Molecular and serological detection of H5N1 avian influenza viruses in poultry and ducks from Bangladesh

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Background

Avian influenza viruses are responsible for several epizootics and pose a continuous threat to humans, animals, and poultry industry globally. Waterfowls are the main reservoir of avian influenza viruses and responsible for the spillover of AIVs to other hosts. Surveillance programs are important to monitor the occurrence of different subtypes among different populations. The present study hereby reports the serological evidence and molecular detection of H5N1 subtype of avian influenza from chickens from backyard poultry, live bird markets, and farms as well as domestic duck population samples from different locations in Bangladesh.

Bangladesh is one of the most densely populated countries in the world, both for human (1072 people/ km^2) and poultry populations (1194 birds/ km^2) , with eight confirmed HPAIV cases including one fatality reported to date (March, 2016) . According to the World Organization for Animal Health (OIE) and the United Nations Food and Agriculture Organization (FAO), Bangladesh and its neighboring countries (Myanmar, Bhutan, Nepal, China, and Indonesia) are endemic for HPAIV . This is a major public health concern and since the poultry industry in an agriculture-based economy like Bangladesh comprises 20% of the livestock sector, the continuous culling of an estimated more than 250 million infected animals is causing an increase in food insecurity as well as affecting economic growth.

The epidemiology of avian influenza is complex. Live bird markets (LBM) are considered to be man-made reservoirs of AIVs, as backyard chickens and ducks play a significant role in the epidemiology and transmission of these viruses.
Diagnosis of AI in animal populations is based on the use of both molecular and virus isolation (VI) techniques which can detect both infectious and noninfectious viral particles respectively are widely utilized for AIV surveillance programs.

The dynamics and transmission of AIVs in asymptomatic infected wild migratory birds is complex and is not yet fully understood. Consequently, in the present study we used RT-PCR, virus isolation, and serological analysis for the ongoing surveillance of AIVs at different locations in Bangladesh.

Matrix and H5N1 specific RT-PCR results of C and OP samples collected from different districts in Bangladesh.

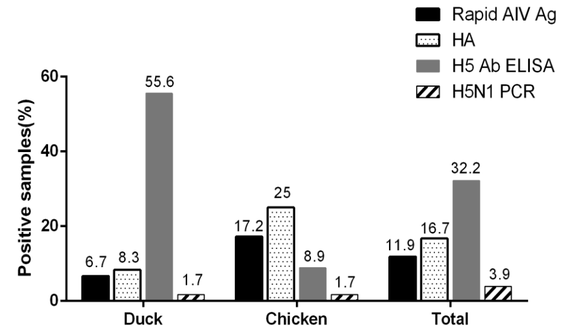
District	Total pooled C + OP samples n	M gene positive pooled samples n (%)	Number of H5N1 AIV positive samples n
Kishorgonj	405	8 (2.0)	5
Netrokona	1500	25 (1.7)	23
Sherpur	157	3 (1.9)	1
Rajshahi	90	5 (5.6)	2
All districts combined	2152	41 (1.9)	31

Materials and Methods

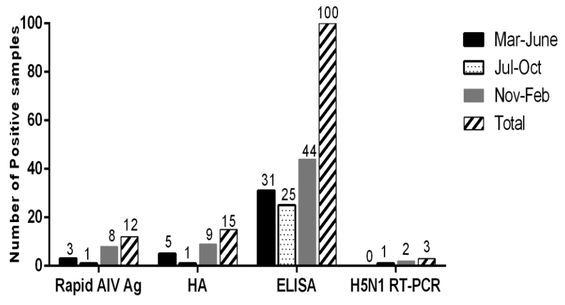
A total of 6,228 samples consisting of cloacal (n = 2169) and oropharyngeal (n = 2191) swabs, and blood samples (n = 1868) from live bird markets chickens, backyard poultry, broilers, layers, and ducks from several locations in different districts in Bangladesh. Cloacal and oropharyngeal samples were screened for the presence of influenza A viruses nucleoprotein rapid antigen test. Positive C and OP samples were tested for live viruses using virus isolation methods. Positive samples were subtyped using molecular methods of conventional RT-PCR. Serum samples from vaccinated and unvaccinated chicken and ducks were screened for antiH5N1 antibodies using ELISA methods

Results

Forty one (0.94%) samples were positive for influenza A viruses. Thirty one samples could be subtyped and were found H5N1 and 21 H5N1 live viruses were isolated. It was found that 545 (34%, 545/1603) serum samples were found positive for H5 Ab. Alarmingly, analysis of 221 serum samples collected from vaccinated birds in four districts revealed that only 18 samples (8.14%) were seropositive for anti H5 antibodies, compared to unvaccinated birds (n = 105), where 8 samples (7.61%) were
seropositive.



Virological test results for the detection of H5N1 AIV in chicken (CK) and duck samples (n=360) from four different locations between November 2013 and October 2014.



AIV test results of (A) duck (n=180) samples collected from Bangladesh between November 2013 and October, 2014.

Conclusion

Molecular detection methods and serological surveillance in Bangladesh shows evidence of presence of H5N1 influenza viruses among live domestic poultry and ducks and that ducks play a major role in the transmission of AIV to domestic poultry. The high prevalence of H5N1 influenza viruses in backyard poultry and LBM suggests that there is an urgent need to enhance surveillance program to insure the proper implementation of pandemic preparedness plans in the future. In addition, the finding that there is no difference in anti H5 seropositivity between vaccinated (8.14%) and unvaccinated chickens (7.61%) indicates a failure of the vaccination program and calls for the use of updated poultry vaccines. Several factors such as the vaccination scheme applied in Bangladesh poultry, the number of vaccination interventions, vaccines used and licensed (vaccine content and type), and age of birds at vaccinations are required to be addressed. Public health education, the application of strict biosecurity, and proper hygienic practices by all personnel dealing with poultry are important and well help mitigate the possible endemicity, reduce the spread and occurrence of seasonal epidemics due to AIVs. Further studies are required to provide a clear picture on the possible sources of these viruses including a national surveillance among wildlife and migratory birds. We are currently screening these samples collected in the present study for other possible existing AIVs subtypes as well as Newcastle disease virus (NDV) to better understand the epidemiology and dynamics of viral transmission and to help provide effective preventive and control measures to contain the spread of AIV subtypes and NDV among animals and humans.

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