

# Unusual Crow (*Corvus Splendens*) and Indian Flying Fox (*Pteropus Medius*), Fruit Bats Death in Dhaka, Bangladesh: A One Health Approach to Outbreak Investigation

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## Introduction

- Highly pathogenic avian influenza (H5N1) caused  $\geq 500$  outbreaks in poultry and wild birds in Bangladesh since 2007.
- In early 2011 and 2016, H5N1 (2.3.2.1) spread among wild crows in several districts of Bangladesh, including the capital, Dhaka.
- Studies demonstrate that domestic poultry sold in the live bird markets (LBMs) throughout Bangladesh carry a wide range of avian influenza virus (AIV) strains.
- Responding to reports of a crow mortality event in Mohakhali, Dhaka, a multidisciplinary One Health team including epidemiologists, veterinarians, and anthropologists investigated crow mortalities in Dhaka city between January 15<sup>th</sup> to February 20<sup>th</sup>, 2017.
- Objectives:** To identify the etiologic agent, source of the infection, and characterize the extent of the outbreak.



Figure 1: Moribund crow



Figure 2: Study sites



Figure 3: Crow feeding on poultry offal in LBM

- All crow samples were negative for H7, H9 and Flavivirus.
- None of the LBM samples were positive for H7.
- All H5 positive crows and LBM samples were tested for N1
  - 70% H5 crows (n=78; 95%CI: 62-77) and 74% H5 LBMs (n=45) samples were positive for N1.
  - the remaining were negative which indicates that other N types might be co-circulating in both crows and LBMs.
  - therefore early outbreak response can potentially detect novel influenza and HPAI subtypes.
- Both fruit bats were PCR positive for influenza A/H5N1.
- Phylogenetic analysis based on partial sequence of the HA gene suggests that strain is similar to the Bangladeshi 2.3.2.1a clade that circulated in 2011.

## Methods

- The team visited all crow roosts with 7 km of reported roosts, Mohakhali, Dhaka.
- The team collected cloacal and oro-pharyngeal swabs from moribund and dead crows (N=171) from all sites
- Environmental samples (N=149) from 19 neighboring LBMs, where crows were observed feeding from within a 7 km radius surrounding the crow roosts of the reported outbreak and LBMs in Dhaka.
- Swabs and tissue samples were collected from two dead Indian flying foxes which were found in the outbreak roosts.
- All samples were tested by specific q-PCR for influenza A (M gene) H5/H7/H9/N1, and sequenced following isolation from virus culture.
- All crow samples were tested using consensus PCR for Flavivirus.
- PCR products were sequenced to confirm positive PCR results.



