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Determinants for the presence of avian influenza virus in live bird markets in Bangladesh: Towards an easy fix of a looming one health issue

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ABSTRACT

Highly pathogenic avian influenza virus subtype H5N1 endangers poultry, wildlife, and human health and is enzootic in large parts of Asia, with live bird markets (LBMs) as putative hotspots for their maintenance, amplification, and spread. To mitigate the extent of these and other avian influenza viruses (AIV) of concern, we aimed to increase our quantitative understanding of the factors determining the presence of avian influenza virus in LBM stalls. Between 2016 and 2017, we collected fecal or offal samples from 1008 stalls in 113 LBMs across the Dhaka and Rajshahi districts in Bangladesh. For each stall, samples were pooled and tested for the AIV matrix gene, followed by H5 and H9 subtyping using rRT-PCR. We detected Influenza A viral RNA in 49% of the stalls. Of the AIV positive samples, 52% and 24% were determined to be H5 and H9 viruses, respectively, which are both subtypes of considerable health concern. We used generalized linear mixed effect modelling to study AIV presence in individual stalls within LBMs as a function of 13 out of the 20 risk factors identified by FAO. We found that small and feasible improvements in cleaning and disinfection frequency, installing running water in stalls, and not mixing different breeds of chicken in the same cages had large impacts on the presence of AIV in stalls (Odds ratios 0.03–0.05). Next, cleaning vehicles used in poultry transport, not selling waterfowl with chickens in the same stall, buying stock directly from commercial farms, separating sick birds from healthy ones, and avoiding access by wild birds like house crows, also had major effects on lowering the risk of stalls having AIV (Odds ratios 0.16–0.33). These findings can be directly used in developing practical and affordable measures to reduce the prevalence of AIV in LBMs. Also, in settings with limited resources like Bangladesh, such mitigation may significantly contribute to reducing AIV circulation amongst poultry and spillover to wildlife and humans.

1. Introduction

Poultry is one of the most important sectors within the global livestock industry, ensuring economical animal protein production and creating employment and income for millions of people [1–3]. In Bangladesh, commercial poultry farming has developed into one of the country's fastest-growing industries over the past few decades [4,5], with live bird markets (LBMs) forming the primary hubs for poultry trade [6,7]. Ninety percent of poultry in the country passes through LBMs [8]. LBMs have previously and repeatedly been identified as primary hotspots for the maintenance, amplification, and spread of AIV, not only in Bangladesh [9,10] but in many countries throughout the East and south-east Asia [11–13]. Multiple subtypes of low pathogenic avian influenza (LPAI) and high pathogenicity avian influenza (HPAI) viruses have been detected in both LBMs as well as farms around Bangladesh, with H9N2 and H5N1 being the most prevalent [14–16]. Both H5N1 and H9N2 are also considered enzootic in Bangladesh as well as many other countries in the South and East Asia [17–19].

The enzootic presence of HPAI H5N1 and LPAI H9N2 in LBMs has raised concerns over the potential for these viruses to evolve, reassort, and spill over to humans, livestock, and wild birds [20,21]. Human cases of H5N1 infections with a history of poultry exposure in LBMs have been

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documented in Vietnam [22], Indonesia [23], Hong Kong, China [24], and Cambodia [25]. In Bangladesh, three human cases of H5N1 have thus far been linked to LBM workers who were likely exposed to infected poultry at LBMs [10]. In addition to spillover from poultry to humans, there have been occasional reports of spillover of AIV from poultry to house crows [26,27] and evidence of AIV in captive birds at zoos and parks [28,29]. In North America and Europe, spillover of H5N1 from poultry to wild mammals has been observed [30–32]. Given their prominent role in AIV dynamics, LBMs are in urgent need of biosecurity improvements to reduce virus circulation amongst poultry and spillover to wildlife and humans [33–35]. Most of the LBMs in Bangladesh are deemed to have poor biosecurity standards, often lacking running water facilities and flooring systems that are easy to clean, with mixing of multiple poultry species and slaughtering of birds at point of sale as additional factors that have been suggested to increase AIV prevalence in LBMs [7,36,37]. Accordingly, many studies have identified avenues to reduce the occurrence of AIV in LBMs, both in Bangladesh and elsewhere ([38–40]. However, these studies focused on a limited number of potential risk factors at a time or were largely qualitative. Moreover, a Bangladeshi study comparing the effect of cleaning and disinfecting on AIV contamination across LBMs found no significant differences across markets [10,38], which could suggest that these interventions have either no effect or be overruled by other factors that could not be controlled for in the comparison. In other words, a large-scale study, in which a comprehensive set of practices are being compared across a large number of vendors within a range of LBMs, will be required to assess the effects and advise on the prioritization of potential interventions.

