

One Health Investigation of a Crow mortality event linked to Potential Circulation of Highly Pathogenic Avian Influenza Virus at Live Bird Markets in Northwestern Bangladesh

Ariful Islam¹, Shariful Islam^{1,2}, Mohammed Abdus Samad³, Mallick Masum Billah², Faisal Talukdar², Md Enayet Hossain⁴, Md Ziaur Rahman⁴, Md Rezaul Karim³, Melinda K. Rostal¹, Emily Hagan¹, ASM Alamgir², Peter Daszak¹, Jonathan H Epstein¹, Meerjady Sabrina Flora²

1. EcoHealth Alliance, New York, USA, 2. Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh 3. National Reference Laboratory for Avian Influenza (NRL-AI), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, 4. International Centre for Diarrhoeal Disease Research, Bangladesh

Introduction

- Highly pathogenic avian influenza (AI) viruses cause extremely rapid deaths in wild, non-anseriformes birds.
- Epidemiologically linked with diet, foraging behavior, habitat use, migratory pattern, population size, and frequency of aggregation.
- In January 12-18, 2017 there were multiple mass media reports on crow die offs in the vicinity of Rajshahi Medical College Hospital (RMCH), Bangladesh.
- Reports of crows suddenly falling out of trees, unable to fly, torticollis and mortality.
- A multi-disciplinary One Health team responded to the outbreak.
- Objectives:** To identify the etiologic agent, characterize the extent of the outbreak, and identify the source of the infection as well as possible associated human infections.



Figure 1: Crow roost



Figure 2: Dead crows

Methods

Outbreak Investigation:

- The crow die-off was reported from RMC Hospital areas.
- The wildlife team surveyed crow roosts in the area and observed crows feeding on poultry offal and wastage in neighboring Live Bird Markets (LBMs) and roosting with other species of wild birds.
- The investigation included sampling crows, LBMs (environmental samples, offal), poultry feces on farms and patients as part of existing hospital based influenza surveillance in RMCH.



Figure 3: Crows feeding at LBM



Figure 4: Collecting samples from dead crows

Data and sample collection procedures:

- The clinical presentation of a subset of infected crows was recorded.
- The team collected cloacal and oral-pharyngeal swabs from moribund and dead crows (N=58), environmental samples from 12 LBMs (N=137) and 14 poultry farms (N=65) as well as samples from 28 Severe Acute Respiratory Infection (SARI) patients at the hospital.
- During sampling appropriate personal protective equipments were used.

Laboratory testing:

- All specimens were tested at icddr,b and BLRI using real-time RT-PCR.
- After screening for influenza A (matrix (M) gene), all positive specimens were further subtyped as H5, H7 and H9 and N1
- Characterization of the Hemagglutinin (HA) surface protein was completed by phylogenetic analysis.
- A ct value of 35 was used as the cut-off point for positivity.
- All crow samples were tested using consensus PCR for Flavivirus.
- PCR products were sequenced to confirm positive PCR results.

Results

- Dead and moribund crows
 - 91% (n=58; 95%CI:81-97) positive for A/H5N1
 - 2% (n=1; 95%CI: 0-9%) for influenza A/untypable.
- Among LBMs samples
 - 45% positive for influenza A (n=61; 95% CI: 36-53)
 - Among influenza A positive samples
 - 59% positive to H5N1 (n=36; 95% CI: 46-71)

- 10% positive for A/H9 (n=6; 95% CI: 4-20)
- 5% had a co-infection with both A/H5 and H9 (n=3; 95% CI: 1-14)
- 26% for influenza A/untypable (n=16; 95% CI: 16-39).

- All crow samples were negative for H7, H9 and Flavivirus.
- None of the LBM samples were positive for H7.
- Crows were observed feeding on poultry waste at the LBM and roosting with other species of wild birds.
- Phylogenetic analysis based on partial sequence of the HA gene suggests it is similar to the Bangladeshi 2.3.2.1a clade, being circulated from 2011.
- All the human samples and poultry farm samples were negative for influenza A.

