Risk Areas for Influenza A(H5) Environmental Contamination in Live Bird Markets, Dhaka, Bangladesh

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We evaluated the presence of influenza A(H5) virus environmental contamination in live bird markets (LBMs) in Dhaka, Bangladesh. By using Bernoulli generalized linear models and multinomial logistic regression models, we quantified LBM-level factors associated with market work zone–specific influenza A(H5) virus contamination patterns. Results showed higher environmental contamination in LBMs that have wholesale and retail operations compared with retail-only markets (relative risk 0.69, 95% 0.51–0.93; p = 0.012) and in March compared with January (relative risk 2.07, 95% CI 1.44–2.96; p<0.001). Influenza A(H5) environmental contamination remains a public health problem in most LBMs in Dhaka, which underscores the need to implement enhanced biosecurity interventions in LBMs in Bangladesh.

Live bird markets (LBMs) have long been identified as major sites for the maintenance, transmission, amplification, and dissemination of influenza A(H5) virus (*1*,*2*). Studies in the United States, China, Indonesia, and Vietnam have shown that LBMs can pose a public health risk for zoonotic spill-over to humans through environmental contamination (*2*–*8*). In Bangladesh, the first evidence of zoonotic transmission of influenza A(H5) virus emerged in 2012; LBMs in Dhaka were considered the main source of exposure for all 3 human cases reported (*9*,*10*). The relatively low level of influenza A(H5) endemicity found in studies conducted in LBMs in Bangladesh since 2012 (e.g., ≤10% prevalence at live bird sampling level) (*11*–*13*) have contributed to a false sense of security regarding contamination risk. Indeed, since 2013, several influenza A(H5) outbreaks in poultry (9 outbreaks), wild birds (5 outbreaks), and humans (2 outbreaks) have occurred in Bangladesh (*14*,*15*). During March 2007–December 2020, Bangladesh reported 556 outbreaks of influenza A(H5) virus in poultry (*14*) and 8 cases in humans (*15*).

Environmental sampling in LBMs for the purposes of avian influenza virus surveillance was first introduced in the United States in 1986 (*16*). A recent study evaluated the effectiveness of environmental sampling for influenza A surveillance and described multiple sampling sites in an LBM (*17*). Earlier studies from Bangladesh primarily focused on collecting samples from market environment sites (such as market floor, stall floor, slaughter area, waste bin, poultry cage, water, fecal material on or underneath the poultry cage, blood, and poultry offal) to understand the LBM environment status for influenza A (*11*,*12*,*18*–*25*).

Few studies to date—1 in Indonesia and 3 in Guangdong, China—have performed simultaneous sampling in different LBM work zones, such as the poultry delivery, poultry holding, poultry slaughter, poultry sale, and waste disposal zones (*26*–*29*). These studies indicated that the poultry slaughter and sale zones were the 2 most contaminated LBM work zones for influenza A(H5N1) in Indonesia (*27*) and influenza A(H7N9), (H5), and (H9) in China (*26*,*28*,*29*). To date, no studies have been performed in Bangladesh on influenza A environmental contamination within different LBM work zones. The results from China and Indonesia have provided additional justification to evaluate the influenza A surveillance program of

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the Food and Agriculture Organization of the United Nations (FAO) in Bangladesh. Given the costs of maintaining influenza surveillance programs, epidemiologic evidence on within-market risk areas for contamination would help fine-tune current surveillance approaches in Bangladesh.

Implementing biosecurity practices in LBMs reduces environmental contamination with influenza A (*30*). For example, weekly market closures (≥1 day) and everyday cleaning and disinfecting interventions were reported to reduce market contamination with avian influenza virus (H7N2) in the United States and influenza (H7N9) and (H9N2) in China (*5*,*31*,*32*). In Bangladesh, improved biosecurity practices at the market level have not effectively reduced environmental contamination for influenza A(H5) virus in Dhaka and Chittagong LBMs during 2012–2014 (*22*,*25*). Since 2014, no study has comprehensively reported the effect of market-level biosecurity practices on the probability of influenza A(H5) environmental contamination in Dhaka. Although the 2 administrative areas of the Dhaka metropolitan area (Dhaka North City Corporation [DNCC] and Dhaka South City Corporation [DSCC]) are known for their distinct demographic and urban features (*33*), no studies to date have investigated how biosecurity practices and influenza A(H5) contamination rates differ in relation to market-level characteristics of LBMs located in different parts of Dhaka. To inform the development of effective environmental sampling strategies for influenza surveillance in LBMs, our study sought to characterize the differences in the proportion of influenza A(H5) environmental contamination in markets in DNCC and DSCC, to identify and quantify market-level factors associated with the probability of influenza A(H5) contamination in specific work zones (i.e., arrival, slaughtering and processing, and consumer exposure or sales), and to identify and quantify market-level factors associated with work zone–specific contamination patterns within LBMs.

Materials and Methods

Study Design for Influenza A(H5) Virus Surveillance in LBMs in Dhaka Metropolitan

We focused our investigation on the Dhaka metropolitan area, which has the highest population density (30,551 residents/km^2) of all metropolitan areas in Bangladesh (*34*). We selected 104 LBMs within metropolitan Dhaka (Figure 1), which were part of the influenza surveillance initiative of the FAO and Department of Livestock Services (DLS) (Appendix, https://wwwnc.cdc.gov/EID/article/27/9/20-4447-App1.pdf) (*35*). Sampling targeted the months of January–March, which are known for a higher level of circulation of influenza A(H5) virus in poultry in Bangladesh (*36*).

We used data on market-level characteristics collected during the Dhaka LBM census to quantify the association between influenza A(H5) environmental contamination in LBMs and within specific market work zones adjusted for market-level characteristics (Appendix). Three market work zones (poultry arrival [A], poultry slaughtering and processing [S], and consumer exposure or sales [E]) and environmental sites in each work zone were selected for sampling on the basis of the findings from Indrani et al. (Appendix) (*27*).

Collection, Preservation, and Transportation of Environmental Samples

Sample collectors from DLS, DNCC, and DSCC performed monthly collection of environmental samples from the selected LBMs. In a given visit, a pool of 6 samples were collected from each work zone using standard polyester-tipped swabs and stored separately in a 3 mL viral transport medium (Becton Dickinson, https://www.bd.com). Pooled samples were kept in ice boxes and transported to the DLS Central Disease Investigation Laboratory and Livestock Research Institute laboratory for temporary storage at 4°C. All samples were then transported in ice boxes to the National Reference Laboratory for Avian Influenza at Bangladesh Livestock Research Institute (Savar, Dhaka) and stored at −80°C before testing.