Figure 4: Sample collection from dead crow

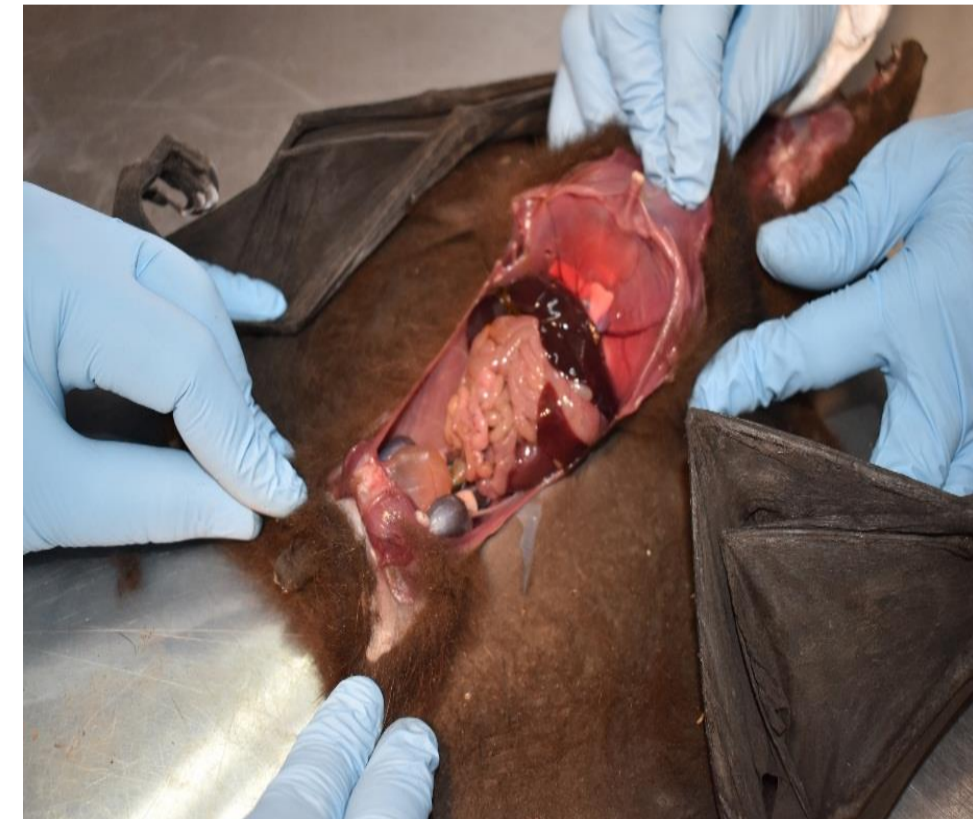


Figure 5: Post-mortem of dead bat

## Results

- 4 crow roosts had evidence of crow morbidity and mortality approximately 7 km from the reported outbreak site.
- Additional 7 apparently healthy crow roosts where there was no evidence of crow mortality or unusual illness.
- The team observed crow feeding on poultry offal and wastage in neighboring LBMs and sharing the roost site with other wild birds and fruit bats.
- 87% of the sampled crows (n=149; 95% CI 81-92) were positive for Influenza A/H5 and 3% for Influenza A/untypable (n=5; 95%CI: 1-7).
- Among LBMs samples,
  - 70% positive to Influenza A (n=105; 95%CI: 62-78)
  - Among influenza A positive
    - 58% were positive to A/H5 (n=61; 95%CI: 48-68)
    - 9% for A/H9 (n=9; 95%CI: 4-16)
    - 7% had co-infection with both A/H5 and H9 (n=7; 95%CI: 3-13)
    - 27% for influenza A/untypable (n=28; 95%CI: 19-36).

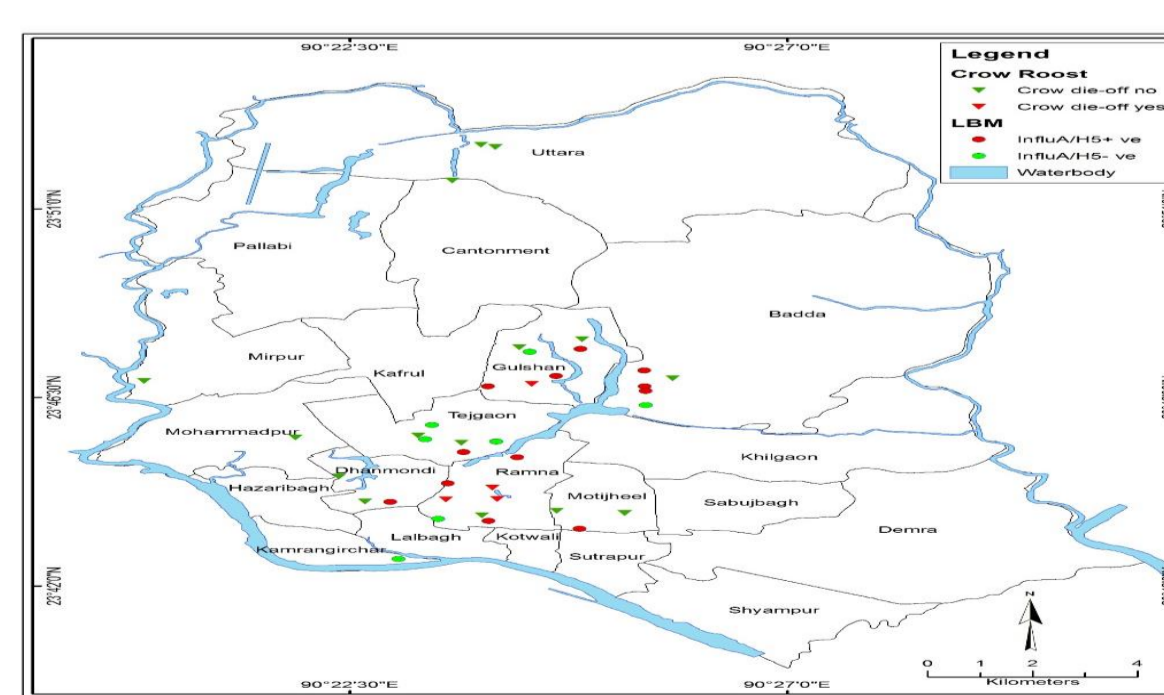


Figure 6: Spatial distribution of AI in crow roosts and LBMs

Table 1: Prevalence of AI from crow samples of Dhaka

	Number of crows	Inf. A (%)	95% CI	Inf. A/H5 (%)	95% CI	Inf. A/Untypable (%)
Mohakhali wireless	89	80 (90)	82-95	75 (84)	75-91	5 (6)
Dhaka University	16	16(100)	79-100	16 (100)	79-100	-
Ramna Park	50	45 (90)	78-97	45 (90)	78-97	-
Sohrawardy Uddan	16	13 (81)	54-96	13 (81)	54-96	-

