Potential risk factors of avian influenza virus infection in asymptomatic commercial chicken flocks in selected areas of Bangladesh during 2019

Md Zulfekar Ali, Mahmudul Hasan, Md Giasuddin
National Reference Laboratory for Avian Influenza, Animal Health Research Division, Bangladesh Livestock Research Institute, Dhaka, Bangladesh

ABSTRACT

Objective: Avian influenza is a zoonotic disease with a pandemic potential that can infect avian and mammalian species, including humans. Studies aimed at investigating avian influenza virus (AIV) status in asymptomatic chickens and their shedding are uncommon in Bangladesh. Therefore, the current study aimed to examine the distribution of AIV subtypes in asymptomatic commercial chicken flocks and to identify the possible risk factors associated with this infection in two selected sub-districts of Bangladesh.

Materials and Methods: A total of 582 oropharyngeal swabs were collected from 23 chicken farms during 2019 and evaluated for the presence of AIV and its subtypes by real-time reverse transcription PCR assays. Risk factors associated with AIV infection were analyzed from questionnaire data.

Results: Overall, AIV prevalence was 7.73% (n = 45) with 7.39% and 7.92% in Dhamrai and Gazipur Sadar sub-districts, respectively. In AIV-positive samples, the prevalence of A/H5N1, A/H5N2, A/H9N1, and A/H9N2 was 31.11%, 28.89%, 6.67%, and 8.89%, respectively. None of the samples were positive for N6 and N8. The odds ratio (OR) of AIV infection was 1.15 in broiler versus layer and 2 in Sonali versus layer chickens. The OR was 1.95 for medium versus small, 2.6 for large versus small flock size, 1.5 for moderate versus good biosecurity, and 2.92 for poor versus good biosecurity practicing farms.

Conclusion: The results demonstrated that A/H5N1, A/H5N2, A/H9N1, and A/H9N2 are circulating in asymptomatic chickens of selected areas. Strict farm biosecurity practices and avoiding higher flock density are recommended to prevent AIV spread in the study.

Introduction

Avian influenza virus (AIV) is a type A influenza virus belonging to the Orthomyxoviridae family, which is highly fatal, economically important, and with public health implications [1]. The significant clinical signs of AIV are acute respiratory problems and cause high morbidity and high mortality [2,3]. Besides avian species, this disease can affect a wide range of mammalian species, including humans, horses, pigs, dogs, and sea mammals [2]. The combination of two groups of proteins, 16 hemagglutinin (HA) and 9 neuraminidase (NA), produces many subtypes of AIV, but most of them are non-pathogenic or causing mild clinical symptoms [4]. According to pathogenicity, AIV has been divided into two groups: highly pathogenic avian influenza viruses (HPAIV) and low pathogenic avian influenza viruses (LPAIV) [5]. The HPAIV (H5N1 and H7N9) is characterized by severe illness and 100% death in infected birds [6]. HPAI H5N1 was first identified in Bangladesh in February 2007, and still several waves of the outbreak occur every year with high mortality in the poultry sector [7,8]. The prevalence of HPAI H5N1 has a seasonal pattern in Bangladesh, and outbreak waves start registering in late autumn, peaking in spring, and then decrease frequency gradually during the summer season [9]. Now, it is deeply entrenched in almost all countries of Asia, including China, India, Indonesia, and Vietnam [10]. The circulating HPAI H5N1 in Bangladesh was under goose/Guangdong lineage clade 2.2.2 from 2007 to 2010, and clade 2.3.2.1 and clade...
2.3.4.2 were introduced during early 2011, and from 2014 clade 2.3.2.1a is circulating exclusively in the poultry sector [11,12]. LPAI H9N2 is the dominating subtype of LPAIV in Bangladesh and is associated with mild clinical signs in poultry, impacting production performance and a low mortality rate [13]. The co-circulation of HPAI H5N1 and LPAI H9N2 is registered in Bangladesh poultry since 2007, and there is an increased probability that the evolution of viruses could require additional prevention measures [14].

The major propagation factors associated with AIV infections are farm biosecurity practices, flock density, seasonal variation, presence of Anseriformes (duck), and wild or migratory birds [15]. A few studies were conducted in Bangladesh to explore possible risk factors of AIV in backyard chickens and duck farms [16–18]. But there is a lack of risk factors analysis regarding AIV in commercial asymptomatic chickens. Therefore, the study's objective was the surveillance of AIV in asymptomatic commercial chickens and identifying possible risk factors in the two selected regions of Bangladesh.

Materials and Methods

Ethical approval

The study was approved by the Animal Experiment Ethics Committee, Bangladesh Livestock Research Institute (No.: BLRI1002).