Laboratory Testing

We tested for influenza A(H5) virus 18-swab pools from each selected market (i.e., 6 swabs/3 work zones) using real-time reverse transcription PCR (rRT-PCR). When an 18-swab pool of a market tested positive, further testing was carried out using rRT-PCR to confirm influenza A(H5) virus in the 6-swab pool of a specific work zone (Figure 2). We used MagMAX viral RNA isolation kit and KingFisher mL Purification System extractor (ThermoFisher Scientific, https://www.thermofisher.com) for RNA extraction. The rRT-PCR testing protocols followed the procedures recommended by the Australian Centre for Disease Preparedness quality assurance manual with influenza A(H5) primers (IVA D148 H5, IVA D149 H5, IVA D204f, and IVA D205r) and probes (IVA H5a and IVA D215P) produced at Australian Animal Health Laboratory and AgPath-ID One-Step RT-PCR Reagents (ThermoFisher Scientific). A pool sample was considered positive for influenza A(H5) if the cycle threshold value was <40 (*37*).

Data Analyses

Our study included markets with information on both infection status and market-level characteristics (n = 97) and those with information on market-level infection status only (n = 7). In our analyses, we considered 2 outcomes of interest: presence or absence of influenza A(H5) virus environmental contamination in specific work zones and LBM–level zone-specific influenza A(H5) environmental contamination patterns. Work zone–specific environmental contamination patterns were classified as negative if all 3 work zones tested negative; ASE–positive when all 3 work zones tested positive; S only–positive when only the slaughtering and processing zone tested positive; SE– or AS–positive when the slaughtering and processing zone and 1 other work zone (E or A) tested positive; and other when the market tested positive for A only, E only, or both A and E.

We summarized DNCC and DSCC market-level biosecurity characteristics by using descriptive statistical analyses. Market-level biosecurity characteristics considered in the investigation included market location, market type, species sold, number of vendors, number of poultry species sold, dominant species (by comparing the poultry headcount), poultry headcount, electricity in the facility, presence of roof, running water in the facility, sale of poultry to other vendors, weekly market closure ($\geq$1 day), direct sale of poultry to consumers, sale of products other than poultry (i.e., fish, red meat, vegetables, groceries), daily cleaning protocol (at minimum with detergent), poultry slaughtering locations, and number of slaughtering facilities. We used a univariable Fisher exact test with a significance level of $p<0.05$ to identify differences in influenza A(H5) recovery by the geographic location of Dhaka markets. We then ran Bernoulli generalized linear models and multinomial logistic regression models to quantify risk factors associated with the probability of influenza A(H5) environmental contamination and work zone–specific contamination patterns (Appendix). The goodness-of-fit of the final multivariable model was assessed by Akaike information criterion (AIC), and the lowest AIC among all competing models was identified as the best fitting model in the study (*38*). We used Stata 15 (StataCorp LLC, https://www.stata.com) for statistical analyses.

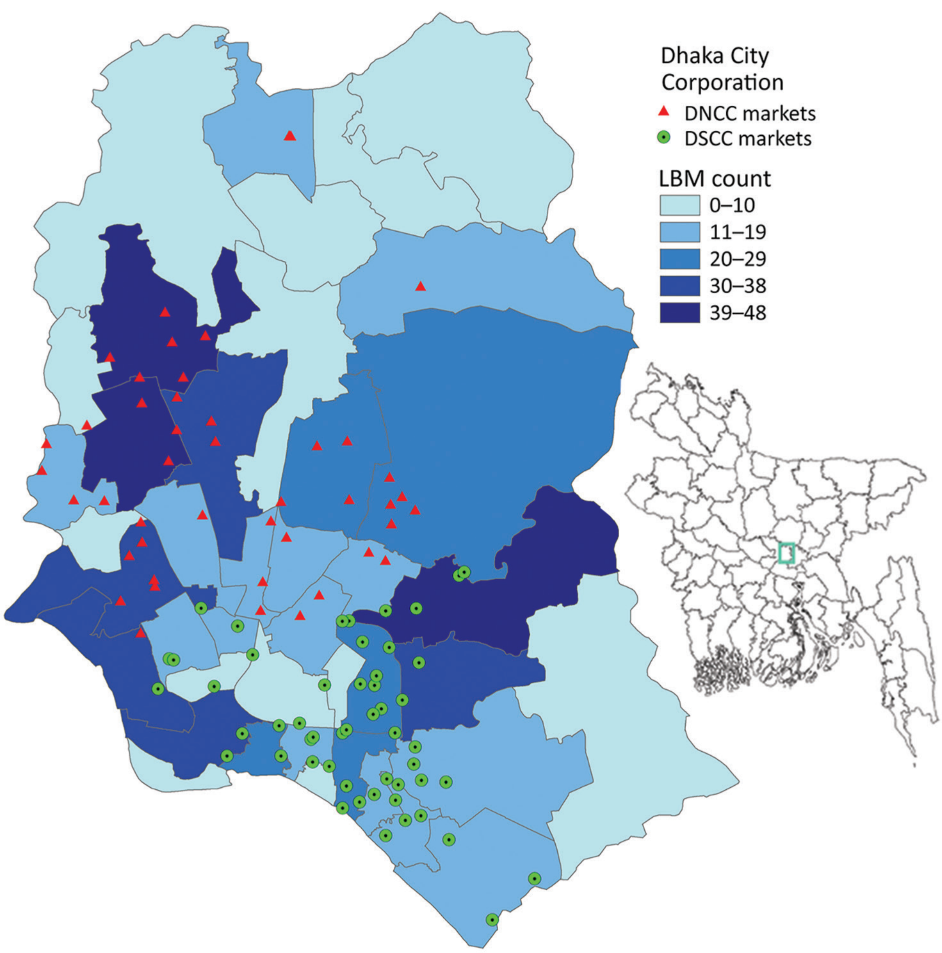

Figure 1. Locations of LBMs in the Dhaka metropolitan area, Bangladesh, January–March 2016. Inset map shows location of Dhaka in Bangladesh. DNCC, Dhaka North City Corporation; DSCC, Dhaka South City Corporation; LBM, live bird market.