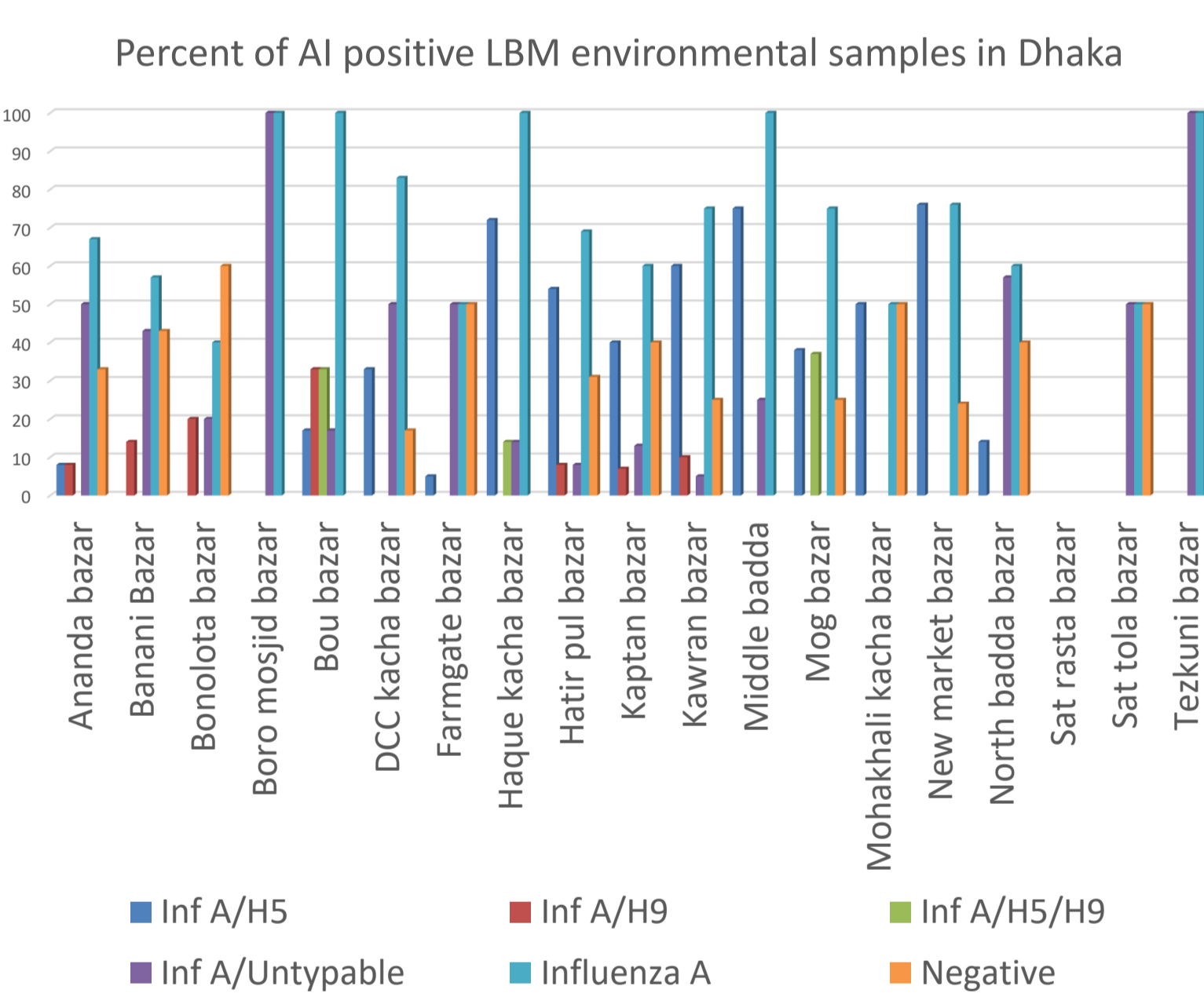


Figure 7: Prevalence of AI positive LBM environmental samples in Dhaka

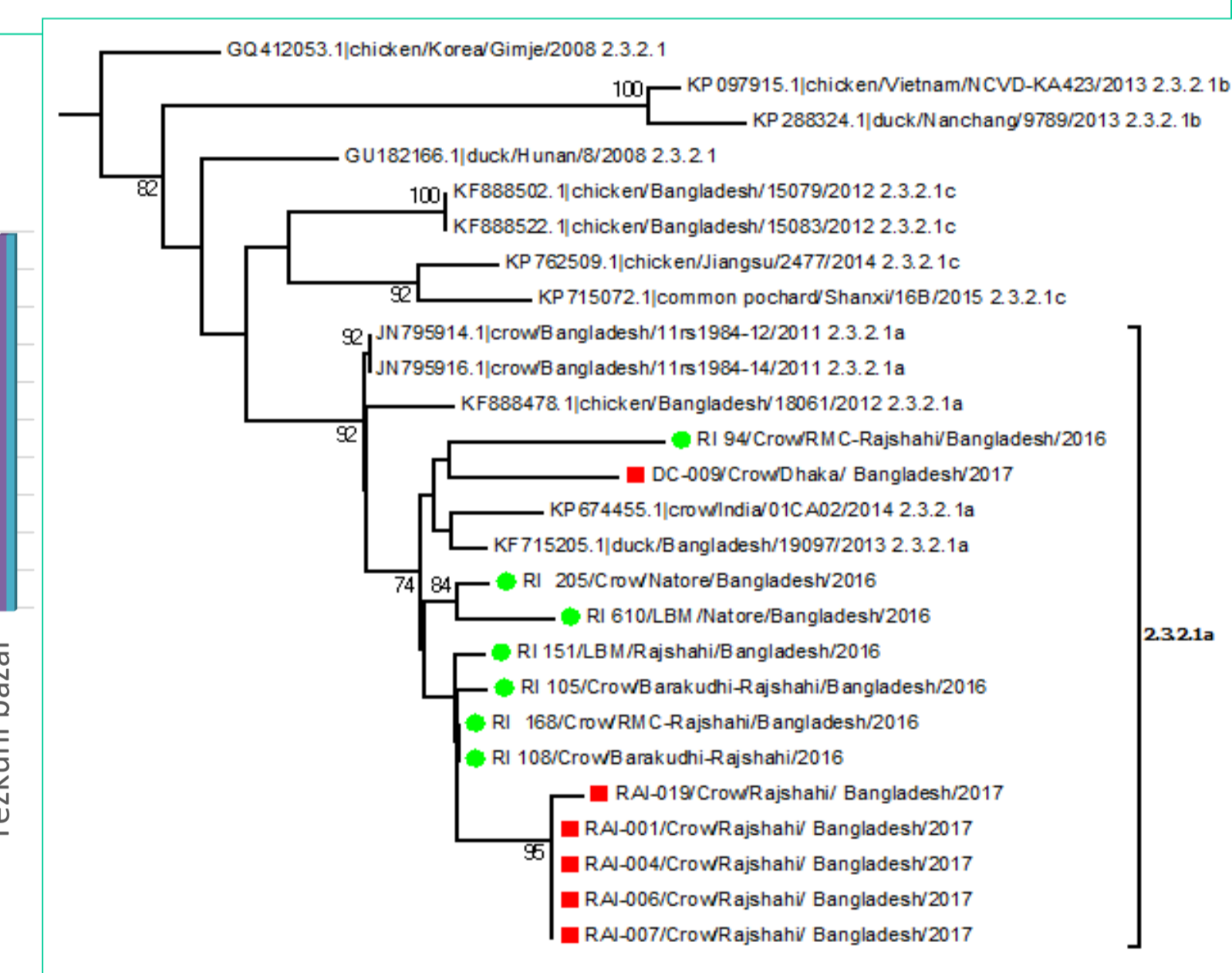


Figure 8: Phylogenetic tree analysis of HA & NA genes of AI isolated from crow, Dhaka

## Conclusions

- Data findings suggest that highly pathogenic AIV is present in the LBMs in Bangladesh, and may have been transmitted to crows while they were feeding on poultry waste in the LBM.
- Bats may have acquired infection while roosting with the infected crows.
- These findings improve our understanding of avian influenza viral diversity in a key wild bird species.
- We also report the unusual finding of bat mortality potentially linked to HPAI infection.
- Improved LBMs biosecurity measures are needed to reduce the risk of influenza virus spillover to wild birds or people in Bangladesh.

## Acknowledgement

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