Data and sample collection

A cross-sectional study was conducted from July to December 2019 in the Dhamrai sub-district of Dhaka district and Gazipur Sadar sub-district of Gazipur district. A total of 582 (25–26 samples per flock) individual oropharyngeal swab samples were collected in virus transfer media from 23 chicken flocks, including ten broilers, five layers, and eight Sonali chicken (cross-breed between Rhode Island Red cocks and Fayoumi hens) flocks. The flocks were classified into three categories according to the number of chickens per flock, counting small (500–1,000 chickens), medium (1,001–1,500), and large (>1,501) flocks. A structured and validated questionnaire was developed and administered to the chicken farmers to record farmers’ demographic information, followed by farm demography, biosecurity practices, and management practices. Samples were labeled and placed into an insulated icebox and transferred to the National Reference Laboratory for Avian Influenza, Bangladesh Livestock Research Institute, Dhaka, and stored at –80°C for testing.

Laboratory testing

The magnetic bead-based RNA isolation technology was applied for RNA extraction from collected samples individually using MagMAX™-96 AI/ND Viral RNA Isolation Kit (Applied Biosystems™, San Francisco, CA) in KingFisher™ Flex 96-well robot (Thermo Scientific™, Waltham, MA) according to the manufacturer's protocol. The samples were screened first for M gene presence by real-time reverse transcription PCR (rRT-PCR) test using reference primers and probes. Then, M gene positive samples were further assessed for H5, H9, N1, N2, N6, and N8 sub-typing using primers and probes by rRT-PCR test. The primers and probes are listed in Table 1.

Statistical analysis

Data from laboratory analysis and questionnaire were recorded and coded in Microsoft Excel 2013 spreadsheet (Microsoft Corporation, Redmond, WA) and exported into STATA-13 (STATA Corp., 4905, Lakeway drive, College Station, TX) for statistical analysis. Descriptive statistics were carried out to calculate the M gene’s overall prevalence and subtypes of AIV (H5, H9, N1, N2, N6, and N8). The distribution of AIV subtypes was then analyzed according to the study area, type of chicken, flock size, and biosecurity practice. The odds ratios (OR) were calculated to determine the risk factors between individual chickens' positivity with independent variables by multivariable logistic regression. Variables included in the multivariable logistic model were chicken types (layer vs. broiler; layer vs. Sonali), flock size (small vs. medium, small vs. large), and biosecurity practice (good vs. moderate and good vs. poor). The results are presented as OR, 95% confidence interval (95% CI), and p < 0.05 was used for the statistical significance level.

Results

Overall, 7.73% (45/582) oropharyngeal swabs were found positive for avian influenza (AIV) M gene. By locations, the samples were found AIV positive as 7.39% (95% CI: 4.19–11.89; n = 203) and 7.92% (95% CI: 5.40–11.11; n = 379) of Dhamrai and Gazipur Sadar, respectively (Table 2). In chickens, the Sonali-type chickens were significantly (p = 0.035) higher and AIV positive than broiler and layer chickens. By flock size, the AIV RNA prevalence was significantly higher in larger flock size (10%; 95% CI: 6.22–15.02; n = 200) compared to medium and small flock sizes (4.10%; 95% CI: 1.34–9.31; n = 122). The AIV prevalence also found to be significantly higher in poor biosecurity flock (10.66%; 95% CI: 7.26–15.95; n = 272) compared to moderate and good biosecurity (3.92%; 95% CI: 1.08–9.74; n = 102) practicing flocks (Table 2).

Furthermore, the HA and NA types of 45 AIV positive samples were analyzed (Table 3). There were four combinations of H and N-type that were found, including A/H5N1 (31.11%; n = 14), A/H5N2 (28.89%; n = 13), A/H9N1 (6.67%; n = 3), and A/H9N2 (8.89%; n = 4). But there was
8.89% (n = 4) AIV matrix gene-positive samples that could not be identified from this combination, and N6 and N8 subtypes were not detected in any of the samples tested.

The AIV prevalence varied significantly (p < 0.05) among the categories of type of chicken, flock size, and biosecurity practices in univariable analysis. These three significant variables were then used for multivariable analysis. The multivariable regression model showed that Sonali chickens were 2.0 (95% CI: 0.83–4.83; p = 0.023) times more likely to be AIV positive than layer chickens. In flock size, a larger flock size was 2.6 (95% CI: 0.95–7.11; p = 0.041) times more likely to be AIV prevalent than a smaller flock size. Also, flocks in poor biosecurity practices were found at 2.92 (95% CI: 1.00–8.53; p = 0.042) times more likely to suffer from AIV infection than flocks with practicing good biosecurity (Table 4).