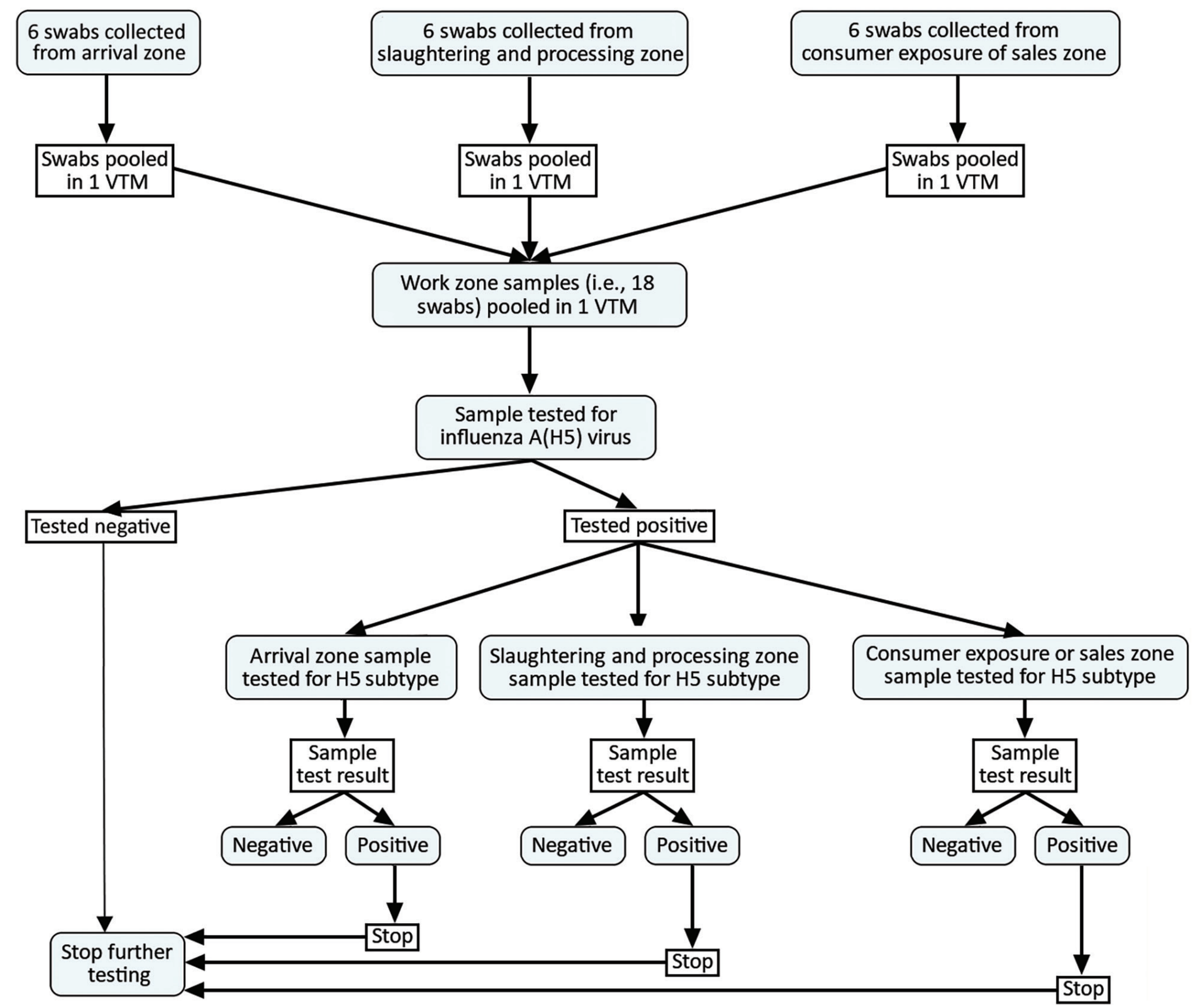

Figure 2. Sampling and laboratory testing protocol for influenza A(H5) in live bird markets, Dhaka, Bangladesh, January–March 2016. VTM, viral transport medium.

Results

Characteristics of LBMs

Of 104 enrolled LBMs, a total of 97 markets (52 from DSCC and 45 from DNCC) had complete questionnaire information on their biosecurity characteristics (Appendix Table 1). The retail type of LBM was predominant in DSCC (84.62%, 45/52) and DNCC (64.44%, 29/45) of Dhaka. Most markets in DSCC (88.46%, 46/52) and DNCC (97.78%, 44/45) sold multiple species of poultry. The broiler chicken was the main species at LBMs in DSCC (69.23%, 36/52) and DNCC (80.00%, 36/45).

Market-level daily cleaning (at minimum with detergent) and weekly market closure (≥1 day) practices varied among DNCC and DSCC markets. These 2 practices were reported to be more common in DSCC markets (75.00% [39/52] for daily cleaning and 45.15% [24/52] for weekly closure) compared with DNCC markets (31.11% [23/45] and 17.78% [8/45]). Most markets reported slaughtering poultry at vendor stalls (78.85% [41/52] in DSCC and 93.33% [42/45] in DNCC) (Appendix Table 1).

Differences in the Proportion of Influenza A(H5) Virus Environmental Contamination and Market Characteristics

Our analysis indicates that the proportion of influenza A(H5) virus environmental contamination was significantly higher in March than the previous 2 months (p≤0.001) (Appendix Table 2). The trend of LBM work zone–specific influenza A(H5) environmental contamination was similar in March in DSCC and DNCC markets, and the highest level of environmental contamination was in the slaughtering and processing zone (Figure 3). Of all market-level characteristics, only 3 characteristics were found to be significantly associated with proportions of influenza A(H5) environmental contamination: market type (p = 0.036) and location of poultry slaughtering (p = 0.014) in DNCC markets and weekly market closure of ≥1 day (p = 0.006) in DSCC markets (Appendix Table 2).

Factors Associated with Influenza A(H5) Virus Environmental Contamination within LBMs

Factors Associated with the Probability of LBM Influenza A(H5) Environmental Contamination Risk

We demonstrated by univariable analysis that the probability of influenza A(H5) environmental contamination was significantly higher in slaughtering and processing zones (relative risk [RR] 1.22, 95% CI 1.01–1.49; p = 0.041) than in market arrival zones. The probability of contamination was significantly higher in March (RR 1.90, 95% CI 1.36–2.65; p≤0.001) than January (Table 1).

In the final multivariable analysis (model 2), after adjusting for market-level biosecurity factors, we demonstrated that the probability of influenza A(H5) environmental contamination remained 2-fold significantly higher in March than January (RR 2.07, 95% CI 1.44–2.96; p<0.001). Our findings also demonstrated that slaughtering and processing zones had an increased risk for influenza A(H5) recovery compared to the arrival zone, but this effect was not statistically significant (RR 1.21, 95% CI 0.99–1.49; p = 0.067). In addition, the probability of influenza A(H5) environmental contamination was significantly associated with market type: retail markets were at lower risk than dual-purpose markets (RR 0.69, 95% CI 0.51–0.93; p = 0.012) (Table 1). Model 2 presented a better fit to the data than model 1 (i.e., without adjusting for market-level biosecurity factors). The AIC of model 1 was 1020.6 and in model 2 was 932.9. Effect modification and confounding were not found among pairs of biologically plausible LBM predictor variables.

Factors Associated with Work Zone–Specific Influenza A(H5) Virus Environmental Contamination Patterns

Our univariable and multivariable model of the multinomial analysis showed a significant increased risk in all LBM work zone–specific influenza A(H5) environmental contamination patterns except "slaughtering and processing zone area only" in March (relative risk ratio [RRR] >1; null value not contained within 95% CI) compared with January (Table 2, https://wwwnc.cdc.gov/EID/article/27/9/20-4447-T2.htm). After multivariable adjustment, no market-level factors were significantly associated with work zone–specific influenza A(H5) virus environmental contamination patterns.

Discussion

Our analyses provide the most comprehensive account of the recovery of influenza A(H5) virus in specific LBM work zones over 3 months across a large sample of LBMs (n = 104) within the Dhaka metropolitan area of Bangladesh. This study overcomes many of the limitations seen in previous studies of LBMs in Dhaka in the context of within-market measurement of environmental contamination (*11*,*12*,*19*,*20*,*22*,*25*).

