 수의학과, **방글라데쉬 농업대학교 미생물 및 위생학과
***서울대학교 수의과대학 수의학연구소 수의내과학연구실

요 약 : 연구는 방글라데시에서 조류인플루엔자와 뉴캐슬 질병 바이러스가 국내의 현장 샘플에서 혼합항원키트의 민감도와 특이성을 평가하기 위하여 육계 및 산란계와 토종 닭 그리고 사육 야생오리, 거위, 비둘기와 메추라기로부터 야외 샘플을 수집하였다. 샘플은 방글라데시의 5 지역에서 발생한 조류인플루엔자 자연감염된 것으로 의심되는 닭과 야생사육조수로부터 수집되었다. 각 지역으로부터 2마리씩 선택적으로 샘플을 수집하였으며, 각 조류에서는 면봉을 이용하여 기도, 총배설강, 구-비강으로부터 3가지 유형의 샘플을 채취하였다. 70 마리의 조류에서 총210개의 야외 샘플이 수집되었으며, 조류인플레인자와 뉴캣슬병 바이러스 혼합항원신속진단키트를 검사하였다. 210개 샘플 중에서 15개(5 마리)가 조류인플루엔자 바이러스, 63개(21 마리)가 뉴캣슬병 바이러스, 27개(9 마리)에서 두 가지의 혼합감염이 나타났으며, 그 외에서는 모두 음성으로 나타났다. 5 곳의 조류인플루엔자 양성 중에서 Mymensingh, Netrokona, Gibandha와 Kurigram의 마켓으로부터 산란계에서, Kurigram의 마켓에서 토종닭에서 양성이 나타났다. 야생사육조수는 뉴캣슬병에 양성이거나 또는 조류인플루엔자와 뉴캣슬병 바이러스에는 감염되지 않았다. 조류인플루엔자 및 뉴캣슬병 바이러스 신속진단키트로 기도, 총배설강, 구-비강으로부터 채집한 샘플에서 동시에 검출할 수 있는 것으로서 효과적으로 사용될 것으로 평가되었다. 조류인플레인자와 뉴캣슬병 바이러스 혼합 항원 검사 결과는 명확히 두 개의 바이러스 검출을 위한 테스트 키트로서 적은 노력과 대규모의 필드 샘플로부터 이들 바이러스의 검출에 효과적으로 사용될 수 있는 최신의 과학적 방법이며, 신속하게 질병을 진단할 수 있었다.

주요어 : 조류인플루엔자, 뉴캣슬병, 조류, 야외샘플, 방글라데쉬