In prioritizing interventions, practicability and affordability are important aspects. To achieve this goal, identifying the best practices amongst already common practices may be a valuable approach. This is not to say that novel strategies are not an option. Several studies concluded that weekly rest days on which markets were closed, and disinfection took place greatly reduced the incidence of AIV in LBMs in both China [41,42] and Vietnam [39]. However, while effective, such interventions may come at a considerable loss of revenue and will find little support amongst vendors and customers, jeopardising successful implementation. Finally, interventions at vendor and stall level, rather than at the level of an LBM, are also likely to be less of an organisational burden and easier to achieve. FAO stipulates 20 market management recommendations to reduce AIV contamination in LBMs [43]. We here quantified the consequences of 13 of these FAO recommendations for the occurrence of AIV within 1008 stalls across 114 LBMs, intending to identify those practices that have the most significant impact on reducing AIV presence in stalls and are already widespread, practical, and affordable such that they are likely to be most acceptable and implementable by authorities, vendors and customers alike. Thereby, the results of this study can assist in developing and prioritizing management mitigation strategies aimed at reducing AIV prevalence amongst poultry sold at LBMs in Bangladesh and other countries in the region and contribute to mitigating the ongoing risk of spillover of AIV to humans, livestock, and wildlife.

2. Material and methods

2.1. Biological samples and data collection

LBMs are facilities where vendors sell live poultry (chickens and ducks, pigeons, and quails) that are usually slaughtered on-site. Birds typically remain at the market until they are sold. A stall owner or stall keeper buys poultry from farms or middlemen to sell to other vendors or directly to consumers. Stalls are small entities (approximate median size 5 m^2) within LBMs to keep, process, and sell poultry. Our study involved 1008 representative stalls from 114 LBMs, of which 16 were in the Rajshahi and 98 in the Dhaka districts of Bangladesh (Figure-2B). All vendors and collaborating stall workers explicitly consented to participate in our study. We collected fecal or fresh offal swabs from recently slaughtered birds from each stall. Per stall, we obtained freshly defecated four fecal or four offal swab samples from chickens, waterfowl, quails, or pigeons. The four samples were subsequently pooled into a single sample for each stall. We did not collect swab samples from adjoining stalls to avoid similar rates of contamination. Pooled swab samples were stored in a uniquely marked, 3.6 ml cryovial containing viral transport medium (VTM), consisting of Hank's balanced salt solution (ICN Biomedicals, Inc., USA), 2% bovine albumin, pH 7.4) containing amphotericin B (15 μg/ml), penicillin G (100 units/ml) and streptomycin (50 μg/ml) [44]. While sampling within LBMs, we kept the swabs in VTM in a cool box at approximately 4 °C, after which they were transferred to a portable dry shipper (−196 °C) within 4 h. of collection. After transport to the laboratory, we stored the samples at −80 °C until further processing.

For each stall from which a (pooled) sample was collected, we collected data on 13 "risk" factors pertaining to hygiene and sanitation practices (Table 1) in relation to the presence or absence of AIV contamination. The order in which categories were ranked within these risk factors is from hypothesized worst to best practice.

2.2. Lab testing procedure

We extracted viral RNA from the pooled swab samples using the MagMAX 96 AI/ND Viral RNA isolation kit (Ambion, Inc. Austin, TX) using the magnetic bead-based RNA isolation technique in a KingFisher Flex 96-well robot (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. Viral RNA was screened for the presence of the AIV Matrix (M) gene using real-time reverse transcriptase PCR (rRT-PCR) with reference primers and probes, followed by the procedures as reported [45,46]. Then, we tested M-gene-positive samples for subtypes H5, H7, and H9 using subtype-specific primers using rRT-PCR as previously described [46,47]. We considered a sample as AIV positive for

Table 1
Hygiene and sanitation practices of vendors at the stall level in LBM.