Our descriptive results indicated vulnerabilities in LBMs in Dhaka associated with increased proportions of influenza A(H5) virus environmental contamination. Previous studies have shown that dual-purpose LBMs (i.e., markets conducting both wholesale and retail operations) in Dhaka were at higher risk for influenza A contamination (*11*). This previous finding suggests that markets in DNCC would be at greater risk for influenza A(H5) contamination. Our analyses confirmed this suggestion, demonstrating a larger proportion of influenza A(H5) recovery in dual-purpose DNCC markets than in retail-only markets. Poultry slaughtering has been consistently found to be a significant risk factor for LBM environmental contamination with influenza A(H5), and studies in Indonesia (*2*,*27*) and Bangladesh (*19*) support this observation. Environmental contamination with influenza A(H5) was significantly higher in DNCC markets without slaughtering facilities than in those reporting poultry slaughtering. Market environmental contamination in the absence of slaughtering facilities could be linked to the sampling procedure, in which sample collectors were instructed to use their sense of perceived risk if suggested sampling sites were not present in the market and other sites had to be chosen. This limitation in the sampling procedure should be corrected in future studies. Biosecurity practices such as cleaning and market closures have been reported to reduce environmental contamination in LBMs and eliminate risk for human infection with influenza A (*39*). Our results indicate that DSCC markets would benefit from higher rates of closures; a higher proportion of influenza A(H5) contamination was found in DSCC markets that did not perform market closures. In 2017, China established the

1110 policy, which involves daily cleaning, weekly disinfection, monthly closure, and no overnight stay of poultry (*40*). This approach has been successful at reducing the level of contamination within LBMs. This suggests that the implementation of a 1110-type policy in Dhaka's LBMs would strengthen LBM biosecurity, thereby reducing the level of influenza A(H5) contamination. Taken together, the observed differences in environmental contamination between markets in DSCC and DNCC can partly be explained by poultry slaughter and market management activities and less so by trader and poultry demographics.

Risk for influenza A(H5) infection in humans and poultry has been shown to be associated with movement of live poultry during national festive periods (*41–43*). In Bangladesh, demand for poultry products is influenced by traditional customs and rituals, including religious and cultural festivals (*44–46*). Our analysis found a 2-fold increase in the probability of environmental contamination in March compared with January, and market-level covariates did not modify this effect. Our analysis indicates the increased probability of influenza A(H5) environmental contamination in March in urban LBMs of Dhaka is likely related to the Bangla new year festival, which occurs in April and is linked to increased demand for poultry products in urban Dhaka LBMs.

We demonstrated that influenza A(H5) environmental contamination was positively associated with 2 market-level covariates: work zone (slaughtering and processing zone compared with arrival zone) and type of market (dual-purpose markets compared with retail-only markets). The higher probability of influenza A(H5) environmental contamination in the slaughtering and processing zone and in dual-purpose markets could be related to the challenge of maintaining adequate sanitation in LBMs with these characteristics. The risk for environmental contamination is known to be increased when slaughtering equipment is not frequently cleaned throughout the day using adequate disinfection protocols (*47*). Market attributes such as the presence of wholesalers in the market (*11*) and within-market trade of asymptomatic poultry between wholesalers and retailers (*44*) explain the higher levels of influenza A(H5) environmental contamination in dual-purpose markets compared with retail markets. Our analysis uncovered biosecurity characteristics that could partially explain these higher levels of influenza A(H5) environmental contamination. For example, dual-purpose markets have greater heterogeneity in poultry species

Table 1. Risk factors associated with the probability of influenza A(H5) environmental contamination at specific live bird market work zones, Dhaka, Bangladesh, January–March 2016

	Univariable analysis			Multivariable model 1			Multivariable model 2		
Risk factor	RR (95% CI)	p value	Overall p value	RR (95% CI)	p value	Overall p value	RR (95% CI)	p value	Overall p value
Market work zones of sample collection; reference: arrival									
Slaughtering and processing	1.22 (1.01–1.49)	0.041	0.110	1.23 (1.01–1.50)	0.040	0.103	1.21 (0.99–1.49)	0.067	0.180
Consumer exposure or sales	1.05 (0.84–1.31)	0.647		1.05 (0.84–1.32)	0.655		1.09 (0.86–1.37)	0.487	
Month of sample collection; reference: January									
February	1.24 (0.87–1.77)	0.233	<0.001	1.24 (0.87–1.76)	0.239	<0.001	1.33 (0.91–1.94)	0.138	<0.001
March	1.90 (1.36–2.65)	<0.001		1.90 (1.36–2.65)	<0.001		2.07 (1.44–2.96)	<0.001	
Market type; reference: dual-purpose†									
Wholesale	0.79 (0.57–1.10)	0.161	0.042				0.79 (0.571.10)	0.161	0.042
Retail	0.69 (0.51–0.92)	0.012					0.69 (0.510.93)	0.012	
Species being sold (reference: multiple species)†	0.57 (0.30–1.08)	0.084							
Electricity in facility†	1.50 (0.87–2.60)	0.148							
Market sells poultry to other vendors†	1.21 (0.92–1.58)	0.176							
Weekly market closure (≥1 day)†	0.79 (0.55–1.14)	0.207							
Akaike information criterion					1,020.588			932.9017	

*Blank cells indicate variables not included in model. RR, relative risk.
†Univariable results adjusted for month of sample collection and market work zones of sample collection.

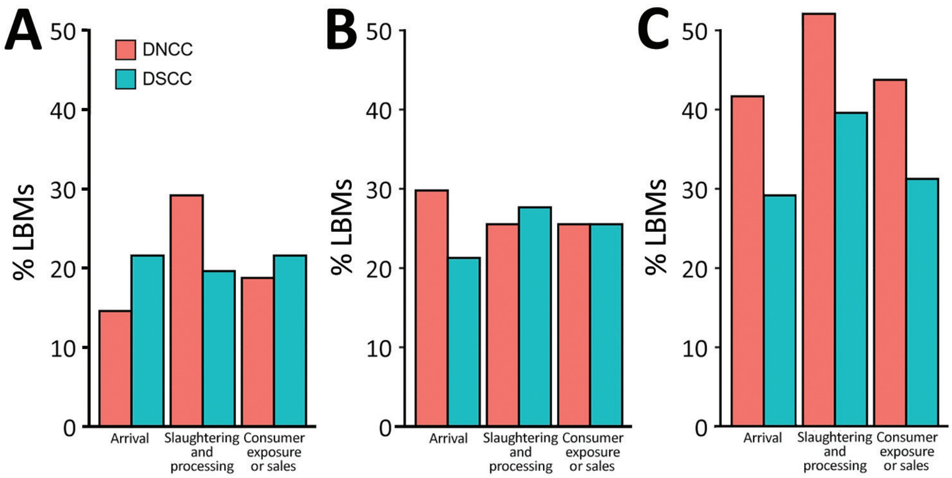

Figure 3. Distribution of influenza A(H5) virus environmental contamination in specific work zones in LBMs of DNCC and DSCC, Dhaka, Bangladesh, January–March 2016. A) January; B) February; C) March. DNCC, Dhaka North City Corporation; DSCC, Dhaka South City Corporation; LBM, live bird market.

than retail-only markets (Appendix Table 3), which could promote virus introduction. Furthermore, our data suggest that the *Sonali* chicken crossbreed was dominant in dual-purpose markets compared with other markets (Appendix Table 3); this crossbreed has previously been shown to have a higher bird-level influenza A(H5) prevalence (*11*).