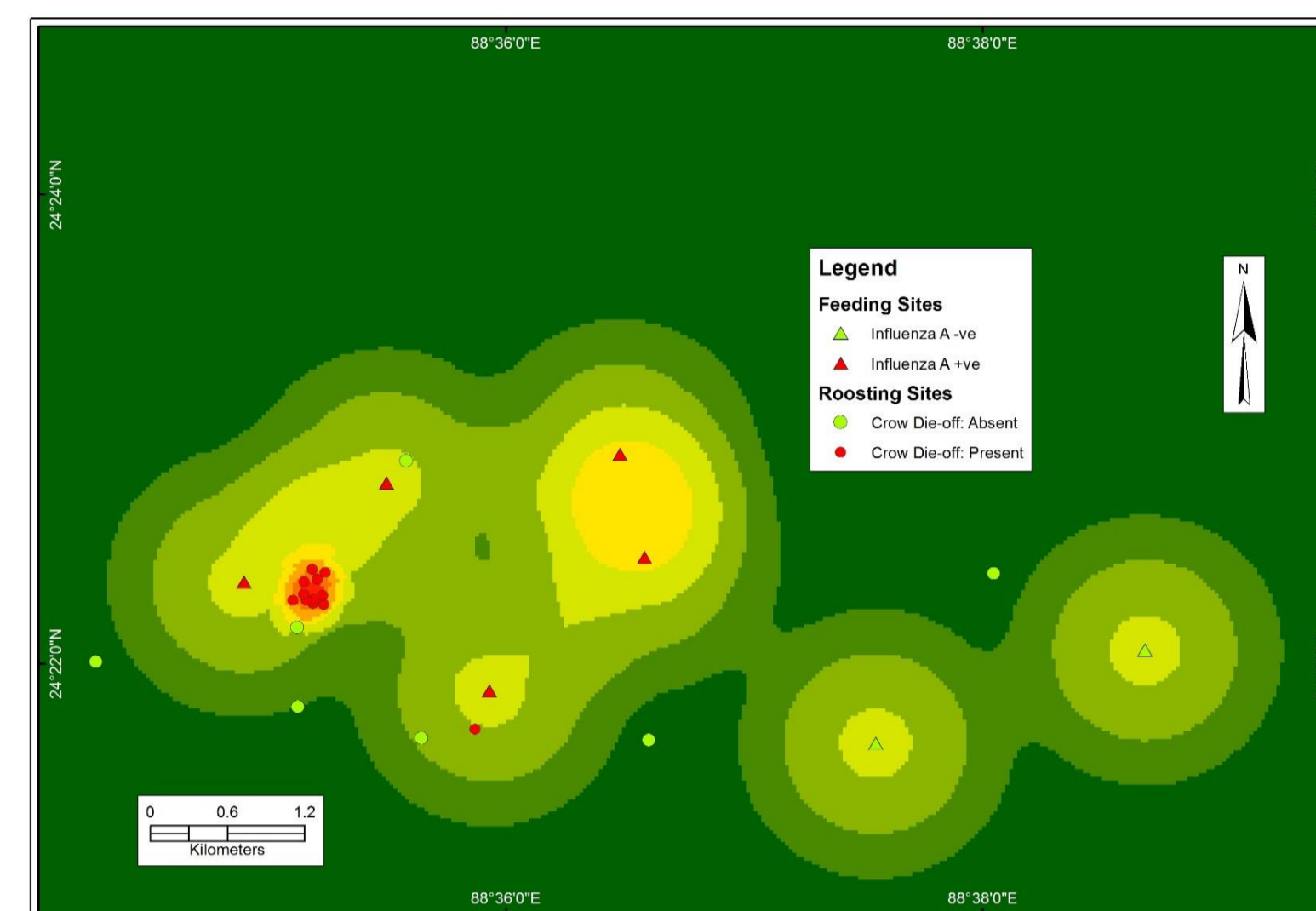


Figure 5: Influenza +ve roosts are centralized around the RMC campus and density of +ve roosts is decreasing with periphery of the RMC areas and nearest feeding sites are +ve for influenza