Discussion

AIV has a substantial economic impact on Bangladesh’s poultry industry and has been considered a threat to human health [1,14,19]. Several subtypes of AIV have been identified in Bangladesh in various species, such as A/H1N1, A/H1N2, A/H1N3, A/H2N5, A/H3N3, A/H3N6, A/H3N8, A/H4N2, A/H5N1, A/H5N6, A/H7N5, A/H7N9, A/H9N1, A/H9N2, A/H10N7, and A/H11N3 [20–22]. By considering these, the present study was conducted to identify the risk factors of the study of AIVs of commercial chickens in Dhamrai and Gazipur regions of Bangladesh as highly poultry-populated areas.

The study investigated the unexplained epidemiological features of avian influenza in asymptomatic commercial chickens in Bangladesh. Overall, the AIV RNA was

Table 1. List of primers and probes used for identification of Matrix (M) gene of avian influenza and its subtypes.

| Target gene | Item | Name  | Sequence | Reference |
|-------------|------|-------|----------|-----------|
| M           | Forward | IVA D161M | 5’ AGATGAGYCTTTCAACCGAGGTGCG 3’ | [46] |
|             | Reverse | IVA D162M1 | 3’ GTCTCTGACTCTACCAAAAAGT 5’ |
|             |         | IVA D162M2 | 3’ GTCTCTGACTCTACCAAAAAGT 5’ |
|             |         | IVA D162M3 | 3’ GTCTCTGACTCTACCAAAAAGT 5’ |
|             |         | IVA D162M4 | 3’ GTCTCTGACTCTACCAAAAAGT 5’ |
|             | Probe   | IVA-MA    | 5’-FAM-TCAAGCCCTCAAAGCGA-TAMRA-3’ |
| H5          | Forward | IVA D148H5 | 5’ AAACAGAGAGAAATAAGTGGGAATTAATT 3’ |
|             | Forward | IVA D204f  | 5’ ATGGCTCTCGGAAACCC 3’ |
|             | Reverse | IVA D149H5 | 3’ CGTTAGATCCATCGACGGAGATGAAGA 5’ |
|             | Reverse | IVA D205r  | 3’ GCCTACGAGATGCTACCTYTT 5’ |
|             | Probe   | IVA H5a   | 5’-FAM-TCAACAGTGGGAGTTCCTCAGA-TAMRA-3’ |
|             | Probe   | IVA D215P  | 5’-FAM-ATGTGAGGAAATTATTATTATAT 3’ |
| N1          | Forward | IAV-N1-3-F | 5’ AGCCCTTGGYTTCGTTGTTGA 3’ |
|             | Reverse | IAV-N1-3-R | 3’ ACCAGAACCAGTGGCCAA 5’ |
|             | Probe   | IAV-N1-3-FAM | 5’-FAM-ATGTGAGGAAATTATTATTATAT 3’ |
| N2          | Forward | IAV-N2-1367F | 3’ AGCCCTTGGYTTCGTTGTTGA 5’ |
|             | Reverse | IAV-N2-1488R | 3’ ACCAGAACCAGTGGCCAA 5’ |
|             | Probe   | IAV-N2-1444.1FAM-MGB | FAM-CCA TCA GCC CAT GAG CCT-MGB |
| N6          | Forward | IAV-N6-10F | 5’ AGG GTG AAR ATG AAT CCA AAY CA 3’ |
|             | Forward | IAV-N6-14F | 3’ TGA ARA TGA ATC CAA ATC AGA AGA TAA 5’ |
|             | Reverse | IAV-N6-97R | 3’ CATCATCGACGCACTATACGT 5’ |
|             | Probe   | IAV-N6-43FAM | 5’-FAM-TGC ATH TCA GCH ACA GGA ATG ACA CTA TC-BHQ1-3’ |
| N8          | Forward | IAV-N8-1296F | 5’ TCCATGTTTTGGTGTGATGAT 3’ |
|             | Reverse | IAV-N8-1423R | 3’ ACCAGAACCAGTGGCCAA 5’ |
|             | Probe   | IAV-N8-1354FAM | 5’-FAM-TCHAGYAGGCTCCATTGTRAGTGAGT-BHQ1-3’ |
found in 7.73% of the chickens with no visible abnormalities. The results of the study indicated AIV is prevalent in commercial chickens in Bangladesh. Estoepangestie et al. [23] isolated highly pathogenic avian influenza virus (HPAI) A/H5N1 clade 2.3.2.1 from an asymptomatic duck in Indonesia in 2019, and ducks can act as a potential spreader of AIV when shedding virus. The report agreed with the study that indicated asymptomatic birds could carry A/H5N1.