Explanatory variables	Categories
Frequency of cleaning	Daily
	Twice a week
	Weekly
	Weekly
	Twice a month
Frequency of disinfection	Infrequent
	Yes
Clean running water facility	No
	Yes
A mixture of chicken breeds held in one cage	No
	Yes
Cleaning of poultry vehicles at marketplace	No
	Yes
Presence of waterfowls	No
	Commercial farm
	Wholesale market
	Middlemen
Source of poultry	Multiple sources
	Yes
Separate sick birds	No
	Yes
Access of crows and other wild birds	No
	Smooth surfaced
	Partially surfaced
Flooring system	Unsurfaced
	Yes
Slaughtering and selling are done at the same stall	No
	Yes
Unsold poultry mixed with new stock	No
	Discard indiscriminately
	Disposed in dedicated bin
	Sell
Disposal of offal and dead birds at vendors level	Both sell and bin

the M-gene if the cycle threshold (Ct) was <40 and as H5, H7, and H9 positive if Ct < 37 [48]. M gene-positive samples that tested negative for H5, H9, and H7 were classified as AIV HA/untyped.

2.3. Statistical analysis

All statistical analyses were conducted using R software version 4.2.0 in Rstudio 2022.02. We used Generalized Linear Mixed Effect Models (GLMM) in R package lme4 to investigate AIV contamination status (positive or negative irrespective of subtype) in each stall as a function of the 13 recorded risk factors for each stall. We included the ID of the LBM, species type (chicken, waterfowl, quail, and pigeon) at stall level, and sample type (fecal or offal) as random effects in the model. Given a large number of explanatory variables (13), we checked these explanatory variables for possible high associations or collinearity between variables using Cramer's V [49]. This procedure did not identify particularly high collinearities that would warrant reducing the set of 13 explanatory variables in the GLMM (Figure. SM1). After GLMM, we conducted posthoc pairwise comparisons using the emmeans package [50]. Since all categories within the explanatory variables were ranked starting with the hypothesized worst practice (i.e., practice yielding the supposedly highest chance of a stall being AIV positive), the worst scenarios were

