A CONTROLLED FIELD EXPERIMENT TO EXPLAIN AMPLIFICATION OF AVIAN INFLUENZA VIRUSES ALONG POULTRY MARKETING CHAINS IN BANGLADESH

L. Kohnle[1,2], T. Das[3], Md. H. Uddin[4], S. C. Nath[4], Md. A. S. Mohsin[4], R. Mahmud[4], P. K. Biswas[3], Md. A. Hoque[4], D. U. Pfeiffer[1,5], G. Fournié[5]

[1]City University of Hong Kong, Department of Infectious Diseases and Public Health, Hong Kong, China ▪ [2]European Food Safety Authority (EFSA), Parma, Italy ▪ [3]Chattogram Veterinary and Animal Sciences University, Department of Microbiology and Veterinary Public Health, Chattogram, Bangladesh
[4]Chattogram Veterinary and Animal Sciences University, Department of Medicine and Surgery, Chattogram, Bangladesh ▪ [5]Royal Veterinary College, Department of Pathology and Population Sciences, Hatfield, United Kingdom

Background

- The H9N2 and H5N1 subtypes of avian influenza A viruses are endemic in the Bangladeshi poultry population.
- Live bird markets [1] showed a more than tenfold higher prevalence than production sites upstream of marketing chains [2], especially for chickens. This suggests an amplification of avian influenza viruses along poultry marketing chains.
- Chickens may not spend enough time in market stalls to get infected and start shedding avian influenza viruses in the live bird market [3].

We therefore hypothesise that the high prevalence observed in live bird markets results from infection of chickens upstream of marketing chains.

Methodology

STUDY DESIGN

To test our hypothesis, we designed and incorporated a marketing chain configuration (the 'intervention') into a controlled field experiment. The primary response variable was the infection status of chickens, which was measured by diagnostic testing of oropharyngeal swab samples at bird level. We assessed the effect of the intervention on reducing viral shedding in the live bird market by comparing the proportions of positive chickens between intervention and control groups. In the first part (Figure 1a), for each of these batches, five birds were purchased from either a farm (in case of broilers) or rural household (in case of backyard chickens), sampled and exposed to the intervention (the 'intervention group'). We transported each intervention group to our research facilities and stored the birds for 2.5 days before delivering them to the live bird market. We did not mix birds of different origins and rigorously implemented high levels of biosecurity along all stages of the configurated marketing chain. Having undergone the usual transport and trade processes, another five birds were purchased from traders on arrival at the live bird market (the 'control group'). Both groups then were sampled, matched and caged together in a market stall. In the second part, we followed and serially sampled each batch over a period of 84 hours (Figure 1b). Stallholders of the respective market stalls were instructed to store our birds over the entire period of time and not to treat them differently than other poultry in the live bird market.

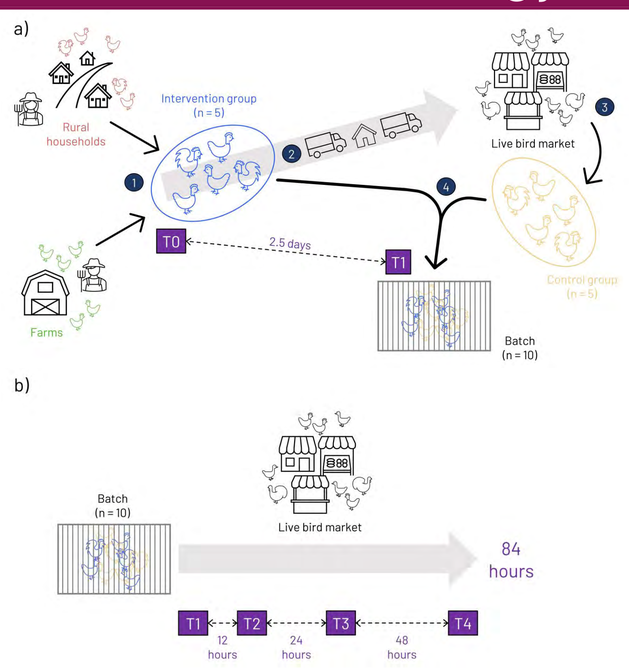

Figure 1. Structure and components of the field experiment. a) first part: (1) recruitment of intervention groups and pretest, (2) transport and storage of intervention groups, (3) recruitment of control groups, (4) posttest and matching of both groups; b) second part: serial sampling.

OBJECTIVE

We aimed to identify the stage of poultry marketing chains at which amplification of avian influenza viruses occurs. By implementing a marketing chain configuration, we assessed whether reducing the risk of infection along all stages reduces viral shedding of chickens in market stalls.