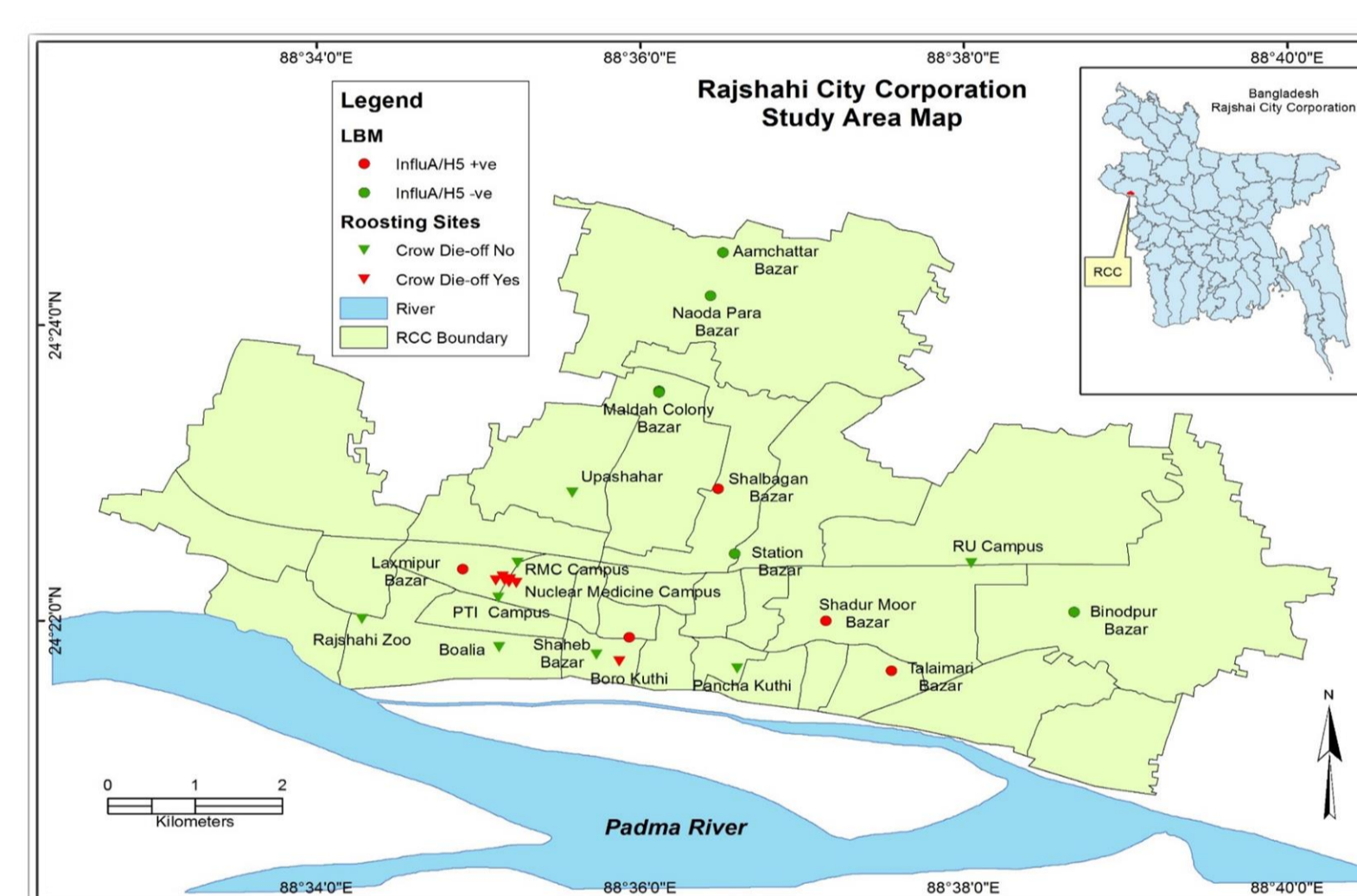


Figure 6: Distribution of AI at crow roosts and LBMs in Rajshahi