Our study revealed a significantly increased probability of influenza A(H5) environmental contamination in March in 3 of the 4 site-specific influenza A(H5) environmental contamination patterns. Our results also extend those from a recent study by demonstrating that, outside the month of March, the slaughter area was the environmental site most contaminated with influenza A(H5) in LBMs (*25*). Our findings suggest that to increase the probability of detection of influenza A(H5) environmental contamination, those conducting surveillance should consider the slaughtering and processing zone as the candidate sampling site within LBMs during the months leading up to the increased demand for poultry in April. Furthermore, our results suggest that market-level biosecurity characteristics did not influence the temporal variation in work zone–specific influenza A(H5) environmental contamination patterns (Appendix Figure 1).

Of note, only 1 market-level characteristic (market sells poultry to other traders) was reported to be marginally associated with the probability of S-only environmental contamination pattern. This relationship could be partly explained by the fact that LBM contamination level is not simply the result of continuous introductions of infected birds, but a consequence of virus recirculation and amplification within them (*1*). To further elucidate the market work zone–specific influenza A(H5) environmental contamination patterns identified in this study, follow-up studies into the social network of poultry trade in LBMs are needed to clarify the effect.

The first limitation of our study is that, although we triangulated information on Dhaka LBM characteristics from data collectors with that from market managers through telephone call data validation, the use of secondary data might have introduced undue reporting bias. Second, we focused our analyses on the 3-month period of the winter season (January–March); further analyses should consider expanding the temporal scope of the investigation to better understand the seasonal trends identified in this study. Third, we used a sample pooling strategy (i.e., 18-swab pools collected in 5 mL of viral transport medium), which has not been validated for the presence of serial dilution effect and should be evaluated in future studies. However, despite the 18-swab pooling, we found a significant positivity rate in pooled samples. Fourth, because of budgetary limitations, our study was only conducted in LBMs in the Dhaka metropolitan area without consideration of other cities in Bangladesh. Thus, caution should be taken in interpretation, because the environmental contamination of LBMs in Dhaka might not reflect the local idiosyncrasies of LBMs in other cities in Bangladesh. Finally, despite our efforts to address confounding effects, we could not consider other factors that could be associated with contamination levels, including the poultry trade network between LBMs and source farms and the presence of other infection reservoirs in LBMs.

In conclusion, this study demonstrates that LBMs located in DNCC of Dhaka are qualitatively more vulnerable to influenza A(H5) virus environmental contamination. The probability of influenza A(H5) environmental contamination is equally likely across all within-LBM sites investigated and particularly higher in the month of March. The slaughtering and processing zones of LBMs could serve as candidate zones for active surveillance programs. Future work also should evaluate the effects of poultry movement and LBM biosecurity in the epidemiology of influenza A (H5) virus. Sanitation practices, market closures, and slaughtering and processing practice interventions within LBMs would help to reduce market-level influenza A contamination.

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Risk Areas for Influenza A (H5) Environmental Contamination in Live Bird Markets, Dhaka, Bangladesh

Appendix

The Rationale of Live Bird Market Selection

In collaboration with the Department of Livestock Services (DLS) in Bangladesh, 2015 data from earlier projects on the presence of live bird markets (LBMs) in Dhaka were made available to the in Bangladeshi office of the Emergency Centre for Transboundary Animal Diseases (ECTAD) of the Food and Agriculture Organization of the United Nations (FAO-UN). LBM data from these earlier projects were merged and duplicate LBMs were identified and removed, yielding a total of 230 LBMs in Dhaka. Of the 230 LBMs, 110 had location information available; influenza surveillance ("AI Sink Surveillance" in LBMs) was initiated in January 2016 in these 110 LBMs to detect influenza A (H5) virus contamination (M.G. Osmani, FAO Bangladesh and Department of Livestock Services, pers. comm., 2016 Nov). In our study, we included 104 markets (of the 110 markets surveilled) in which influenza A (H5) environmental contamination was laboratory-confirmed (*1*). All 104 markets targeted for sampling were visited multiple times during the study period (i.e., each market visited once a month) (*1*).

Background of LBM Census and Market-Level Characteristic Selection

A LBM census in Dhaka was designed and carried out by the ECTAD of FAO-UN Bangladesh in collaboration with DLS to develop a comprehensive database of all LBMs in Dhaka that would be freely shared with participating and interested parties. The specific objectives of the census were to collect information on the number and type of poultry, available facilities and location information for all poultry markets in Dhaka; and to develop a database containing all validated LBM data to be made available to collaborators and interested parties.

During January–March 2016, a LBM census in the Dhaka metropolitan area was conducted by veterinary students of the Sher-E-Bangla Agricultural University (SAU) of Dhaka with support from the staff of Dhaka North City Corporation (DNCC) and Dhaka South City Corporation (DSCC). The role of DNCC and DSCC staff was to confirm the location of the market in Dhaka. Markets were identified by walking all roads in a ward (an administrative subdivision unit in the Dhaka city) and asking local traders and consumers if they knew of any poultry sellers in the area. A market was defined as a place in which $\geq$1 traders sell live poultry at least once a week to end-users, other traders, or both. Market management and biosecurity questionnaires were prepared and pretested by Bangladesh FAO-UN ECTAD technical staff by interviewing a small set of market vendors within Dhaka. Students and staff were trained on census standard operating procedures and forms through a 2-day workshop held at SAU. Each LBM was visited once in the census period and data were collected by questionnaires. The census included data collection on the market type (retail, wholesale, or dual-purpose [both wholesale and retail]), poultry-trading statistics (number of vendors and volumes and species of poultry), available facilities (such as running water, electricity, and roof), and biosecurity characteristics (such as daily cleaning protocol, market closure schedule, and poultry slaughtering locations) (FAO Bangladesh and Department of Livestock Services, pers. comm., 2016). The LBM census reported 659 LBMs in the Dhaka metropolitan area; 326 were located in Dhaka North and the remainder in Dhaka South.

In our study, we compared the LBM census database with the infection database (influenza surveillance) of January–March 2016. By comparing market addresses, we identified 97 markets that appeared in both databases. A total of 7 markets from the infection database were not identified through the census. We used the LBM census information (i.e., market-level characteristics) for 97 markets along with market infection data for 104 markets to quantify risk factors associated with the probability of influenza A (H5) virus environmental contamination in specific market work zones, as well as to work zone–specific environmental contamination patterns.

