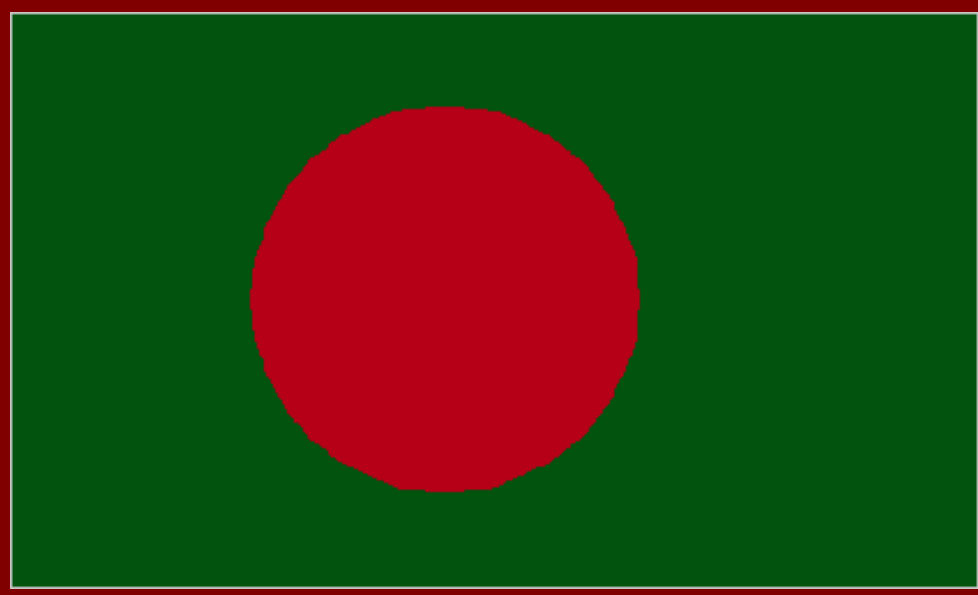


EPIDEMIOLOGICAL ASSESSMENT OF HYGIENIC CONDITIONS OF LIVE BIRD MARKETS ON AVIAN INFLUENZA IN CHITTAGONG METROPOLITAN CITY, BANGLADESH

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ABSTRACT

Live Bird Market (LBM) is a place where live bird traders and consumers are interacted in respect to live bird trading. The environment of LBM might be contaminated with various infectious diseases like Avian Influenza Virus. No systematic study has previously been attempted for investigation of Avian Influenza status in LBM which could pose threats to the economy and public health. Therefore, a cross sectional study was conducted on hygienic status and Avian Influenza in LBMs under Chittagong Metro in Bangladesh. The overall objective of the study was to assess the LBM demographic information and hygienic status in contrast with AI prevalence followed by subtype distribution and associated risk factors. A total of 290 pooled environmental samples along with questionnaire based identity information, participant's demography, market structure followed by management and hygienic status based data were obtained from 290 stalls under 40 different LBMs. At each stall swab samples were collected from up to 9 different sites. The samples were evaluated by Real time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) for detection of M gene followed by subtypes of H5, H7 and H9. The prevalence of AIV in LBM was 40% (95% CI: 20-60%; N=40) whereas the prevalence of avian influenza was 20.3% (95% CI: 10-30%, N=290) at stall level. Again, the prevalence of H5, H7 and H9 at stall level was 2.8% (95% CI: 1-5%), 0% and 3.1% (95% CI: 1-6%) respectively. Generalized Estimating Equation was applied to identify potential risk factors associated with Avian Influenza in LBMs. Selling of species (OR=2.5: Chicken and non-duck species versus Duck with other species.), Bird holding area (OR=1.9: Cage versus Floor) and Hygienic score (OR=3.1: Score 3 or more versus score less than 3) were identified as the risk factors for AI in LBMs. The present study has been identified the risk factors associated with the occurrence of AIV at stall level of LBMs. Knowledge obtained from this study could provide new understanding of the distribution and transmission of AI through LBM in Bangladesh. The findings could be used to develop a proof based programme concerning environmental sanitation along with development of a strong surveillance system to reduce the AI transmission through LBMs in Bangladesh.