HPAI H5N1 is a significant cause of poultry mortality and human infections worldwide [24]. The world faced a century ago the Spanish flu in 1919 (H1N1) with 50 million mortalities and sickness of one-fourth of the world’s total population [25]. Since the first identification of HPAI H5N1 in Bangladesh in 2007, several AIV outbreaks have occurred every year [8,26]. The study identified 31.11% found in 7.73% of the chickens with no visible abnormalities. The results of the study indicated AIV is prevalent in commercial chickens in Bangladesh. Estoepangestie et al. [23] isolated highly pathogenic avian influenza virus (HPAI) A/H5N1 clade 2.3.2.1 from an asymptomatic duck in Indonesia in 2019, and ducks can act as a potential spreader of AIV when shedding virus. The report agreed with the study that indicated asymptomatic birds could carry A/H5N1.

HPAI H5N1 is a significant cause of poultry mortality and human infections worldwide [24]. The world faced a century ago the Spanish flu in 1919 (H1N1) with 50 million mortalities and sickness of one-fourth of the world’s total population [25]. Since the first identification of HPAI H5N1 in Bangladesh in 2007, several AIV outbreaks have occurred every year [8,26]. The study identified 31.11% found in 7.73% of the chickens with no visible abnormalities. The results of the study indicated AIV is prevalent in commercial chickens in Bangladesh. Estoepangestie et al. [23] isolated highly pathogenic avian influenza virus (HPAI) A/H5N1 clade 2.3.2.1 from an asymptomatic duck in Indonesia in 2019, and ducks can act as a potential spreader of AIV when shedding virus. The report agreed with the study that indicated asymptomatic birds could carry A/H5N1.

HPAI H5N1 is a significant cause of poultry mortality and human infections worldwide [24]. The world faced a century ago the Spanish flu in 1919 (H1N1) with 50 million mortalities and sickness of one-fourth of the world’s total population [25]. Since the first identification of HPAI H5N1 in Bangladesh in 2007, several AIV outbreaks have occurred every year [8,26]. The study identified 31.11% found in 7.73% of the chickens with no visible abnormalities. The results of the study indicated AIV is prevalent in commercial chickens in Bangladesh. Estoepangestie et al. [23] isolated highly pathogenic avian influenza virus (HPAI) A/H5N1 clade 2.3.2.1 from an asymptomatic duck in Indonesia in 2019, and ducks can act as a potential spreader of AIV when shedding virus. The report agreed with the study that indicated asymptomatic birds could carry A/H5N1.

HPAI H5N1 is a significant cause of poultry mortality and human infections worldwide [24]. The world faced a century ago the Spanish flu in 1919 (H1N1) with 50 million mortalities and sickness of one-fourth of the world’s total population [25]. Since the first identification of HPAI H5N1 in Bangladesh in 2007, several AIV outbreaks have occurred every year [8,26]. The study identified 31.11% found in 7.73% of the chickens with no visible abnormalities. The results of the study indicated AIV is prevalent in commercial chickens in Bangladesh. Estoepangestie et al. [23] isolated highly pathogenic avian influenza virus (HPAI) A/H5N1 clade 2.3.2.1 from an asymptomatic duck in Indonesia in 2019, and ducks can act as a potential spreader of AIV when shedding virus. The report agreed with the study that indicated asymptomatic birds could carry A/H5N1.

HPAI H5N1 is a significant cause of poultry mortality and human infections worldwide [24]. The world faced a century ago the Spanish flu in 1919 (H1N1) with 50 million mortalities and sickness of one-fourth of the world’s total population [25]. Since the first identification of HPAI H5N1 in Bangladesh in 2007, several AIV outbreaks have occurred every year [8,26]. The study identified 31.11%
samples out of total AIV positive samples were A/H5N1 positive. Surveillance conducted by the National Reference Laboratory for Avian Influenza Bangladesh on HPAI H5N1 reported 323 cases till 2009 [27], and there were 44% cases from small commercial farms [28]. The live bird market surveillance also reported that 47.4% samples were positive for AIV where 21.26% had HPAI H5N1 and distribution on a different type of chickens was including 36.3% in chickens (broiler 47.4%, Sonali 31.7%, and Deshi 39.4%), and 18.7% in waterfowl (duck 19.3% and geese 16.7%) [29]. Negovetich et al. [30] reported that 23% of the live bird markets were positive for AIV, where 94% of infections were low pathogenic avian influenza (LPAI). After the first identification of HPAI in Bangladesh, both A/H5N1 and A/H5N2 were co-circulating in the poultry farms, environments, and LMBs [31]. HPAI H5N1 was also identified in various species other than chickens in Bangladesh like crow, bats, monkey, waterfowl, goose, quails, and pigeons [26,32,33]. A/H9N1 and A/H9N2 are a low pathogenic avian influenza bearing low mortality with the continuous loss of egg and meat production in the poultry industry and significant economic losses [34,35]. Here, 6.67% of the samples were found to be A/H9N1 positive and 8.89% to be A/H9N2 positive. The previously published report identified 16.5% of A/H9N2 in selected poultry farms in Bangladesh [30]. The LPAI causes 90% of morbidity in chickens, with continuous production loss occurring in the poultry industry in Bangladesh [14].

