



Investigation of an Unusual Crow Mortality Event in Jessore, Bangladesh, December 2018

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Abstract

After a report of an unusually high number of crow deaths in Jessore, Bangladesh, a multidisciplinary team investigated the event in December 2018 to identify the etiologic agent, and the source and extent of the outbreak. We interviewed students, teachers, live bird sellers, poultry farm owners and cleaners for fever and cough symptoms. We reviewed the hospital records for acute respiratory distress syndrome and chronic obstructive pulmonary disease. We observed live bird market practices, crow roosts and their feeding behavior, and collected cloacal and oropharyngeal swabs from moribund and dead crows, and pooled environmental samples from live bird markets (LBMs) and farms. All samples were tested for influenza A/H5, H7 and H9 by RT-PCR. The H5 prevalence was 77.4% in samples obtained from crow roosts. Among environmental samples from the LBMs, 11.1% were positive for H5 and 5.5% had co-infections with H5 and H9. Our results indicate that the H5 influenza virus is circulating in LBMs and was transmitted to crows through their feeding on the waste. We recommend that continuous surveillance in wild birds and LBMs is required to understand the virus's evolution, transmission pathways and potential source of infection. Improved waste management practices in LBMs and public awareness are needed to reduce the risk and stop spillover of avian influenza virus to humans in Bangladesh.

Keywords: Bangladesh, live bird market, crow, influenza, poultry

Introduction

Avian influenza is a highly contagious viral disease with a high fatality that affects poultry as well as wild and domesticated birds.¹ Bangladesh has reported H5N1 infections in domestic poultry since February 2007 and 64 Highly Pathogenic Avian Influenza (HPAI) outbreaks occurred in commercial poultry farms from

February to December 2017.² Influenza A/H5 caused deaths in crows during 2012–2014 in Bangladesh.³ The majority of the influenza A/H5-positive samples were from apparently healthy waterfowl in 2012. Multiple subtypes, including H1N1, H1N3, H3N2, H3N6, H3N8, H4N1, H4N2, H4N6, H5N1, H5N2, H6N1, H7N9, H9N2, H11N2, H11N3, and H11N6 were detected in waterfowl and environmental samples in Bangladesh.⁴

The World Health Organization has stated that animal influenza viruses are distinct from human seasonal influenza viruses and do not easily transmit from animals to human. However, influenza viruses from animals occasionally infect humans through direct or indirect contact and can cause disease in humans.⁵ Generally, most human zoonotic influenza cases occurred due to exposure of the influenza A/H5 viruses through contact with infected poultry or contaminated environments, including live bird markets (LBMs).⁶ From 2016–2020, only 14 human influenza A/H5 cases were reported and in 2018 no influenza A/H5 case was reported in the world.¹ Vaccine and hygienic measures can prevent transmission of human influenza and there is effective treatment with neuraminidase inhibitors.⁷ Development of medium- and long-term capacities of the veterinary and public health systems are needed to strengthen the emergency response and “One Health” approach to ensure inter-sector coordination in control of HPAI outbreaks.⁸

During January to December 2011, the Forest Department and the Department of Livestock Services of Bangladesh received multiple reports of

crow deaths from at least two administrative divisions (Rajshahi and Dhaka).⁹ The Public Health Emergency Operation Centre (PHEOC) of Institute of Epidemiology Disease Control and Research (IEDCR) reported an unusually high number of crow deaths at Shankarpasha Secondary School, Abhaynagar, Jessore on 21 Dec 2018. PHEOC verified the event by telephone conversation with a news reporter, school teacher and Upazila Livestock Officer. A multidisciplinary investigation team included IEDCR, Department of Livestock Services (DLS) and Food and Agricultural Organization investigated the crow deaths to confirm and characterize the outbreak and to identify the etiologic agent and the source of the infection as well as possible associated human infections.

Methods

We used a mixed methods design and a One Health approach to determine the scope and magnitude of avian influenza outbreaks in humans and crows and to identify linkages between these occurrences. The outbreak occurred in Abhaynagar Upazila, Jessore District (population 262,434 in 2020) in the southwestern part of Bangladesh (Figure 1).