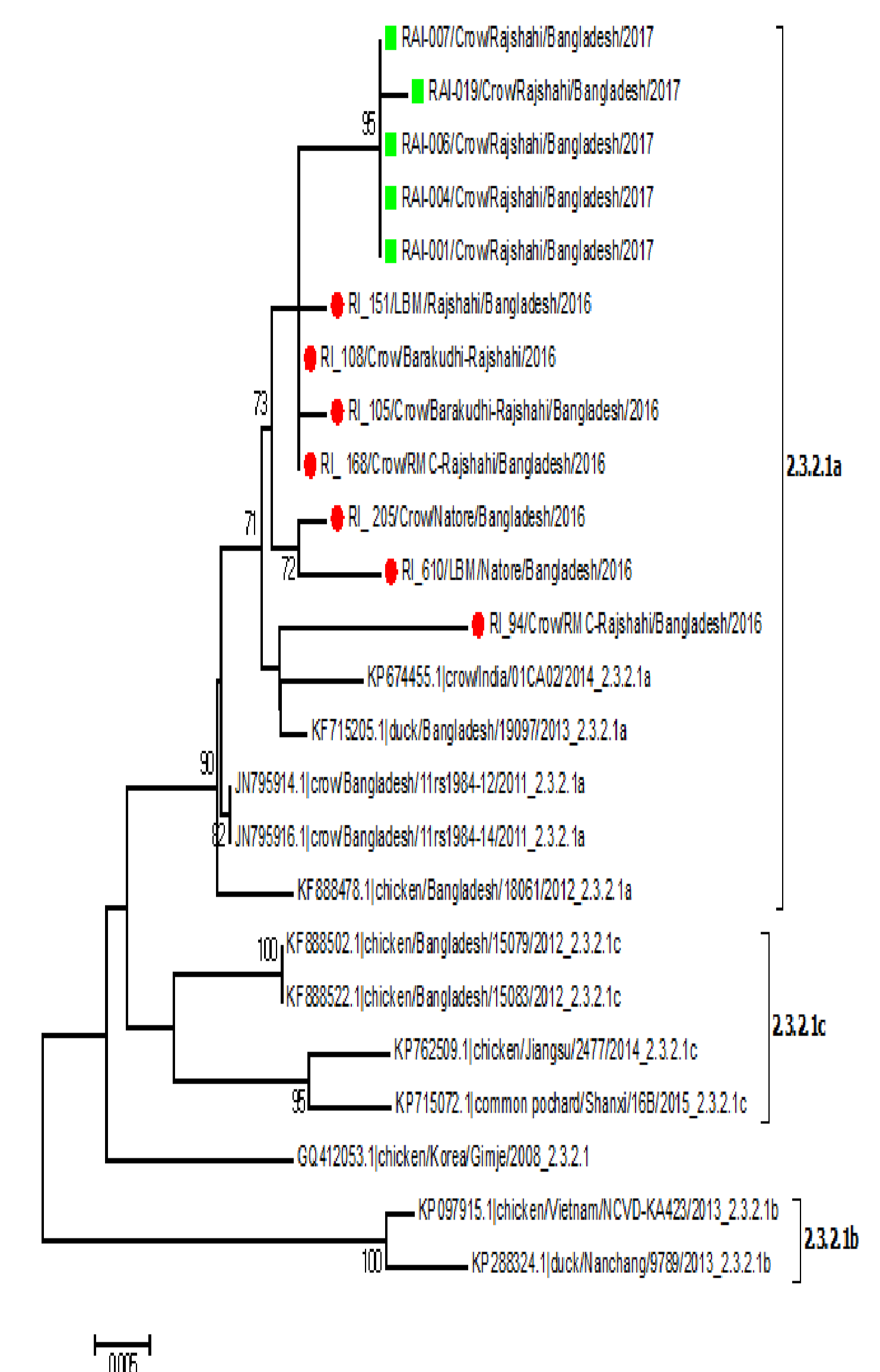


Figure 7: Phylogenetic analysis of HA gene sequences of AI from dead crows of Rajshahi