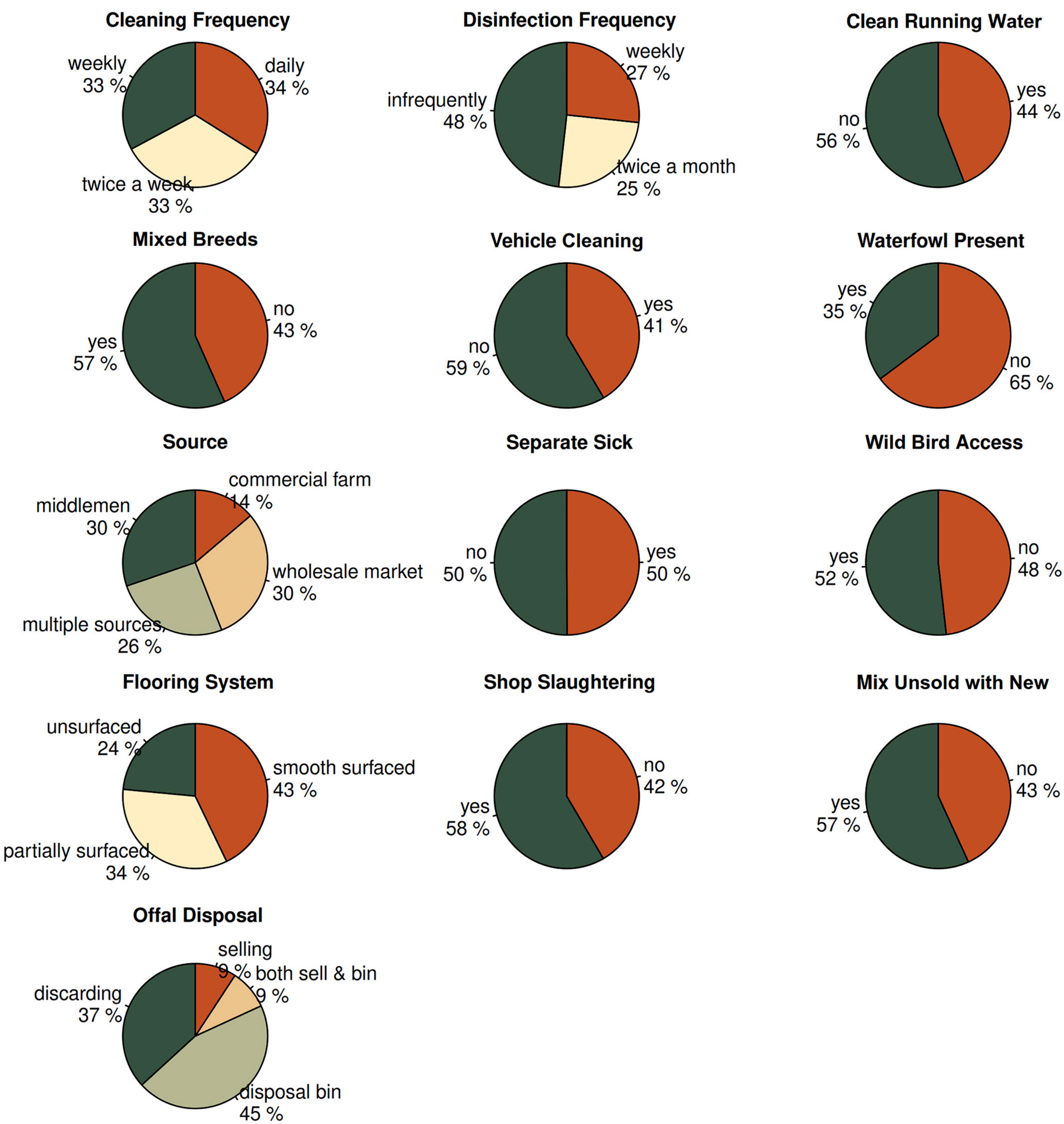

Fig. 1. Frequency distribution of the 13 explanatory risk-factor variables used in investigating the presence/absence of AIV across 1008 LBM stalls.

included in the intercept, thus having an odds ratio of 1.

2.4. Ethics approval

The study protocol was approved by Chattogram Veterinary and Animal Sciences University-Animal Experimentation Ethics Committee (protocol: CVASU/Dir (R&E) AEEC/2015/751) and by the Institutional Animal Care and Use Committee at the University of California at Davis (protocol number 16048).

3. Results

Characteristics of the stalls and the sanitation practices by their vendors varied greatly across the investigated stalls; for nearly all 13 explanatory variables, the different categories were near-uniformly distributed across the 1008 stalls, with the notable exception of offal disposal (Fig. 1).

For offal disposal, the categories *selling* (9%) and *selling & disposal bin* (9%) were underrepresented relative to *discarding* (37%) and *disposal bin* (45%), but still sufficiently large to allow their inclusion in the statistical analyses. This underrepresentation of the two *selling* categories is likely because of the limited demand for offal. Worryingly, but possibly unsurprisingly, given the high prevalence of poor biosecurity standards, we found that AIV was present in as many as 49% of all stalls investigated (Fig. 2A). Moreover, in more than three-quarters of the positive cases AIV subtypes of concern were found; half of the cases (i.e., 27% overall) were H5 and a quarter (i.e., 12% overall) were H9.

Of the 13 explanatory variables investigated, as many as 12 showed significant effects on the observed AIV prevalence in the investigated stalls (Table 2, Fig. 3). The only explanatory variable not having an effect was offal disposal. Of those having an effect, the strongest effects were observed by disinfection frequency, cleaning frequency, and having running water available in the stall with odds ratios as low as 0.03 (Table 2, Fig. 3).

As aptly illustrated in the marginal means plots; we found that the predicted AIV prevalence for weekly cleaning was as high as 81%, dropping quickly to 39% when cleaning frequency increased to twice a week and as little as 11% when cleaning was done on a daily basis (Fig. 4A).

Similarly effective in decreasing the prevalence of AIV, if the frequency of disinfection was increased from infrequent via twice a month to weekly, the predicted prevalence of AIV dropped from 84% to as low as 13% (Fig. 4B). Also of major importance, we determined that providing clean water in the stalls reduced the risk from 79% to 11% (Fig. 4C). The fourth most important factor with an odds ratio of 0.05 was whether vendors kept multiple breeds of poultry in the same cage within their stall, with keeping single rather than multiple breeds in cages reducing the AIV risk from 76% to 14% (Fig. 4D). Next, there were five factors for which the effect size was somewhat more limited but still considerable, with odds ratios for their most effective categories ranging from 0.16 to 0.33. In this group, the most effective practice of cleaning poultry vehicles in marketplaces decreased the risk of contamination from 63% to 22% (Fig. 4E). Not selling chickens and waterfowl in the same stall similarly reduced the likelihood of AIV contamination of the stall from 62% to 23% (Fig. 4F). If vendors purchased poultry from commercial farms directly rather than through a middleman, wholesale market, or from multiple sources, AIV contamination reduced from a predicted range of 46 to 51% to as little as 20% (Fig. 4G). Separating sick birds from healthy birds reduced the likelihood of AIV in the stall from 57% to 26% (Fig. 4H). Finally, if crows or other wild birds could not enter the stall areas, the risk was reduced from 55% to 28% (Fig. 4I).