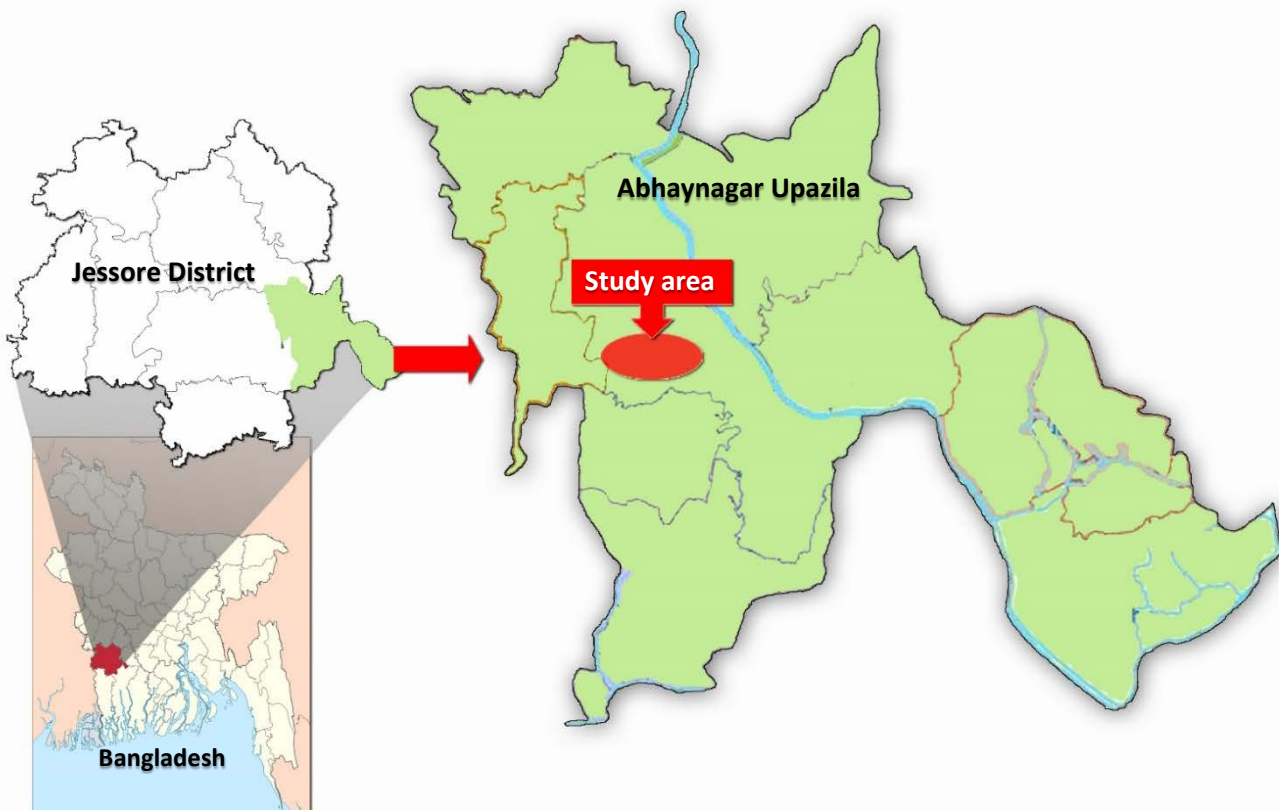


Figure 1. Map showing Abhaynagar Upazila in Jessore, Bangladesh

To verify a human outbreak, we reviewed the records of severe acute respiratory illness (SARI) and influenza like illness (ILI) patients in the 50 bed Upazila Health Complex. We reviewed medical records

for acute respiratory distress syndrome and chronic obstructive pulmonary disease records for SARI and ILI patient with the help of medical officers for the last two months (October to December 2018).

Based on clinical features and review of the literature, we defined a suspected SARI and ILI case as any resident of Abhaynagar, Jessore, with fever, sore throat, or cough and/or sneezing with the onset of illness from 15 to 31 Dec 2018. We performed active case search among the students and teachers of Shankarpasha Secondary School of which there were 304 students and 20 teachers. Because of the winter vacation, only a limited number of students and teachers were present. We also interviewed live bird sellers, poultry farm owners and farm cleaners.