LBM OF BANGLADESH



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INTRODUCTION

Small scale Live Bird Markets (LBM) are commonly seen in every town of Bangladesh whereas wholesale and larger retail LBMs are in larger cities

Majority of LBMs are located mostly in open air markets with exposure to outdoor environmental factors and contaminants

Different species of birds enter into LBMs from different sources through a complex transaction chain, infectious diseases like avian influenza can be easily introduced

Infectious organisms are amplified in the market environment and disseminated across different populations (bird-bird-human) (Cardona et al., 2009)

Many previous AIV studies have used live bird samples to evaluate AIV status in LBMs (Garber et al., 2007)

Environmental sampling is the best option to assess the hygienic status associated with the detection of AIV in LBMs (Indriani et al., 2010; Biswas et al., 2015)

Pooled environmental swab samples from all possible contamination sites of a stall across all 40 LBMs has been collected for this study

Very few previous studies determined potential risk factors for the occurrence of AIV at LBM or stall level (Indriani et al., 2010; Zhou et al., 2015) rather than the proper estimation of hygienic condition

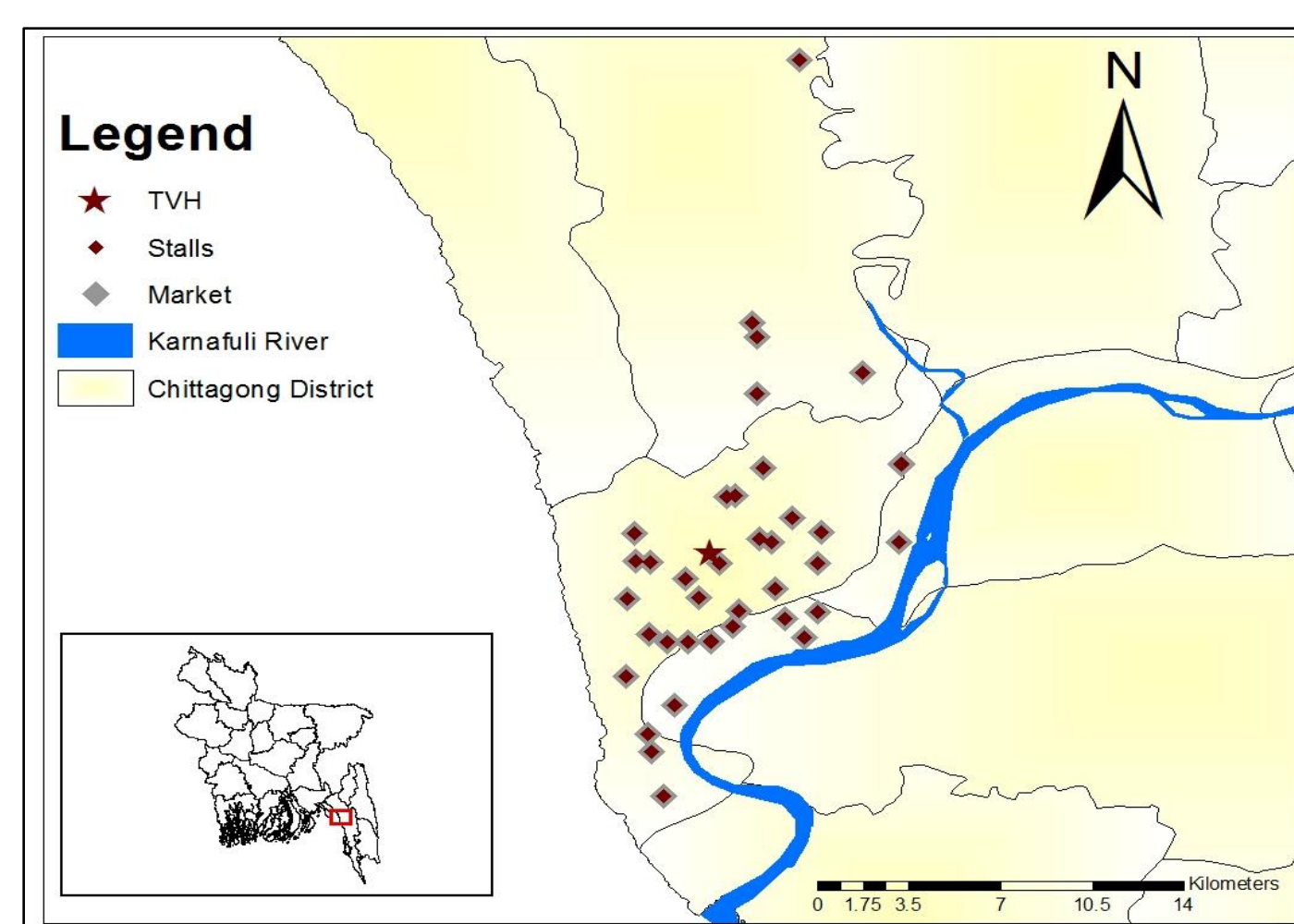
Therefore this study has been conducted targeting the following objectives