The final four explanatory variables in the model had limited or non-

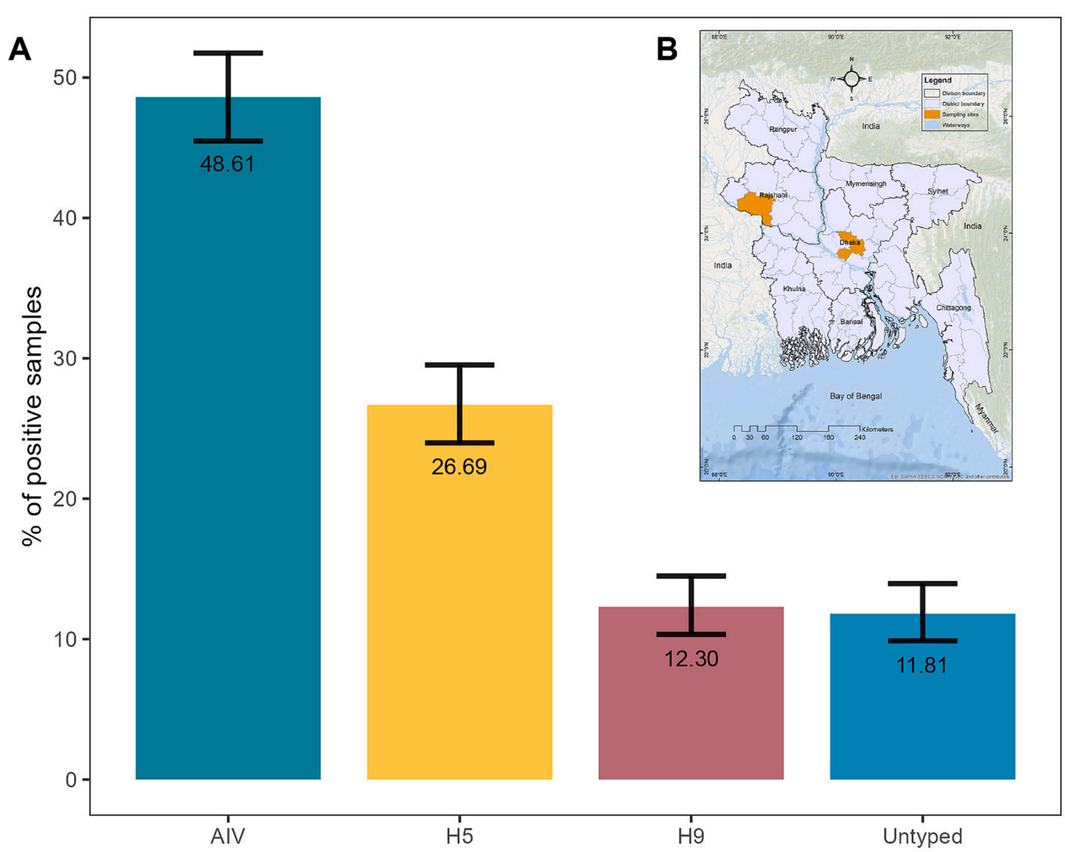

Fig. 2. A) Prevalence of Influenza A virus and HA subtypes (i.e. H5, H9, and untyped) across 1008 LBM stalls. The number inside the graph denotes the percentage of being positive in the given category. B) Investigated LBMS were located within the districts of Rajshahi and Dhaka in Bangladesh.

Table 2
Estimates with standard error and *p*-value of generalized linear mixed effect model to find out the risk factors of AIV contamination in LBM stalls in Bangladesh during 2016–17.

Variable	Category	Estimates	Std. error	Statistic	P value
Frequency of cleaning [Ref: Weekly]	Twice a week	−1.87	0.37	−5.06	<0.01
	Daily	−3.55	0.45	−7.93	<0.01
Frequency of disinfection [Ref: Infrequently]	Twice a month	−2.57	0.44	−5.9	<0.01
	Weekly	−3.63	0.46	−7.8	<0.01
Clean running water facility [Ref: No]	Yes	−3.41	0.54	−6.31	<0.01
A mixture of chicken breeds held in one cage [Ref: Yes]	No	−3.03	0.44	−6.86	<0.01
Cleaning of poultry vehicles at marketplace [Ref: no]	Yes	−1.83	0.39	−4.74	<0.01
Presence of waterfowls [Ref: Yes]	Yes	−1.72	0.4	−4.34	<0.01
Source of poultry [Ref: Middlemen]	Multiple sources	−0.23	0.44	−0.52	0.6
	Wholesale market	−0.03	0.46	−0.08	0.94
	Commercial farm	−1.45	0.63	−2.31	0.02
Separate sick birds [Ref: No]	Yes	−1.33	0.29	−4.57	<0.01
Access of crows and other wild birds [Ref: Yes]	No	−1.1	0.33	−3.39	<0.01
Flooring system [Ref: Unsurfaced]	Partially surfaced	0.74	0.38	1.94	0.05
	Smooth-surfaced	−0.83	0.38	−2.18	0.03
Slaughtering and selling are done at the same stall [Ref: Yes]	Yes	−0.81	0.36	−2.24	0.02
Unsold poultry mixed with new stock [Ref: Yes]	No	−0.68	0.31	−2.2	0.03
Disposal of offal and dead birds at vendors level [Ref: Discarding]	Disposal bin	−0.38	0.34	−1.12	0.26
	Both sell and bin	0.82	0.57	1.44	0.15
	Selling	−0.42	0.59	−0.72	0.47