Selection of Environmental Sites in Work Zones within LBMs

A total of 3 environmental work zones within LBM were selected for sampling on the basis of a previous study in Indonesia that showed significant likelihood of influenza A (H5) contamination (*2*). These zones were sampled once a month during the January–March 2016 study period. Although the environmental work zones were specific, the location of environmental sampling sites per LBM work zone were not always the same at every market follow-up.

The environmental sampling sites were indicated by work zone. For the poultry arrival zone (A), these sites were sampled: swab 1, floor of arrival/holding area; swab 2, cages or pens with live birds; swab 3, waste water; swab 4, waste bins; swab 5, trucks (if present); swab 6, any one of the above (on the basis of perceived risk). For the poultry slaughtering and processing zone (S), these sites were sampled: swab 1, processing table after defeathering; swab 2, baskets or trays holding poultry meat; swab 3, slaughtering boards/area; swab 4, waste bins; swab 5, waste water/blood drain path; swab 6, any one of the above (on the basis of perceived risk). For the consumer exposure or sales zone (E), these sites were sampled: swab 1, table for display; swab 2, chopping boards; swab 3, wet cleaning cloths; swab 4, scales; swab 5, knives/utensils; swab 6, any one of the above (on the basis of perceived risk).

Multivariable Statistical Modeling of LBM–Level Influenza A (H5) Environmental Contamination Risk

To quantify risk factors associated with the probability of influenza A (H5) virus environmental contamination in specific LBM work zones (i.e., arrival, slaughtering and processing, consumer exposure or sales), we performed our analysis in 3 steps. First, we developed univariable Bernoulli generalized linear models by using a log link function and a market random effect to screen variables with $p<0.20$ to be considered in a full multivariable model. Second, before inclusion in the full multivariable model, correlations between selected variables were investigated by using tetrachoric and polychoric correlation methods for binary and ordered-category variables (*3*). The purpose of checking correlation was to reduce or avoid collinearity among predictor variables that can lead to biased estimates and inflated standard errors (*4*). If 2 predictors were highly correlated (correlation coefficient >0.70) (*5*), only 1 of the variables was included in the full multivariable model. Third, we established 2 multivariable models: a multivariable model to understand the effects of month of sample collection and LBM work zone on the probability of environmental contamination risk (model 1), and a multivariable model to understand how market-level factors influence the effect of month of sample collection and LBM work zone level on the probability of environmental contamination risk (model 2). We arrived at the final multivariable model by using a backward elimination variable selection process and retained predictors significant at $p<0.05$ (all reported p values are 2-sided) and those that could be deemed confounders. Confounding was checked by adding and removing a variable from the model and assessing the impact on coefficients of other variables; if the change was >25%, that variable was deemed a confounder and was retained in the final multivariable model. Effect modification was also investigated for pairs of predictor variables on the basis of biologic plausibility. Generalized joint Wald tests were used to test the significance of each fitted categorical variable with >2 levels. Akaike information criterion (AIC) was used to determine the best-fitting multivariable model (*6*). The best-fitting model was the one with the lowest AIC among all competing models (*6*).

To quantify the association between LBM work zone–specific influenza A (H5) environmental contamination patterns and the month of sample collection, we developed univariable and multivariable multinomial logistic regression models, which included a random effect of market to account for repeated measurements at the market level. The univariable model only included the month of sample collection (i.e., month categorized into January, February, and March). The multivariable model was further adjusted for market-level factors to assess the influence of these factors on the relationship between LBM work zone–specific influenza A (H5) environmental contamination patterns and timing of sampling. We arrived at the final and best-fitting multivariable multinomial model following the process outlined above for the Bernoulli generalized linear model without backward elimination variable selection process. Factors with insufficient data across different categories were not considered in the final multivariable model analysis.

We have used a Bernoulli family logistic regression to model the error in the probability of influenza A (H5) recovery as opposed to a binomial logistic regression model specification. Retaining this nomenclature is better to reflect the nature of the model used and will also help differentiate this model from the additional multinomial logistic regression model we developed to provide insight into site-specific infection patterns.

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Appendix Table 1. Characteristics of live bird markets in Dhaka metropolitan area, Bangladesh, January–March 2016*

	No. (%)		
Market level characteristics	Dhaka area (DSCC and DNCC)	DSCC	DNCC
Market type			
Dual purpose (wholesale and retail)	22 (22.68)	6 (11.54)	16 (35.56)
Wholesale	2 (2.06)	2 (3.85)	0
Retail	73 (75.26)	44 (84.61)	29 (64.44)
Species being sold			
Multiple	90 (92.78)	46 (88.46)	44 (97.78)
Single	7 (7.22)	6 (11.54)	1 (2.22)
No. vendors			
>15	23 (23.71)	12 (23.07)	11 (24.44)
11–15	16 (16.49)	9 (17.31)	7 (15.56)
6–10	23 (23.71)	9 (17.31)	14 (31.11)
1–5	35 (36.08)	22 (42.31)	13 (28.89)
No. species being sold			
7–9	8 (8.25)	1 (1.92)	7 (15.56)
4–6	59 (60.82)	30 (57.69)	29 (64.44)
1–3	30 (30.93)	21 (40.39)	9 (20.00)
Dominant species (by comparing poultry headcount)			
Broiler	72 (74.23)	36 (69.24)	36 (80.00)
Deshi	9 (9.28)	8 (15.38)	1 (2.22)
Sonali	16 (16.49)	8 (15.38)	8 (17.78)
Number of poultry head			
>1,000	39 (40.21)	18 (34.61)	21 (46.66)
501–1,000	24 (24.74)	12 (23.08)	12 (26.67)
1–500	34 (35.05)	22 (42.31)	12 (26.67
Electricity in facility			
No	7 (7.22)	4 (7.69)	3 (6.67)
Yes	90 (92.78)	48 (92.31)	42 (93.33)
Presence of roof			
No	16 (16.49)	8 (15.38)	8 (17.78)
Yes	81 (83.51)	44 (84.62)	37 (82.22)
Running water in facility			
No	45 (46.39)	22 (42.31)	23 (51.11)
Yes	52 (53.61)	30 (57.69)	22 (48.89)

Market level characteristics	No. (%)		
	Dhaka area (DSCC and DNCC)	DSCC	DNCC
Sell poultry to other vendors			
No	69 (71.13)	41 (78.85)	28 (62.22)
Yes	28 (28.87)	11 (21.15)	17 (37.78)
Weekly market closure (≥1 day)			
No	65 (67.01)	28 (53.85)	37 (82.22)
Yes	32 (32.99)	24 (46.15)	8 (17.78)
Sell poultry to consumers directly			
No	4 (4.12)	2 (3.85)	2 (4.44)
Yes	93 (95.88)	50 (96.15)	43 (95.56)
Sell products other than poultry (e.g., fish, red meat, vegetables, groceries)			
No	21 (21.65)	17 (32.69)	4 (8.89)
Yes	76 (78.35)	35 (67.31)	41 (91.11)
Daily cleaning protocol (at minimum with detergent)			
No	35 (36.08)	13 (25.00)	22 (48.89)
Yes	62 (63.92)	39 (75.00)	23 (51.11)
Poultry slaughtering location			
Vendor stall	83 (85.57)	41 (78.85)	42 (93.34)
Central facility	2 (2.06)	1 (1.92)	1 (2.22)
Vendor stall and central facility	4 (4.12)	3 (5.77)	1 (2.22)
Vendor stall and outside market	4 (4.12)	4 (7.69)	0
No facility	4 (4.12)	3 (5.77)	1 (2.22)
No. slaughtering facilities			
>10	31 (31.96)	17 (32.69)	14 (31.11)
6–10	26 (26.8)	10 (19.23)	16 (35.56)
0–5	40 (41.24)	25 (48.08)	15 (33.33)

*DNCC, Dhaka North City Corporation; DSCC, Dhaka South City Corporation.