Objectives:

- Evaluate the LBM demography, hygienic status, prevalence of AIV and the selective subtypes of H5, H7 and H9 at stall and LBM levels in the CMA
- Determine potential risk factors associated with the occurrence AIV at stalls of LBMs in the CMA

METHODS AND MATERIALS

Study sites: Tropical zone; 22°22'N and 91°48'E; 29 m up from the sea level; Temperature of 13°C to 32°C and Humidity of 70 to 85%



Study type: Cross sectional study

Study population: Poultry stalls (n=398) under 40 LBM of Chittagong Metro.

Sample size: 290 stalls (12% expected prevalence; ±1% precision; 95% CI; 1% design effect)

Sampling strategy: 100% stalls were sampled if a market had ≤10 stalls, whereas 50% stalls were sampled if a market had >10 stalls

Collection, Preservation and Transportation of Samples:

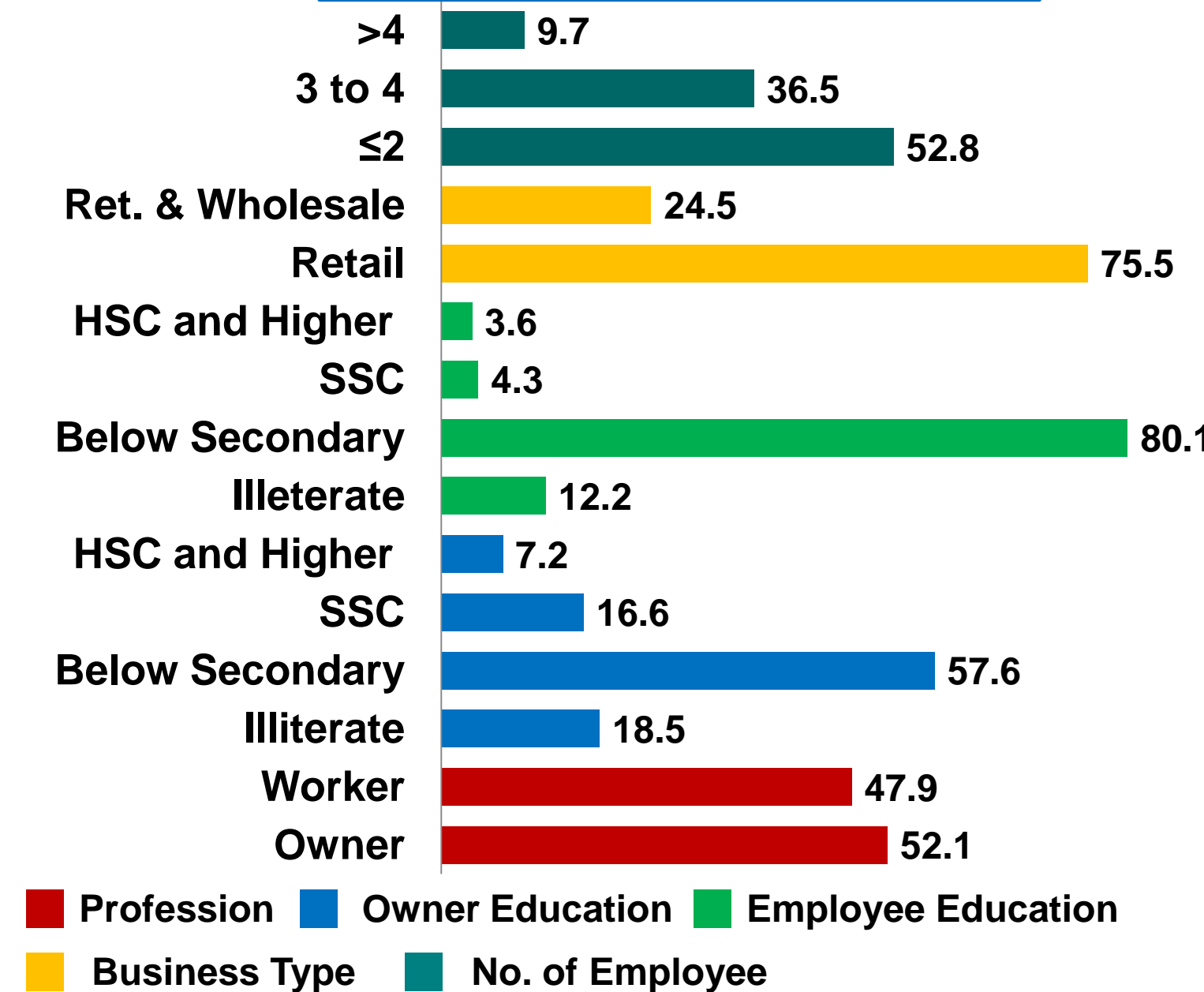
- Pooled environmental swab samples (up to 9) per stall
- Placed in 15 ml sterile Falcon tube containing 5 ml VTM
- Falcon tubes with samples were placed in insulated ice-box and transferred to laboratory
- 2 aliquots per sample with a volume of 2 ml each were made in the lab
- One aliquot was stored in -80°C at the Laboratory
- Another aliquot was forwarded to the National Reference Lab. for Avian Influenza at Bangladesh Livestock Research Institute
- Molecular evaluation was performed by rRT-PCR

Data collection: Questionnaire, interview and observation

Statistical evaluation: STATA 13 Descriptive and Generalized Estimating Equation Model were performed

RESULTS

Graph 1: Stall demography (%)



Graph 2: Stalls hygienic status (%)