The study was identified as a significant risk factor of increasing the prevalence of AIV in the flocks, including the type of chicken, flock size, and biosecurity practices. Sonali chickens were identified as potentially vulnerable to the infection with AIV compared to broiler and layer chickens. Kim et al. [29] demonstrated that Sonali chickens harbor a significant infection load. The husbandry practice of Sonali chickens is under poor biosecurity and larger flock size [36,37]. The breeding policy was also traditional and non-systematic [36]. Other avian viral diseases like infectious bronchitis, avian metapneumovirus, and reticuloendotheliosis virus reported a higher prevalence in selected areas of Bangladesh [38–40]. Therefore, Sonali acts as a potential spreader and harbors AIV infection compared to the other poultry flocks.

Flock density is also a significant risk factor for rapid transmission and higher prevailing of AIV. The AIV can be transmitted from bird to bird by direct contact with secretions, feces, and aerosol droplets [41]. So higher flock density can increase the spread of AIV rapidly from bird to bird [15].

Tiensin et al. [15] reported that high flock density is a potential risk factor to transmit AIV, and they identified AIV risk to be 0.98 times and 1.02 times higher in backyard chickens and fighting cock chickens in Thailand, respectively. Therefore, there is a strong association between flock density and AIV infection in birds.

Poor biosecurity practice is a significant risk factor for the higher prevalence of AIV [42]. Waziri et al. [43] demonstrated that chickens reared in poor biosecurity practices were three times more vulnerable to be spreaders of AIV compared to good biosecurity practicing farms, which is in accordance with the current findings. Osmani et al. [16] conducted a case control study and reported an increased number of farm staffs (OR: 5.2), weekly visit of veterinarians (OR: 3.0), and roaming of village chickens around the farm (OR: 0.6) were the major biosecurity risk factors in commercial poultry farms in Bangladesh. This study agreed with our biosecurity investigation. Large-scale poultry farms usually maintain good biosecurity practices due to standard operation and commercial establishment, but it is relatively poor in small-scale farms. As a result, maintaining good biosecurity is sometimes compromised in small-scale poultry farms and poses a higher risk of AIV [22,44,45]. So, it is obligatory to practice good farm biosecurity to control flocks from AIV.

However, A/H5N1 and A/H9N2 are prevalent and sometimes co-circulating in different chicken types in Bangladesh.

Conclusion

The co-circulation of HPAI H5N1 and LPAI H9N2 is reported in the commercial poultry industry since the first identification of HPAI H5N1 in Bangladesh. Birds carrying A/H5N1 without showing clinical symptoms is a big concern for the poultry and public health sectors. Poor farm biosecurity practices, large flock density, and Sonali chickens are identified as potential risk factors related with AIVs infection in commercial chickens in Bangladesh. Enhancing biosecurity and proper vaccination practices against HPAI H5N1 and LPAI H9N2 could be an effective AIVs control measure in Bangladesh. Further study of amino acids arrangement of the cleavage site of the HA molecule for the determination of HPAIV H5N1 and LPAIV H9N2 is suggested.

List of abbreviations

95% CI: 95% CIs, AIV: Avian influenza virus, HA: Hemagglutinin, HPAIV: Highly pathogenic avian influenza virus, LPAI: Low pathogenic avian influenza virus, NA: Neuraminidase, OR: Odds ratio, RNA: Ribonucleic acid, rRT-PCR: Real-time reverse transcription PCR, LBM: Live Bird Market.

Acknowledgments

We are grateful to the poultry farmers who responded to the surveillance and helped during sample collection.

http://bdvets.org/javar/
We are also thankful to the Director General, Bangladesh Livestock Research Institute, for allocating funds from the core research fund.

Conflict of interest
The authors declare no conflicts of interest.