significant effects, with minimum odds ratios across their categories varying between 0.44 and 0.69. Of these, the one with the biggest effect was the type of flooring with smooth-surfaced stalls having a predicted AIV contamination risk of 24% relative to 42% and 60% for unsurfaced or partially surfaced stalls (Fig. 4J). The risk of AIV contamination at stalls was reduced from 51% if slaughtering and selling took place in the same stall to 32% if these practices were separated (Fig. 4K). The risk of infection was reduced from 49% to 33% when unsold poultry from the day before was not mixed with new poultry (Fig. 4L). The way offal was disposed of had no effect on AIV contamination risk (Fig. 4M).

4. Discussion

Our study provides the most comprehensive overview of the circulation of AIV viruses in LBMs in Bangladesh to date. It reveals alarmingly high levels of AIV in Bangladeshi LBMs, with 49% of stalls found to sell AIV-infected poultry, with 27% of stalls being positive for H5 AIV and 12% positive for H9. At the same time, our study identified large and systematic variations in AIV presence across the 1008 stalls investigated, offering insights that open up simple avenues to potentially mitigate the problem and dramatically reduce the high prevalence of AIV in LBMs. Notably, relatively small increases in cleaning and disinfection frequency, the installation of running water in stalls, and keeping different breeds and species of birds in separate cages had large impacts on the presence of AIV in stalls. Next, cleaning transport vehicles, not selling waterfowl with chickens in the same stall, buying stock directly from commercial farms, separating sick birds from healthy ones, and avoiding access by wild birds like house crows also had major effects on lowering the risk of stalls having AIV. Smaller risk-reducing effects were also found for stalls with smooth flooring, where no slaughtering of birds took place, and unsold birds and new stock were not mixed.

The high prevalence of AIV contamination within the studied LBMs is comparable to what was found in previous studies of AIV contamination in Bangladeshi LBMs [48,51]. That a large proportion of these AIVs found in LBMs belong to H5 and H9, subtypes are also in accordance with previous studies in LBMs in Bangladesh [6,35,52,53]. These are remarkable findings against the background of vaccination against H5N1 and H9N2, having been implemented in Bangladesh in 2012 and 2019, respectively. This suggests that vaccination is inadequate in that it does not adequately reduce infection risk and merely suppresses disease symptoms leading to silent spread [54]. Also, >50% of commercial poultry farms were found to be AIV positive for H5, H9, and other HA subtypes [47,55], further supporting the notion that silent spread in Bangladesh is widespread. In any case, our finding of high AIV presence in LBMs highlights that to control these viruses, vaccination alone is insufficient, and improved biosecurity measures are needed to combat these viruses from spreading and infecting other poultry [7,16]. Such improved biosecurity is also required to avoid occasional spillover into wildlife [56] and humans [10,57].

In the following discussion, we interpret the here investigated 13 risk factors in order of importance from most to least important in explaining AIV presence and absence in LBM stalls. The ***frequency of cleaning*** and ***disinfection*** proved to have the most significant impacts on stalls, with daily cleaning and weekly disinfection having odds ratios as small as 0.03. While cleaning with water is an excellent first step, cleaning with water alone cannot disinfect surfaces properly, and the use of detergent is essential to inactivate the AIV virus further [58,59]. Regular disinfection in LBMs has been reported to reduce environmental contamination with AIV in a range of studies in e.g., China, Indonesia, Vietnam, and the USA [12,40,60–62]. It has also been recognized previously that cleaning and disinfection activities, as well as personal hygiene practices, are critical in reducing the likelihood of AIV contamination and transmission from poultry to humans in the LBMs [61,63]. However, to our knowledge, quantitative assessments of the effect of cleaning and disinfection frequencies have not previously been conducted. As many as 34% of vendors clean their stalls on a daily basis, and 27% disinfect their stalls weekly (Fig. 1), implying that these are apparently neither impractical nor overly costly procedures. There may thus be great potential to significantly decrease viral contamination risk by campaigning for vendors to implement daily cleaning and weekly disinfection routines. Having access to ***clean running water*** was another major factor in reducing AIV contamination. In a previous study in Cambodia, 75% of poultry carcass wash water samples were found positive for AIV [25]. Obviously, and notably, if the same source of water is used for multiple purposes, this increases the risk of cross-contamination. This a problem that could be mitigated if clean running water would be readily available and used. We found that as many as 44% of stalls already had running water facilities, implying that extending these facilities to other vendors might be feasible.