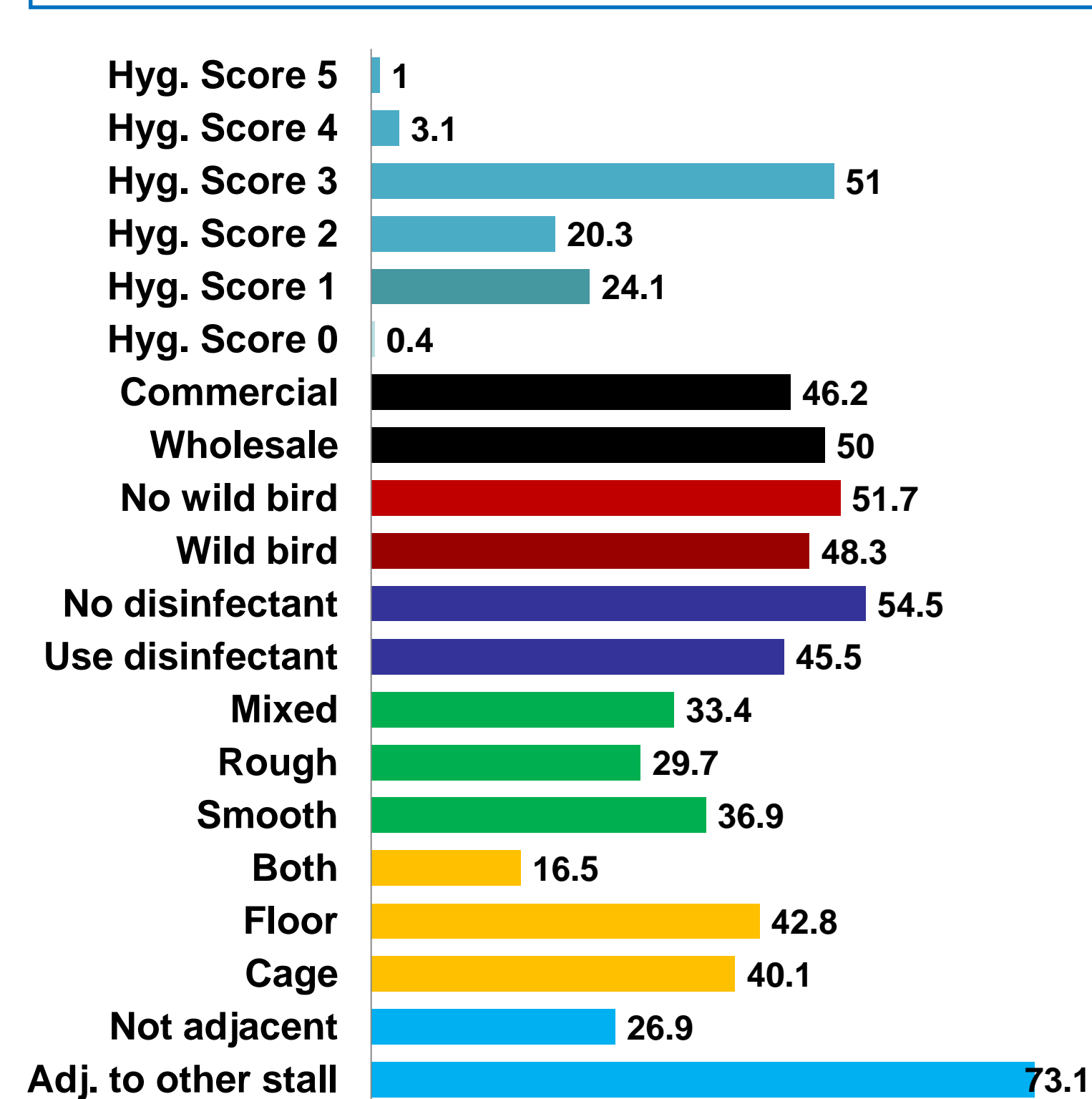