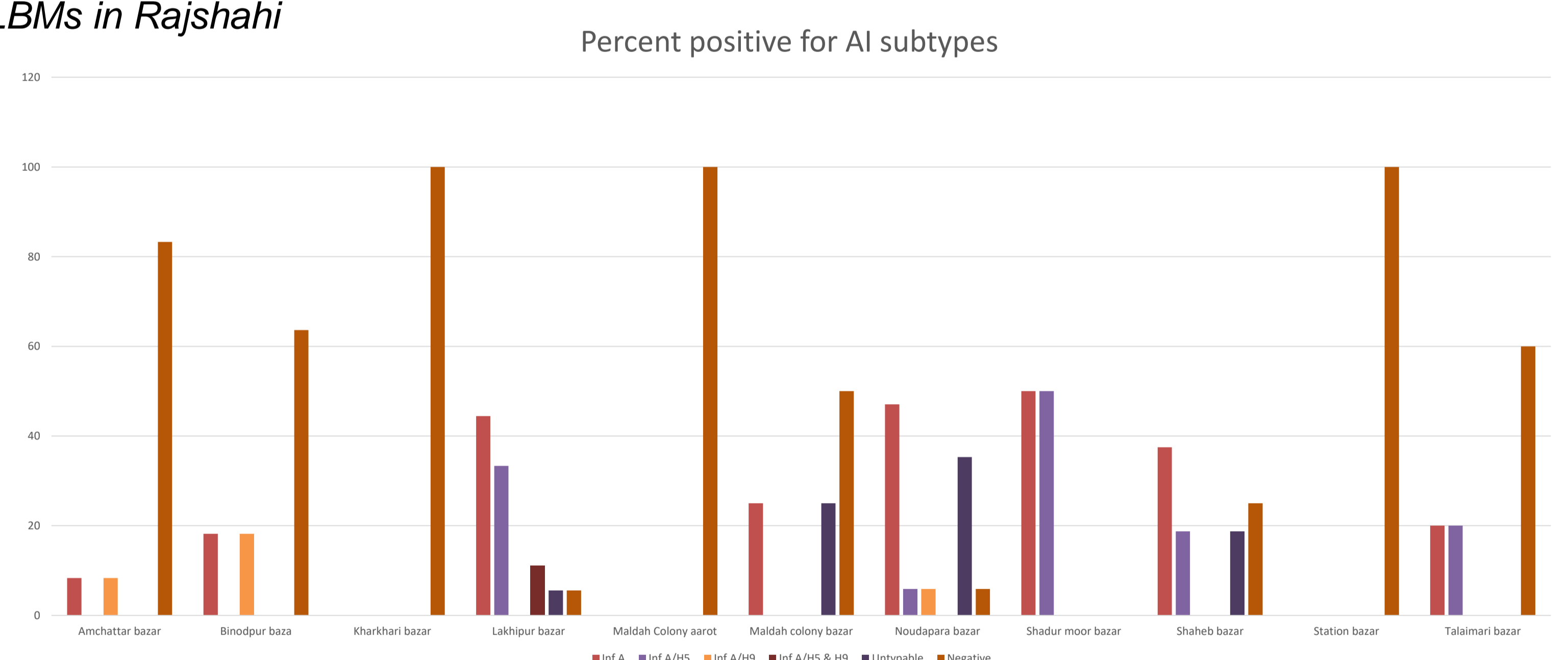


Figure 8: Prevalence of avian influenza in LBM

Conclusions

- This study provides evidence of an influenza contaminated LBM environment. Suggesting that influenza viruses are circulating in LBMs and crows may have been infected by consuming dead poultry and their waste.
- Continued surveillance for AI in wild birds and live bird markets is recommended to better understand the evolution of influenza viruses, transmission pathways, and potential sources of infection in crows and the risk of spillover of highly pathogenic influenza viruses to people.
- Improved LBM biosecurity measures are needed to reduce the risk of influenza virus spillover to birds or people in Bangladesh.

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