Keeping multiple breeds in the same cage has previously been suggested to contribute to the spreading of AIV infection in stalls yielding a three times higher risk of AIV [64]. In our study, we found this to be as high as 20 times. However, it needs to be considered that the much bigger effect that we found may, at least in part, be due to vendors selling single breeds of poultry only. Irrespectively, this highlights that mixing of breeds (and possibly species) is a practice that should be avoided as much as possible as it greatly reduces the risk of AIV contamination. Poultry vendors and intermediaries frequently visit

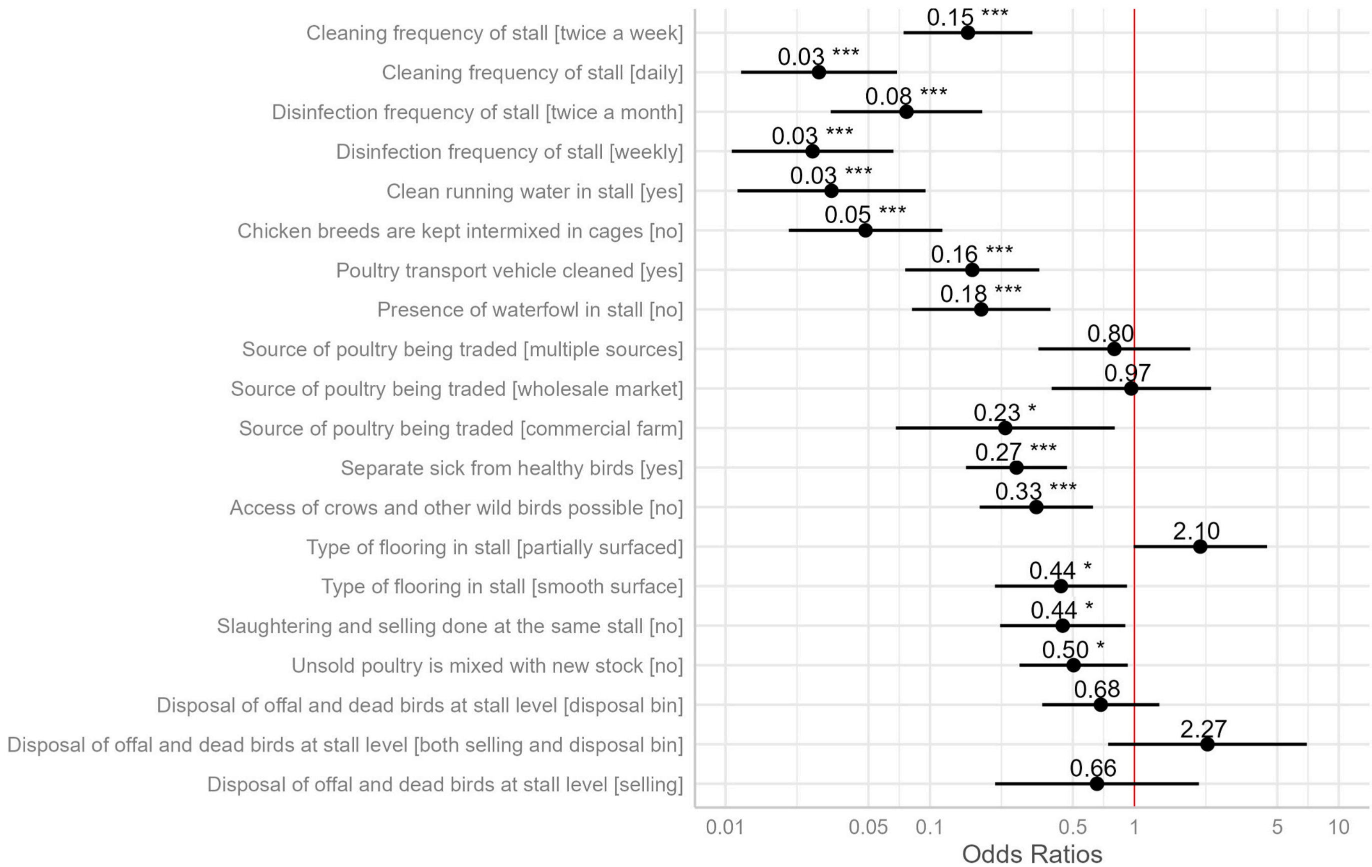

Fig. 3. Estimated odds ratios (± 95% confidence interval) for the 13 risk factors predicting AIV contamination in LBM stalls. For each risk factor, the category that was a priori considered to yield the highest risk of contamination is included in the intercept (i.e., equivalent to an odds ratio of 1). Significance levels indicated using * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Data from Table 2.

multiple farms in a single day to collect a small number of live birds, which they subsequently transport to wholesale markets or other vendors in vehicles that are rarely thoroughly cleaned [65], promoting viral transmission between birds from various sources [66]. Therefore, possibly unsurprisingly, we found that ***cleaning poultry transport vehicles*** after each transport reduced the AIV contamination rate, similar to what was previously reported in both Vietnam and Bangladesh [37,40]. However, in our study, the effect was large (odds ratio 0.16), strongly calling to make this a standard practice, which should be feasible given that this already happened in 41% of the cases (Fig. 1). The ***presence of waterfowl*** in poultry stalls increases the risk of AIV contamination have found in previous studies in LBMs in Bangladesh [16,51]. However, they reported a doubling of that risk rather than the fivefold increase in risk found in our study. Waterfowl are considered the natural reservoir for most subtypes of influenza A virus [67,68], shedding the virus asymptomatically, which, as shown here, is a significant risk factor increasing the circulation of AIV within LBMs.