Appendix Table 2. Differences in the proportion of influenza A (H5) virus environmental contamination in live bird markets, Dhaka metropolitan area, January–March 2016*

	Dhaka area (DSCC and DNCC)			DSCC			DNCC		
Selected market-level factors	N	No. (%)	p value†	N	No. (%)	p value†	N	No. (%)	p value†
Month of sample collection									
January	297	62 (20.88)	<0.001	153	32 (20.92)	0.049	144	30 (20.83)	<0.001
February	282	73 (25.89)		141	35 (24.82)		141	38 (26.95)	
March	288	114 (39.58)		144	48 (33.33)		144	66 (45.83)	
Market work zone									
Arrival	289	76 (26.30)	0.268	146	35 (23.97)	0.665	143	41 (28.67)	0.401
Slaughtering and processing	289	93 (32.18)		146	42 (28.77)		143	51 (35.66)	
Consumer exposure or sales	289	80 (27.68)		146	38 (26.03)		143	42 (29.37)	
Market type									
Dual-purpose (wholesale and retail)	174	66 (37.93)	0.006	42	16 (38.10)	0.150	132	50 (37.88)	0.036
Wholesale	18	5 (27.78)		18	5 (27.78)		0	0 (0.00)	
Retail	615	157 (25.53)		369	90 (24.39)		246	67 (27.24)	
Weekly market closure (≥1 day)									
No	552	168 (30.43)	0.044	237	74 (31.22)	0.006	315	94 (29.84)	0.300
Yes	255	60 (23.53)		192	37 (19.27)		63	23 (36.51)	
Species being sold									
Multiple	744	217 (29.17)	0.057	375	102 (27.20)	0.134	369	115 (31.17)	0.727
Single	63	11 (17.46)		54	9 (16.67)		9	2 (22.22)	
Electricity in facility									
No	54	10 (18.52)	0.118	33	6 (18.18)	0.408	21	4 (19.05)	0.331
Yes	753	218 (28.95)		396	105 (26.52)		357	113 (31.65)	
Sell poultry to consumers directly									
No	36	9 (25.00)	0.850	18	5 (27.78)	0.789	18	4 (22.22)	0.602
Yes	771	219 (28.40)		411	106 (25.79)		360	113 (31.39)	
Sell poultry to other vendors									
No	573	153 (26.70)	0.143	336	81 (24.11)	0.141	237	72 (30.38)	0.818
Yes	234	75 (32.05)		93	30 (32.26)		141	45 (31.91)	
Sale of products other than poultry (e.g., fish, red meat, vegetables, groceries)									
No	183	46 (25.14)	0.306	147	36 (24.49)	0.728	36	10 (27.78)	0.850
Yes	624	182 (29.17)		282	75 (26.60)		342	107 (31.29)	
Presence of roof									
No	135	36 (26.67)	0.677	69	19 (27.54)	0.765	66	17 (25.76)	0.380
Yes	672	192 (28.57)		360	92 (25.56)		312	100 (32.05)	
Running water in facility									
No	384	111 (28.91)	0.696	192	55 (28.65)	0.268	192	56 (29.17)	0.505
Yes	423	117 (27.66)		237	56 (23.63)		186	61 (32.80)	
Daily cleaning protocol (at minimum with detergent)									
No	294	82 (27.89)	0.871	114	26 (22.81)	0.454	180	56 (31.11)	1.000
Yes	513	146 (28.46)		315	85 (26.98)		198	61 (30.81)	
Poultry slaughtering locations									
Vendor stall only	699	192 (27.47)	0.171	345	87 (25.22)	0.599	354	105 (29.66)	0.014
Other locations (central facility only, vendor stall, and other sites)	75	22 (29.33)		57	15 (26.32)		18	7 (38.89)	
No slaughtering facility	33	14 (42.42)		27	9 (33.33)		6	5 (83.33)	
No. slaughtering facilities									