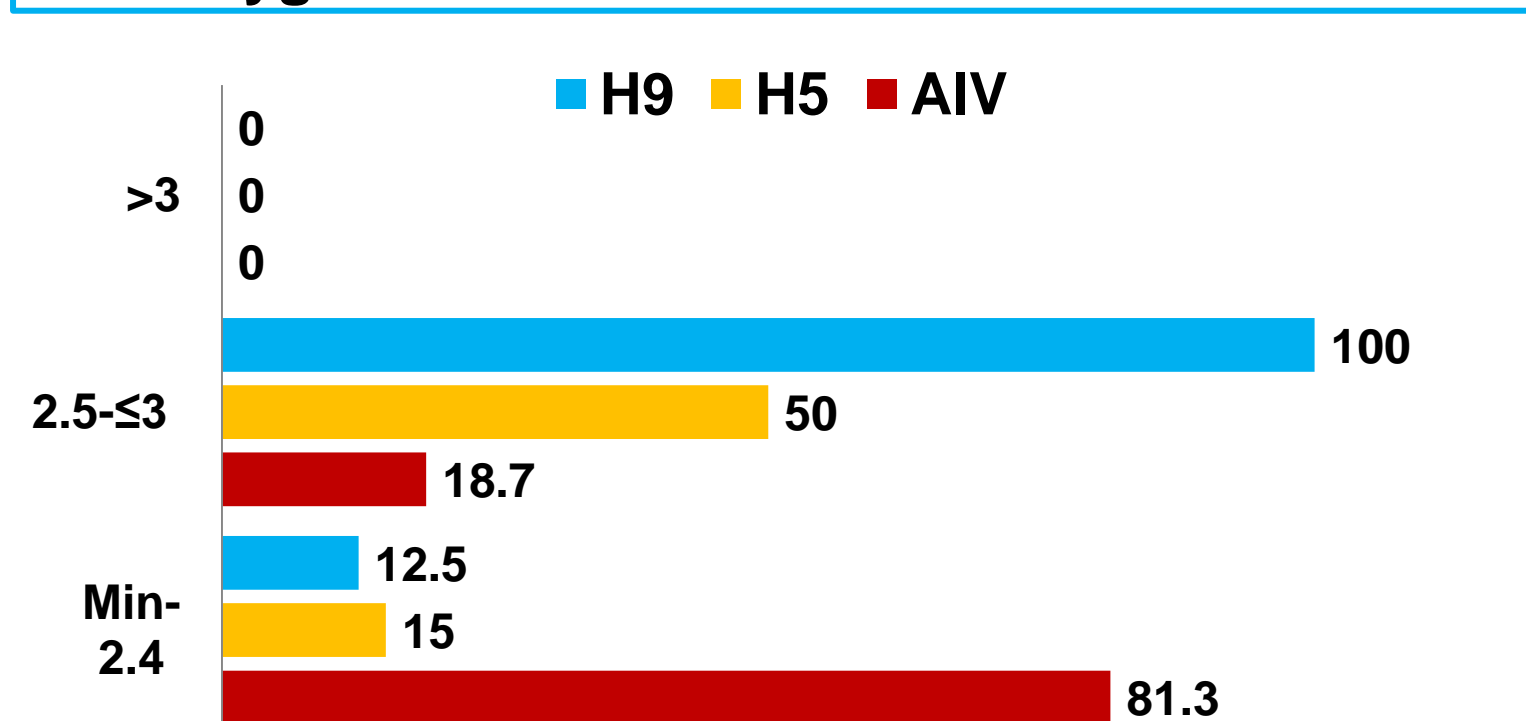