Authors’ contributions
MZ Ali conceived, designed the study, analyzed the data, and wrote the article. M Hasan conducted surveillance and laboratory analysis. M Giasuddin reviewed and finalized the article. All the authors read the final version and approved for publication of the article.

References
[1] Kraïdi O, Langeroudi A, Madargor O, Karimi V. Prevalence of AIV subtype H9 among poultry with respiratory signs in Iraq. Bul J Vet Med 2017; 20(4):367–76; https://doi.org/10.15547/bjvm.2012
[2] Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Mol Biol Rev 1992; 56:152–79; https://doi.org/10.1128/MMBR.56.1.152-179.1992
[3] Webster RG, Guan Y, Poon L,�, Webby R, Govorkova E, et al. The spread of the H5N1 bird flu epidemic in Asia in 2004. Arch Virol 2005; 19:117–29; https://doi.org/10.1007/3-211-29981-5_10
[4] Alexander DJ. Summary of avian influenza activity in Europe, Asia, Africa, and Australasia, 2002–2006. Avian Dis 2007; 51:161-6; https://doi.org/10.3382/avian.dis.06-0260R1
[5] Alexander DJ. A review of avian influenza in different bird species. Vet Microbiol 2000; 74(1–2):3–13; https://doi.org/10.1016/S0378-1135(00)00160-7
[6] Swayne DE, Suarez D. Highly pathogenic avian influenza. Rev Sci Tech Off Int Epizoot 2000; 19:463–475; https://doi.org/10.20506/rtv.19.2.1230
[7] Biswas PK, Christensen JP, Ahmed SS, Barua H, Das A, Rahman MH, et al. Avian influenza outbreaks in chickens, Bangladesh. Emerg Infect Dis 2008; 14(12):1909; https://doi.org/10.3201/ eid1412.071567
[8] Giasuddin M, Ali MZ, Karim MR, Hassan MN, Hasnain M, Islam E. The past and present scenario of avian influenza and its control strategy in Bangladesh: a review. Bangladesh J Livest Res 2018; 21–2124–28; https://doi.org/10.3329/bjl.v0i0.45443
[9] Ahmed SS, Ershbak AK, Biswas PK, Christensen JP, Toft N. Spatio-temporal magnitude and direction of highly pathogenic avian influenza (H5N1) outbreaks in Bangladesh. PLoS One 2011; 6:e24324; https://doi.org/10.1371/journal.pone.0024324
[10] Centers for Disease Control and Prevention (CDC). (2012) CDC Seasonal Influenza (Flu) – H5N1 in Birds and Other Animals [www Document]. Available via http://www.cdc.gov/flu/avianflu/h5n1-animals.htm (Accessed 28 January 2019)
[11] Islam MR, Haque ME, Giasuddin M, Chowdhury EH, Samad MA, Parvin R, et al. New introduction of clade 2.3.2.1 avian influenza virus (H5N1) into Bangladesh. Transbound Emerg Dis 2011; 59(5):460–3; https://doi.org/10.1111/j.1865-1681.2011.01297.x
[12] Haque ME, Giasuddin M, Chowdhury EH, Islam MR. Molecular evolution of H5N1 highly pathogenic avian influenza viruses in Bangladesh between 2007 and 2012. Avian Pathol 2014; 43(2):183–94; https://doi.org/10.1080/00052944.2014.898244
[13] Uyeki TM, Nguyen DC, Rowe T, Lu X, Hu-Primmer J, Huynh LP, et al. Seroprevalence of antibodies to avian influenza A (H5) and A (H9) viruses among market poultry workers, Hanoi, Vietnam, 2001. PLoS One 2012; 7(8):e43948; https://doi.org/10.1371/journal.pone.0043948
[14] Parvin R, Begum JA, Nooruzzaman M, Chowdhury EH, Islam MR, Vahlenkamp TW. Review analysis and impact of co-circulating H5N1 and H9N2 avian influenza viruses in Bangladesh. Epidemiol Infect 2018; 146(10):1259–66; https://doi.org/10.1017/S0950268818001292
[15] Tiensin T, Ahmed SS, Rojanasthien S, Songserm T, Ratankorn P, Chaichoun K, et al. Ecologic risk factor investigation of clusters of avian influenza A (H5N1) virus infection in Thailand. J Infect Dis 2009; 199(12):1735–43; https://doi.org/10.1086/592207