To verify the avian influenza outbreak in birds, we reviewed the mortality records, with a focus on crows, from the Upazila Livestock Office (ULO). The population of birds in Abhaynagar Upazila was 127,842 poultry, 841,520 ducks and 95,845 pigeons (source: ULO Abhaynagar). We actively searched for dead or moribund crows, poultry and pigeons among farms within five kilometers radius of the Shankarpasha Secondary School. We interviewed all commercial poultry and backyard farm owners and pigeon farmers. We interviewed the temporary workers who handled the dead crows (note: paid by school). We also interviewed sellers at the live bird market. A live bird market is a temporary non-structured market in the district, sub-district or village level with between 6-10 vendors and 200–1000 birds. The market is open every day. Most of the shops have no municipal water supply and waste is discharged into open sewers. The bird sellers wear no personal protective equipment and do not have access to first aid for emergencies.

Laboratory investigation was conducted by collecting pooled environmental samples from the poultry cages in the LBMs, fecal and offal samples from poultry in

LBMs, oropharyngeal and cloacal samples from dead and moribund crows in secondary school playgrounds, fecal samples beneath crow roosts, and oropharyngeal and cloacal swabs from poultry and pigeons from commercial and backyard farms.

The collected samples were stored in a dry shipper and transported in a viral transport media to the National Reference Laboratory for Avian Influenza at Bangladesh Livestock Research Institute. In addition, all samples were tested for influenza A/H5, H7 and H9 viruses by RT-PCR and Matrix gene (M-gene). M-gene is the influenza A genome consisting of eight segments of single-stranded, negative-sense RNA. The matrix (M), non-structural (NS), and PB-1 genes, each of which encodes two proteins encoded by the matrix gene. M1 is important in initiating progeny virus assembly, while M2, an integral membrane protein.¹⁰ Therefore, the evolution of the M-gene may reflect host-specific adaptation. Despite the association of the two genes, influenza viruses in wild waterfowl contain distinguishable lineages of M-genes.¹¹

Results

Human Cases

In our investigation, we identified 178 patients suffering from acute respiratory distress syndrome and 290 patients from chronic obstructive pulmonary disease at the Upazila Health Complex. There was no SARI and ILI patients. Next, we interviewed 45 students, 15 teachers, 26 bird sellers, 12 poultry farm owners and two cleaners. None reported that they had SARI or ILI symptoms during the past 15 days (Table 1).

Table 1. Descriptive epidemiology of the participants, Jessore, Bangladesh, 2018

Sl No	Source of participant	Number interviewed or reviewed	Male (%)	Average age (range) in years	Remarks
1	Medical records of patients with acute respiratory distress syndrome	178	47.8	38.0 (3-72)	
2	Medical records of patients with chronic obstructive pulmonary disease	290	62.1	35.5 (4-75)	
3	Students (class 9 and class 10)	45	47.0	15.8 (14-19)	No one met case definition
4	School teachers	15	86.7	46.2 (31-54)	
5	School cleaners	2	50.0	45.0 (42-48)	
6	Live bird market, bird sellers	26	100.0	41.0 (23-51)	
7	Commercial and backyard poultry farm owners	12	16.7	36.5 (35-61)	
Total		568			

Table 2. Sample collected from different types of birds in Jessore and results of laboratory tests, Bangladesh, 2018

Sample site	Type of sample	Number of samples	M-gene reaction ^a	H5 (%)	H9 (%)
Live bird market	Fecal and offal sample of poultry	18	2	11.0	5.5
Crow roost	Swab sample (oropharyngeal and cloacal)	31	24	77.0	0.0
	Fecal and urine sample	17	1 ^b	0.0	0.0
Poultry farm	Commercial farm (oropharyngeal and cloacal)	2	0	0.0	0.0
	Backyard poultry (oropharyngeal and cloacal)	1	0	0.0	0.0
Pigeon farm	Pigeon (oropharyngeal and cloacal)	3	0	0.0	0.0
Total		72	27	37.5	0.01

Note: ^a M-gene Reaction: Matrix gene responsible for influenza A virus, ^b Influenza A/untypable.

Bird Mortality

The team found 31 dead and moribund crows during the investigation period. The crow mortality increased from 19 to 25 December and reached a peak on 24

December (Figure 2). The investigation team assumed that the approximate number of crows was 1,000 at Shankarpasha Secondary School crow roost and 1,500 at Pirbary roost, the two nearest crow roosts to Shankarpasha Secondary School.