Table 1: Distribution of Avian influenza and its subtype specific prevalence by different classes of poultry species traded

Species	No. of Stalls	AIV %	H5 %	H9 %
BC only	80	22.5	2.5	1.3
BC and LC or DC	106	13.2	1.9	2.8
BC, LC and DC	57	5.2	1.8	1.8
BC, DC, LC and P/D	28	10.7	---	---
BC, LC, DC, D and P/Q	58	34.5	5.2	5.2
Only DC	2	50.0	---	50

Graph 3: AIV/H5/H9 prevalence (%) in contrast to mean hygienic score at LBM level



Graph 4: AIV/H5/H9 prevalence (%) at Stall and LBM level

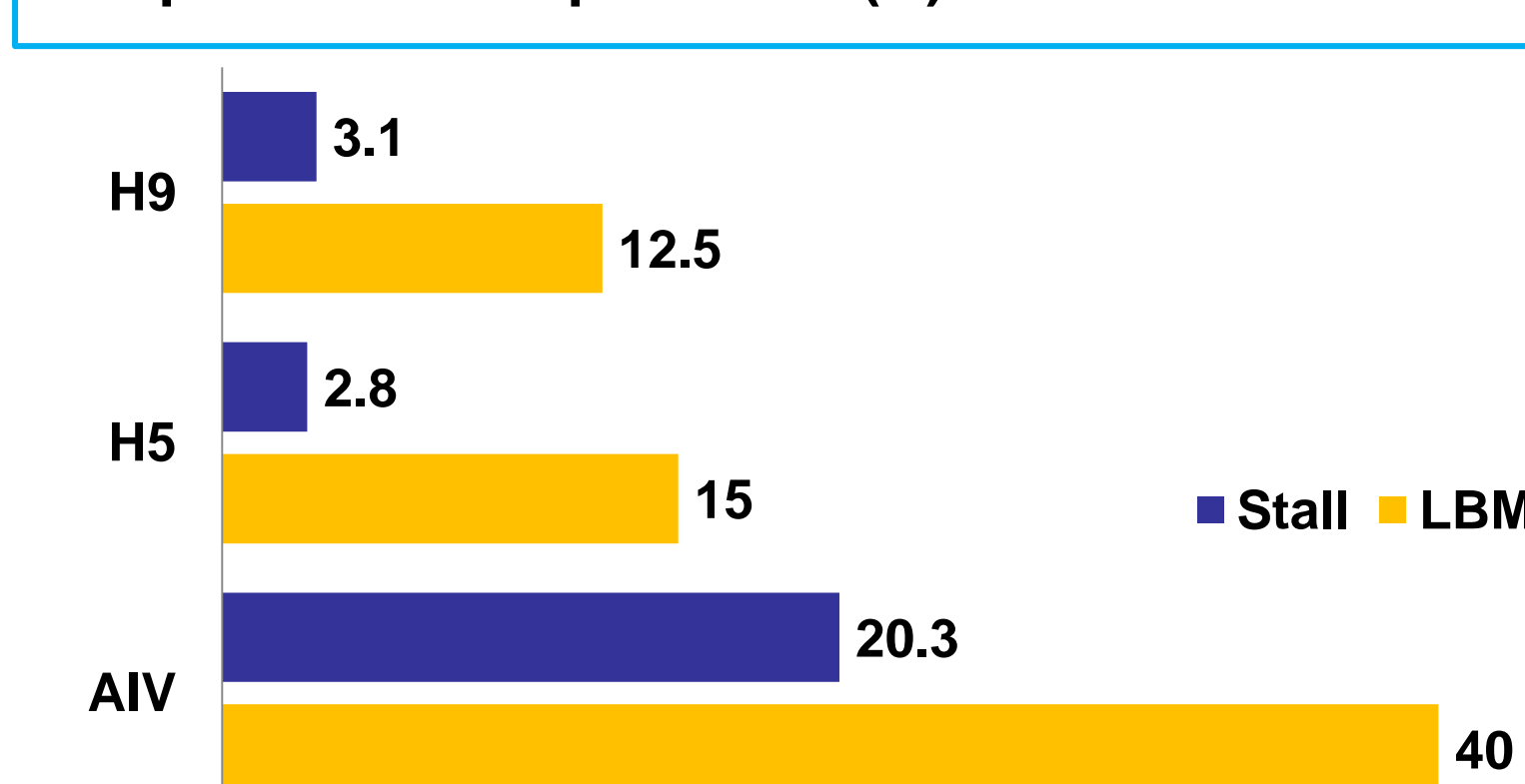


Table 2: Outputs of Generalized Estimating Equation model

Factors	Category	OR	95% CI	p-value
Selling of species	Chicken and non-duck species	1.0		
	Duck with other species	2.5	1.5-4.1	<0.001
Bird holding area	Cage	1.0		
	Floor	1.9	1.1-3.4	0.03
Hygienic score	≥3	1.0		
	<3	3.1	1.7-5.6	<0.001

DISCUSSION

Live Bird Market demography and hygienic status

- More than 50% stall vendors had a HX level of education. So, it would be efficient to educate them on LBM hygiene by providing leaflets or manuals
- A single type of poultry stall and business may limit the spread and contamination of organisms
- Muddy floors of the stall produces unhygienic condition and could be a source of infectious agent
- Poultry stalls having mixes of different species from different sources which create an environment for infectious pathogens to transmit from one bird species to another and also from birds to humans
- Wild birds access may likewise permit roundabout transmission of viral pathogens such as AIV