LABORATORY ANALYSIS

Sample processing → Long-term storage at -80 °C

Viral RNA extraction

RT-qPCR → M, H9 and H5 genes (positive if Ct < 40)

STATISTICAL ANALYSIS

- Descriptive statistics
- Effect of the intervention: Generalised linear mixed models (T1) and conditional logistic regression models (T2-T4) for each of the three genes and both strata separately
- Time until first positive test result by RT-qPCR after entering the live bird market (for each of the three genes): discrete-time survival models

Results

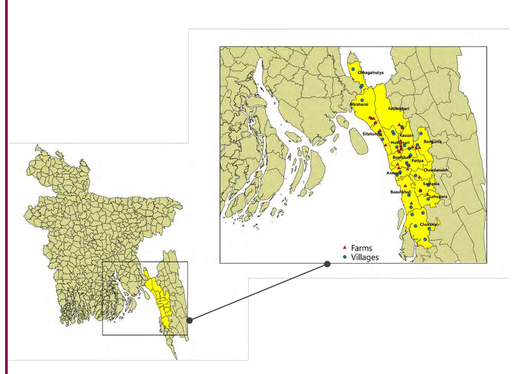

Figure 2. Map of visited sub-districts ('upazilas') (yellow) in 3 different districts of Chattogram Division, Bangladesh. Backyard chickens were purchased from rural households (blue circles), while broilers were purchased from farms (red triangles).

- Few chickens entered the live bird market already shedding avian influenza viruses (12.9% and 6.8% for Ct < 40 and Ct < 33, respectively), while bird-level prevalence substantially increased over time with almost all chickens being positive for the M (92.4% and 86.7% for Ct < 40 and Ct < 33, respectively) and H9 genes (86.1% and 79.6% for Ct < 40 and Ct < 33, respectively) after 84 hours in the live bird market (i.e. at T4).
- Backyard chickens generally showed a slower increase in bird-level prevalence, which did neither reach the same peak levels nor a plateau after 36 hours (i.e. after T3) as it was observed for broilers. However, more backyard chickens than broilers entered the live bird market at an already advanced stage of infection (Ct < 40 for the M gene): 15.3% compared to 9.7% for backyard chickens and broilers, respectively.
- When considering a cycle threshold of 40, there were significant differences in the odds of a chicken testing positive for the M (T2) and H9 (T4) genes between intervention and control groups (OR = 0.47, 95% CI [0.23, 0.99] and OR = 0.27, 95% CI [0.13, 0.55]). These differences became even more significant (OR = 0.43, 95% CI [0.24, 0.78] and OR = 0.29, 95% CI [0.16, 0.51]) when considering a cycle threshold of 33. Moreover, in case of the lower cycle threshold, the odds of retrieving a positive test result for the M (T4) and H9 (T2) genes were significantly lower for chickens in the intervention than in the control group (OR = 0.34, 95% CI [0.17, 0.69] and OR = 0.46, 95% CI [0.25, 0.85]).

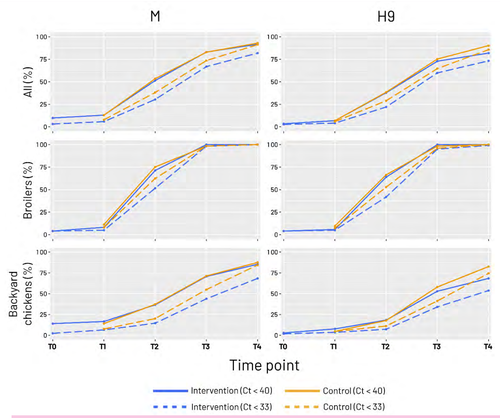

Figure 3. Temporal trend of bird-level prevalence (M and H9 genes) for intervention and control groups considering two different cycle thresholds.

Discussion & Conclusions

- We demonstrated that a substantial proportion of the prevalence of avian influenza viruses observed in Bangladesh's live bird markets results from infection of chickens upstream of marketing chains.
- We showed that reducing the risk of infection during transport and trade reduces viral shedding of chickens in the live bird market.

Trade and transport networks should therefore be targeted to complement already existing prevention and control strategies in live bird markets and farms.

Contact

Lisa Kohnle[1,2], DVM, MSc (One Health)
[1]Department of Infectious Diseases and Public Health, City University of Hong Kong, Hong Kong, China
[2]European Food Safety Authority (EFSA), Parma, Italy
☎ +39 3452105169
lisa.kohnle@my.cityu.edu.hk / lisa.kohnle@efsa.europa.eu

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Jockey Club College of Veterinary Medicine and Life Sciences
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