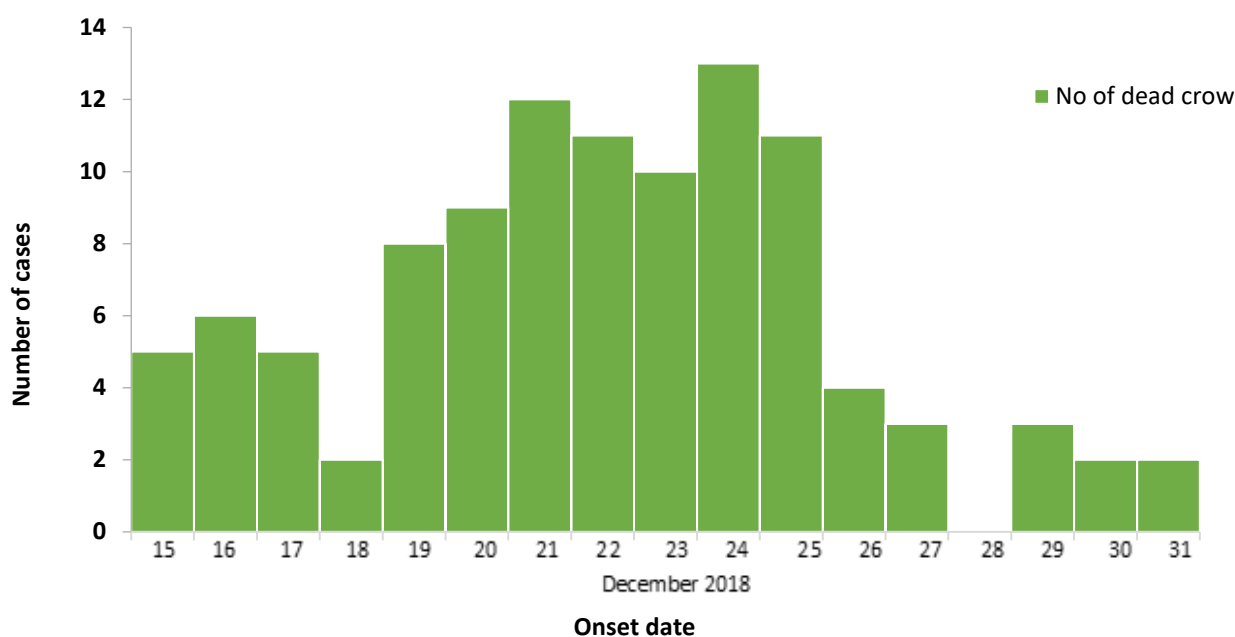


Figure 2. Epidemic curve of crow mortality at reported site premises, Jessore, Bangladesh, 2018 (n=106)

Laboratory Investigation

All crow, poultry and pigeon samples were tested by RT-PCR for influenza A (M-gene) and for H5, H7, H9, and N1. Among all tested crows and poultry from the live bird markets, 77.4% (24/31; 95% confidence interval (CI): 58.9-90.4) of crow and 11.1% (2/18; 95% CI:1.4-34.7) of poultry samples were positive for the influenza A/H5 virus, respectively; all pigeons were negative and 5.5% (1/18; 95% CI: 0.1-27.3) of the poultry had co-infection with influenza A/H5 and H9.

Among fecal samples obtained from the crow roosts, 5.8% (1/17; 95% CI: 0.2-28.7) were positive for influenza A/untypable. All the environmental samples of pigeon and poultry farms were negative for influenza.

Walk Through Survey

Most of the shops in the LBMs have no supply of water from the municipality. Waste is discharged into open sewers. The team observed that the birds sellers threw offal and poultry wastage into nearby rivers and ponds. More importantly, we noticed crows eating the poultry offal and waste materials in the LBMs and areas along the river. We observed that crows shared their roosts with other wild birds.