AIV can be inactivated using detergent treatment, whereas 50% stalls use only water as cleaning agent so consciousness should be build up among the poultry market authority and stall vendors

Avian Influenza prevalence and risk factors

- The prevalence of AIV at LBM level was 40% and stall level was 20.3%, represents the high levels of AIV prevalence in LBMs of Bangladesh
- Selling of ducks alongside other species identified as a risk factor (OR=2.5) for AIV agrees with an earlier study (Zhou et al. 2015)
- Bird holding system (Floor/Cage) in the stall identified as a risk factor for AIV (OR=1.9; Floor versus Cage) that is Supported by (Samaan et al., 2011)
- Low hygienic score (below 3) contributed significantly (OR=3.1) for AIV supported by Fournié et al., (2013)
- Sub-type specific prevalence (H5, H7 & H9)
- AIV prevalence at stall level (2.8% H5 & 3.1% H9) seems a new finding as many previous studies conducted at LBM level (Negovetich et al., 2011)

CONCLUSIONS

Prevalence of avian influenza and other subtype

LBM level	Stall level
•AI: 40%	AI: 20.3%
•H5: 15.0%	H5: 2.8%
•H7: 0%	H7: 0%
•H9: 12.5%	H9: 3.1%

Risk factors for avian influenza
-duck with other species in stalls, birds keeping on floor and hygienic score of stalls

REFERENCES

- benEmbarek, P., Briand, S., Brown, I., Bruscke, C., Domenech, J., F ormenty, P., Fukuda, K., Hamilton, K., Hay, A., King, L., 2009. FAO-OIE-WHO joint technical consultation on avian influenza at the human-animal interface.
- Biswas, P.K., Giasuddin, M., Nath, B., Islam, M., Debnath, N., Yamag e, M., 2015. Biosecurity and Circulation of Influenza A (H5N1) Virus in Live-Bird Markets in Bangladesh, 2012. Transboundary and Emerging Diseases
- Cardona, C., Yee, K., Carpenter, T., 2009. Are live bird markets reservoirs of avian influenza? Poultry Science 88, 856-859.
- Fournié, G., Guitian, J., Desvaux, S., Cuong, V.C., Pfeiffer, D.U., Ma ngani, P., Ghani, A.C., 2013. Interventions for avian influenza A (H5N1) risk management in live bird market networks. Proceedings of the National Academy of Sciences 110, 9177-9182.
- Garber, L., Voelker, L., Hill, G., Rodriguez, J., 2007. Description of live poultry markets in the United States and factors associated with repeated presence of H5/H7 low-pathogenicity avian influenza virus. Avian Diseases 51, 417-420
- Indriani, R., Samaan, G., Gultom, A., Loth, L., Indriani, S., Adjid, R., Dharmayanti, N.L.P.I., Weaver, J., Mumford, E., Lokuge, K., 2010b. Environmental sampling for avian influenza virus A (H5N1) in live-bird markets, Indonesia. Emerging Infectious Diseases 16, 1889-95
- Negovetich, N.J., Feeroz, M.M., Jones-Engel, L., Walker, D., Alam, S., Hasan, K., Seiler, P., Ferguson, A., Fr iedman, K., Barman, S., 2011a. Live bird markets of Bangladesh: H9N2 viruses and the near absence of highly pathogenic H5N1 influenza. PLoS One 6, e19311-e19311.
- Samaan, G., Gultom, A., Indriani, R., Lokuge, K., Kelly, P.M., 2011. Critical control points for avian influenza A H5N1 in live bird markets in low resource settings. Preventive Veterinary Medicine 100, 71-78.
- Zhou, X., Li, Y., Wang, Y., Edwards, J., Guo, F., Clements, A.C., Hua ng, B., Magalhaes, R.J.S., 2015. The role of live poultry movement and live bird market biosecurity in the epidemiology of influenza A (H7N9): A cross-sectional observational study in four eastern China provinces. Journal of Infection 71, 470-479.

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