[16] Osmani M, Thornton R, Dhand NK, Hoque M, Milton SM, Kalam M, et al. Risk factors for highly pathogenic avian influenza in commercial layer chicken farms in Bangladesh during 2011. Transbound Emerg Dis 2014; 61:e44–51; https://doi.org/10.1111/tbed.12071
[17] Loth L, Gilbert M, Osmani MG, Kalam AM, Xiao X. Risk factors and clusters of highly pathogenic avian influenza H5N1 outbreaks in Bangladesh. Prev Vet Med 2010; 96:104–13; https://doi.org/10.1016/j.prevetmed.2010.05.013
[18] Ahmed SS, Ershbak AK, Biswas PK, Christensen JP, Hannan AS, Toft N. Ecological determinants of highly pathogenic avian influenza (H5N1) outbreaks in Bangladesh. PLoS One 2012; 7:e39398; https://doi.org/10.1371/journal.pone.0039398
[19] Chakraboryt A, Rahman M, Hossain MJ, Khan SU, Haider MS, Sultana R, et al. Mild respiratory illness among young children caused by highly pathogenic avian influenzaA(H5N1) virus infection in Dhaka, Bangladesh, 2011. J Infect Dis 2017; 216:5520–8. https://doi.org/10.1093/infdis/jix019
[20] Sarker RD, Giasuddin M, Chowdhury EH, Islam MR. Serological and virological surveillance of avian influenza virus in domestic ducks of the north-east region of Bangladesh. BMC Vet Res 2017; 13(1):180; https://doi.org/10.1186/s12917-017-1104-6
[21] Gerloff NA, Khan SU, Zanders N, Balish E, Haider MS, Islam A, et al. Genetically diverse low pathogenicity avian influenza A virus subtypes co-circulate among poultry in Bangladesh. PLoS One 2016; 11:e0152131; https://doi.org/10.1371/journal.pone.0152131
[22] Parvin R, Begum JA, Chowdhury EH, Islam MR, Beer M, Harder T. Co-subistence of avian influenza virus subtypes of low and high pathogenicity in Bangladesh: challenges for diagnosis, risk assessment and control. Sci Rep 2019; 9(1):1–10; https://doi.org/10.1038/s41598-019-44220-4
[23] Estropangestie A, Rahardjo K, Rahardjo A, Novianti A, Prasetya R, Nastria A, et al. Identification of avian influenza virus A(H5) clade 2.3.2.1 in asymptomatic ducks (Anas species) at a live-poultry market in East Java, Indonesia. International Society for Economics and Social Sciences of Animal Health-South East Asia 2019 (ISESSAH-SEA 2019). Atlantis Press, Amsterdam, Netherlands, 2019.
[24] Swayne DE. The global nature of avian influenza. 2nd edition. John Wiley & Sons, Hoboken, NJ, pp 177–201, 2016; https://doi.org/10.1002/9781118923441.ch8
[25] Lüthy SA, Ritacco V, Kantor IN. One hundred years after the Spanish” flu. Medicina 2018; 78(2):113–8.
[26] Nooruzzaman M, Mumu TT, Hasnatin A, Aktor MN, Rasel MS, Rahman MM, et al. A new reassortant clade 2.3.2.1 a H5N1 highly pathogenic avian influenza virus causing recent outbreaks in ducks, geese, chickens and turkeys in Bangladesh. Transbound Emerg Dis 2019; 66(5):2120–33; https://doi.org/10.1111/tbed.13264
[27] Alam J, Giasuddin M, Samad MA, Taimur MJFA. Recent evidence of avian influenza in Bangladesh: a review. World’s Poult Sci J 2010; 66(3):455–64; https://doi.org/10.1017/S004393391000053X
[28] OIE. Follow-Up Report No 43 (Final Report) 2013. Available via http://www.oie.int/wahis_2/public%5C%5Ctemp%5C%5Crepportsv en_fup_0000014568_20131223_145541.pdf (Accessed 11 May 2019).
[29] Kim Y, Biswas PK, Giasuddin M, Hasan M, Mahmud R, Chang YM, et al. Prevalence of avian influenza A (H5) and A (H9) viruses in live
bird markets, Bangladesh. Emerg Infect Dis 2018; 24(12):2309; https://doi.org/10.3201/eid2412.180879