Discussion

This investigation confirmed that the influenza A/H5 virus was found in sick and dead crows at Jessore, Bangladesh. No transmission to humans occurred. Avian influenza in crows has previously been reported in Bangladesh, Russia, and South Korea. In January

2017, influenza A/H5 infection was found in dead and moribund crows in Rajshahi Medical College Hospital, Bangladesh.¹² In Russia 2007, RT-PCR revealed influenza virus A/H5 in more than half of pigeons and crows and in around 20% of starlings.¹³ In South Korea, the prevalence of avian influenza was 0.6% in wild birds from 2003–2008.¹⁴ The World Organization for Animal Health reported that an outbreak occurred in domestic birds in Bangladesh from January to June 2018, where 385 domestic birds and 600 wild birds died.¹⁵ Globally, avian influenza surveillance has identified many subtypes of influenza A in LBMs, duck farms, and wild birds.¹⁶ The small seller sells poultry to larger LBMs in the city, due to fear of financial loss, so sometimes clinically diseased poultry is sold. Diseased poultry is cheaper, which encourages other villagers to buy these birds. People purchase live poultry and slaughter them at home. The inedible portions from poultry are usually disposed in an unsafe way or are fed to other birds, which grossly enhances incursion risks.¹⁷

No transmission to humans occurred based on clinical observation because there is no direct food chain connectivity from crow to human. Muslims do not eat crows due to religious beliefs. Therefore, it is difficult to transmit the avian influenza virus from crows to human.¹⁸ Humans may be infected with influenza A/H5 from LBMs but there are no reports of transmission of influenza A/H5 from crows. Therefore, we assumed influenza A/H5 can be transmitted to crows but not from crows to humans.

The unprecedented epizootic of influenza A (H5N1) viruses among birds continues to cause human disease with high mortality and poses a threat to future pandemics.¹⁹ According to the World Health Organization there were 456 deaths from H5N1 out of 860 human avian influenza cases (2003 to 2018) in 16 countries.²⁰ The influenza A (H5N1) viruses that have infected humans have been entirely avian in origin, and they reflect strains circulating locally among poultry and wild birds.²¹ If HPAI Asian H5N1 viruses gain the ability for efficient and sustained transmission among humans, an influenza pandemic could result, with potential high rates of illness and deaths worldwide. Therefore, the HPAI epizootic continues to pose an important public health threat.²²

Bangladesh Livestock Research Institute isolated influenza A/H5N1 viruses from crows that ingested the internal organs of infected poultry sold at live bird markets.²³ In Bangladesh, the majority of isolated subtype was non-pathogenic H9N2, but virulent subtypes H1N2, H1N3, H3N6, H4N2, H5N1, H5N6 and H10N7 were also found in LBMs in 2011.^{24,25} The

presence of influenza A/H5 in the samples collected from LBMs suggested that the crows share the viruses with the chickens. The crows, as carrion eaters, could be infected from the offal or wastage of infected poultry. Live bird sellers throw offal and wastage into the river where the crows frequently feed, which might be a source of infection. The crow is the closest wild bird to the human habitat and sometimes it also moves to urban kitchen. House crow deaths appeared to be an indicator of the presence of HPAI viruses in poultry at live bird markets.²⁶ Despite numerous efforts at containment from the World Health Organization, H5N1 influenza viruses and their precursors still circulate among poultry and wild birds in Asia and remain a threat to both veterinary and human public health.²⁷

Limitations

Human cases may have been underreported because the study protocol tested only symptomatic cases while asymptomatic people can be tested positive for avian influenza. Ideally, necropsy should be done soon after the animal has died. However, for these dead crows, necropsy was not feasible because we arrived at the site too late and all of the crows were too decomposed. Generalization of the study is limited because the affected school was on winter vacation and we could only interview the students and teachers that remained.

Public Health Actions and Recommendations

We recommend surveillance of influenza A/H5 in birds to further understand the influenza evolution, transmission pathways and potential source of infection in crows and poultry. This investigation also demonstrated the value of following the One Health strategy to respond and mitigate the zoonotic transmission risk. Live bird market waste management should be improved to reduce the potential risk of transmission of avian influenza. There is a need to strengthen outbreak investigation considering co-infection, toxicity and bacterial infection, and histo-pathological test to identify the etiologic agent and the source of infection in future investigations. Moreover, awareness building and community engagement is important for obtaining accurate information in the shortest possible time.

The investigation findings were shared with all stakeholders to increase awareness and ensure use of personal protective equipment by workers related to disposal of dead birds and to improve bio-security measures in LBMs to reduce the spread of influenza A/H5. Local authorities should improve public awareness to reduce the risk of influenza virus

spillover to humans in Bangladesh. These findings indicate that improvements in hygiene and biosecurity measures are needed in LBMs to reduce exposure to the avian influenza virus.

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Conflict of Interest

The authors declare no conflict of interests.

Suggested Citation

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