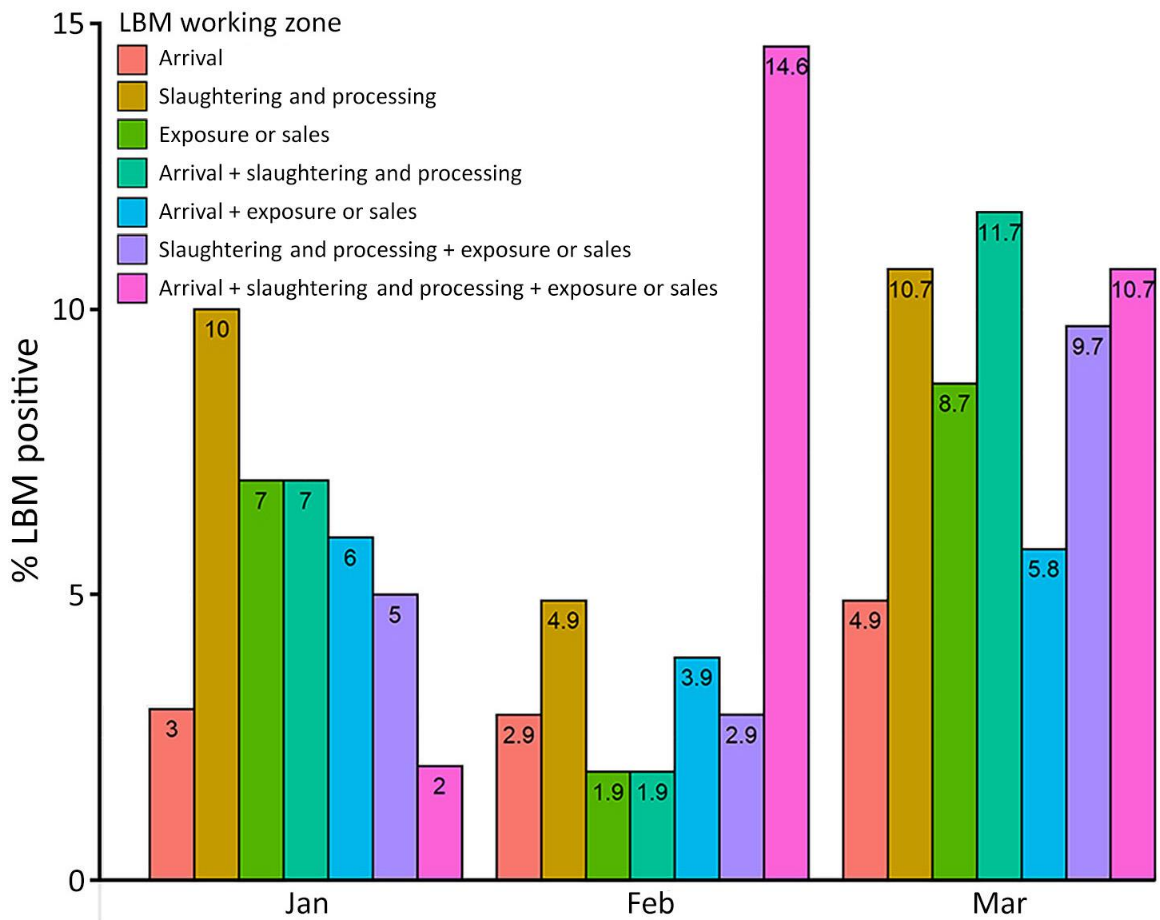
Selected market-level factors	Dhaka area (DSCC and DNCC) N	No. (%)	p value†	DSCC N	No. (%)	p value†	DNCC N	No. (%)	p value†
>10	258	83 (32.17)	0.231	138	43 (31.16)	0.163	120	40 (33.33)	0.714
6–10	210	57 (27.14)		72	14 (19.44)		138	43 (31.16)	
0–5	339	88 (25.96)		219	54 (24.66)		120	34 (28.33)	
No. vendors									
>15	195	56 (28.72)	0.642	102	30 (29.41)	0.166	93	26 (27.96)	0.900
11–15	129	42 (32.56)		69	22 (31.88)		60	20 (33.33)	
6–10	186	49 (26.34)		66	11 (16.67)		120	38 (31.67)	
1–5	297	81 (27.27)		192	48 (25.00)		105	33 (31.43)	
No. species being sold									
7–9	66	20 (30.30)	0.656	9	1 (11.11)	0.617	57	19 (33.33)	0.866
4–6	489	142 (29.04)		237	64 (27.00)		252	78 (30.95)	
1–3	252	66 (26.19)		183	46 (25.14)		69	20 (28.99)	
Dominant species (by comparing poultry headcount)									
Broiler	606	173 (28.55)	0.696	303	76 (25.08)	0.766	303	97 (32.01)	0.266
Deshi	60	14 (23.33)		54	14 (25.93)		6	0 (0.00)	
Sonali	141	41 (29.08)		72	21 (29.17)		69	20 (28.99)	
No. poultry head									
>1,000	327	103 (31.50)	0.239	144	46 (31.94)	0.068	183	57 (31.15)	0.549
501–1,000	195	52 (26.67)		96	18 (18.75)		99	34 (34.34)	
1–500	285	73 (25.61)		189	47 (24.87)		96	26 (27.08)	

*DNCC, Dhaka North City Corporation; DSCC, Dhaka South City Corporation.
†p values by Fisher's exact test.

Appendix Table 3. Characteristics of live bird markets by market types in Dhaka metropolitan area, Bangladesh, January–March, 2016*

Market-level characteristics	No. (%) Dual-purpose (wholesale and retail)	Wholesale	Retail	Total	p value†
No. vendors					
>15	10 (43.48)	2 (8.70)	11 (47.83)	23	0.006
11–15	4 (25.00)	0	12 (75.00)	16	
6–10	5 (21.74)	0	18 (78.26)	23	
1–5	3 (8.57)	0	32 (91.43)	35	
Sell poultry to consumers directly					
No	1 (25.00)	2 (50.00)	1 (25.00)	4	0.001
Yes	21 (22.58)	0	72 (77.42)	93	
Sell poultry to other vendors					
No	5 (7.25)	0	64 (92.75)	69	<0.001
Yes	17 (60.71)	2 (7.14)	9 (32.14)	28	
Sell products other than poultry (e.g., fish, red meat, vegetables, groceries)					
No	3 (14.29)	1 (4.76)	17 (80.95)	21	0.266
Yes	19 (25.00)	1 (1.32)	56 (73.68)	76	
Running water in facility					
No	11 (24.44)	1 (2.22)	33 (73.33)	45	0.904
Yes	11 (21.15)	1 (1.92)	40 (76.92)	52	
Electricity in facility					
No	0	0	7 (100.00)	7	0.308
Yes	22 (24.44)	2 (2.22)	66 (73.33)	90	
Presence of roof					
No	2 (12.50)	0	14 (87.50)	16	0.545
Yes	20 (24.69)	2 (2.47)	59 (72.84)	81	
Daily cleaning protocol (at minimum with detergent)					
No	8 (22.86)	1 (2.86)	26 (74.29)	35	1.000
Yes	14 (22.58)	1 (1.61)	47 (75.81)	62	
Weekly market closure (≥1 day)					
No	14 (21.54)	1 (1.54)	50 (76.92)	65	0.734
Yes	8 (25.00)	1 (3.13)	23 (71.88)	32	
No. slaughtering facilities					
>10	11 (35.48)	0	20 (64.52)	31	0.036
6–10	7 (26.92)	0	19 (73.08)	26	
0–5	4 (10.00)	2 (5.00)	34 (85.00)	40	
Poultry slaughtering locations					
Vendor stall	18 (21.69)	0	65 (78.31)	83	0.001
Central facility	2 (100.00)	0	0 (0.00)	2	
Vendor stall and central facility	1 (25.00)	0	3 (75.00)	4	
Vendor stall and outside market	0	0	4 (100.00)	4	
No facility	1 (25.00)	2 (50.00)	1 (25.00)	4	
Species being sold					
Multiple	22 (24.44)	2 (2.22)	66 (73.33)	90	0.308
Single	0	0	7 (100.00)	7	
No. poultry species being sold					
7–9	5 (62.50)	0	3 (37.50)	8	0.002
4–6	15 (25.42)	0	44 (74.58)	59	
1–3	2 (6.67)	2 (6.67)	26 (86.67)	30	
Dominant species (by comparing poultry headcount)					
Broiler	12 (16.67)	1 (1.39)	59 (81.94)	72	0.018
Deshi	2 (22.22)	0	7 (77.78)	9	
Sonali	8 (50.00)	1 (6.25)	7 (43.75)	16	
Market location					
DSCC	6 (11.54)	2 (3.85)	44 (84.62)	52	0.005
DNCC	16 (35.56)	0	29 (64.44)	45	
Total no. poultry head					
>1000	14 (35.90)	2 (5.13)	23 (58.97)	39	0.004
501–1000	6 (25.00)	0	18 (75.00)	24	
1–500	2 (5.88)	0	32 (94.12)	34	

*DNCC, Dhaka North City Corporation; DSCC, Dhaka South City Corporation.
†p value by Fisher's exact test.



Appendix Figure. Distribution of market work zone–specific influenza A (H5) environmental contamination patterns at live bird markets (LBMs) of Dhaka metropolitan area, Bangladesh, January–March 2016. Exposure or sales refers to consumer exposure.