[30] Negovetich NJ, Feeroz MM, Jones-Engel L, Walker D, Alam SR, Hasan K, et al. Live bird markets of Bangladesh: H9N2 viruses and the near absence of highly pathogenic H5N1 influenza. PloS One 2011; 6(4):e19311; https://doi.org/10.1371/journal.pone.0019311

[31] Parvin R, Heenemann K, Halami MY, Chowdhury EH, Islam MR, Vahlenkamp TW. Full-genome analysis of avian influenza virus H9N2 from Bangladesh reveals internal gene reassortments with two distinct highly pathogenic avian influenza viruses. Arch Virol 2014a; 159:1651–61; https://doi.org/10.1007/s00705-014-1976-8

[32] Parvin R, Kamal AH, Haque ME, Chowdhury EH, Giasuddin M, Islam MR, et al. Genetic characterization of highly pathogenic H5N1 avian influenza virus from live migratory birds in Bangladesh. Virus Genes 2014b; 49(3):438–48; https://doi.org/10.1007/s11262-014-1118-0

[33] Ali MZ. Common respiratory diseases of poultry in Bangladesh: a review. SAARC J Agric 2020; 18(1):1–11; https://doi.org/10.3329/sja.v18i1.48377

[34] Jegede A, Fu Q, Berhane Y, Lin M, Kumar A, Guan J. H9N2 avian influenza virus retained low pathogenicity after serial passage in chickens. Can J Vet Res 2018; 82(2):131–8.

[35] Verhagen JH, Munster VJ, Fouchier RA. Ecology and evolution of avian influenza viruses. In: Tibayrenc M (ed.). Genetics and evolution of infectious disease. Elsevier, Amsterdam, Netherlands, pp 729–49, 2011; https://doi.org/10.1016/B978-0-12-384890-1.00028-5

[36] FAO. Comparative performance of Sonali chickens, commercial broilers, layers and local non-descript (deshi) chickens in selected areas of Bangladesh. Animal Production and Health Working Paper. No. 14, Rome, Italy, 2015.

[37] Ali MZ, Moula MM, Bhuiyan ZA, Javed MT. A cross sectional survey of chicken astroviruses antibody in broiler and Sonali (cross-bred) chickens in selected areas in Bangladesh. Mac Vet Rev 2020; 43(1):75–80. https://doi.org/10.2478/macvetrev-2020-0016

[38] Ali MZ, Park JE, Shin HJ. Serological survey of avian Metapneumovirus infection in chickens in Bangladesh. J Appl Poult Res 2019; 28(4):1330–4; https://doi.org/10.3382/appr/plz050

[39] Bhuiyan ZA, Ali MZ, Moula MM, Giasuddin M, Khan ZJM. Prevalence and molecular characterization of infectious bronchitis virus isolated from chicken in Bangladesh. Vet World 2019; 12(6):909–15; https://doi.org/10.14202/vetworld.2019.909-915

[40] Ali MZ. The seroprevalence study of Reticuloendotheliosis virus infection in chicken in Bangladesh. Egypt J Vet Sci 2018; 49(2):179–86; https://doi.org/10.21608/ejvs.2018.5856.1051

[41] Bouma A, Claassen I, Nath K, Klinkenberg D, Donnelly CA, Koch G, et al. Estimation of transmission parameters of H5N1 avian influenza virus in chickens. PLoS Pathog 2009; 5(1):e1000281; https://doi.org/10.1371/journal.ppat.1000281

[42] FAO. Biosecurity for highly pathogenic Avian Influenza. FAO Anim Prod Heal Pap, Rome, Italy, 2008.

[43] Waziri MI, Abdu PA, Sa’idu L, Bello M. Seroepidemiology and assessment of risk factors for the spread of avian influenza in birds in two Nigerian states. Vet Med Sci 2017; 3(4):227–38; https://doi.org/10.1002/vms3.57

[44] Win H, Mon CS, Aung K, Oo K, Sunn K, Htun T, et al. Retracted: risks of avian influenza (H5) in duck farms in the Ayeyarwaddy Delta region, Myanmar. Zoonoses Public Health 2014; 61:317–23; https://doi.org/10.1111/zph.12073

[45] Yupiana Y, De Vlas SJ, Adnan NM, Richards JH. Risk factors of poultry outbreaks and human cases of H5N1 avian influenza virus infection in West Java Province, Indonesia. Int J Infect Dis 2010; 14:e800–5; https://doi.org/10.1016/j.ijid.2010.03.014

[46] Heine HG, Foord AJ, Wang J, Valdeter S, Walker S, Morrissy C, et al. Detection of highly pathogenic zoonotic influenza virus H5N6 reverse-transcriptase quantitative polymerase chain reaction. Virol J 2015; 12(1):18; https://doi.org/10.1186/s12985-015-0250-3

[47] Hoffmann B, Hoffmann D, Henritzi D, Beer M, Harder TC. Riems influenza a typing array (RITA): an RT-qPCR-based low density array for subtyping avian and mammalian influenza viruses. Sci Rep 2016; 6(1):1–10; https://doi.org/10.1038/srep27211