In Bangladesh, the ***source of poultry*** is diverse. Sources include poultry coming directly from farms and through middlemen, as well as wholesale markets. We found poultry contamination in stalls to be lowest when the poultry comes directly from commercial farms when poultry comes directly from farms (odds ratio 0.23). Notably, in the big cities and metropolitan areas of Bangladesh, wholesale and middlemen are the primary source of supplying live birds to the LBMs [51]. It has been noted previously that complex trade networks enhance AIV transmission in Vietnam [69,70]. Unsurprisingly, being aware of the birds' health status and ***separating sick birds*** from healthy birds decreased the presence of AIV contamination in poultry stalls. A previous study in LBM from Bangladesh [71] reported that separate sick from healthy birds decrease the risk of AIV presence by 24%, whereas we found it to decrease by 74% (odds ratio 0.26). We found that ***wild birds having access*** to the stalls is associated with a threefold higher risk of AIV presence, which is supported by earlier findings of Sayeed, Smallwood [51]. Wild birds frequently scavenge on offal or dead birds that might be infected with AIV, and they could, therewith, be an important vector for AIV dispersal between stalls and LBMs [72,73]. That wild birds are implicated in AIV dynamics and dispersal was evidenced by several studies finding unusually high house crow mortality in Bangladesh due to HPAI H5N1, with LBMs being considered the primary source of infection [26,27,56]. To thoroughly clean and disinfect unsealed surfaces is problematic, resulting in an unhygienic state of stalls that lack smooth surfaces [12,51]. Accordingly, and in accordance with a previous study [71], we found that stalls with an unsurfaced or partially surfaced ***flooring system*** had a more than two times higher risk of AIV contamination than those that had sealed flooring. While the sealed flooring system undeniably comes at a cost, 43% of investigated stalls already had such flooring, while 34% was partially sealed. ***Slaughtering at the point of sale*** is common practice in the LBMs of Bangladesh [74]. Yet, as evidenced in our study and a previous study [71], slaughtering and selling poultry within the same stall roughly doubles the risk of AIV presence within that stall. Similarly, promoting AIV risk at stalls is ***mixing unsold poultry with new stock***, since the practice likely facilitates the continuation of AIV circulation within the stall [51,75,76]. While the way in which ***disposal of offal*** is organized in stalls did not significantly determine AIV presence at those stalls, improper waste disposal management can still facilitate opportunities for environmental exposure to AIV [77]. For instance, if offal and dead birds are discarded in open areas, street dogs and scavenging birds, such as crows, can become exposed and infected, as has been confirmed to be frequently the case in house cows in Bangladesh [26,56].

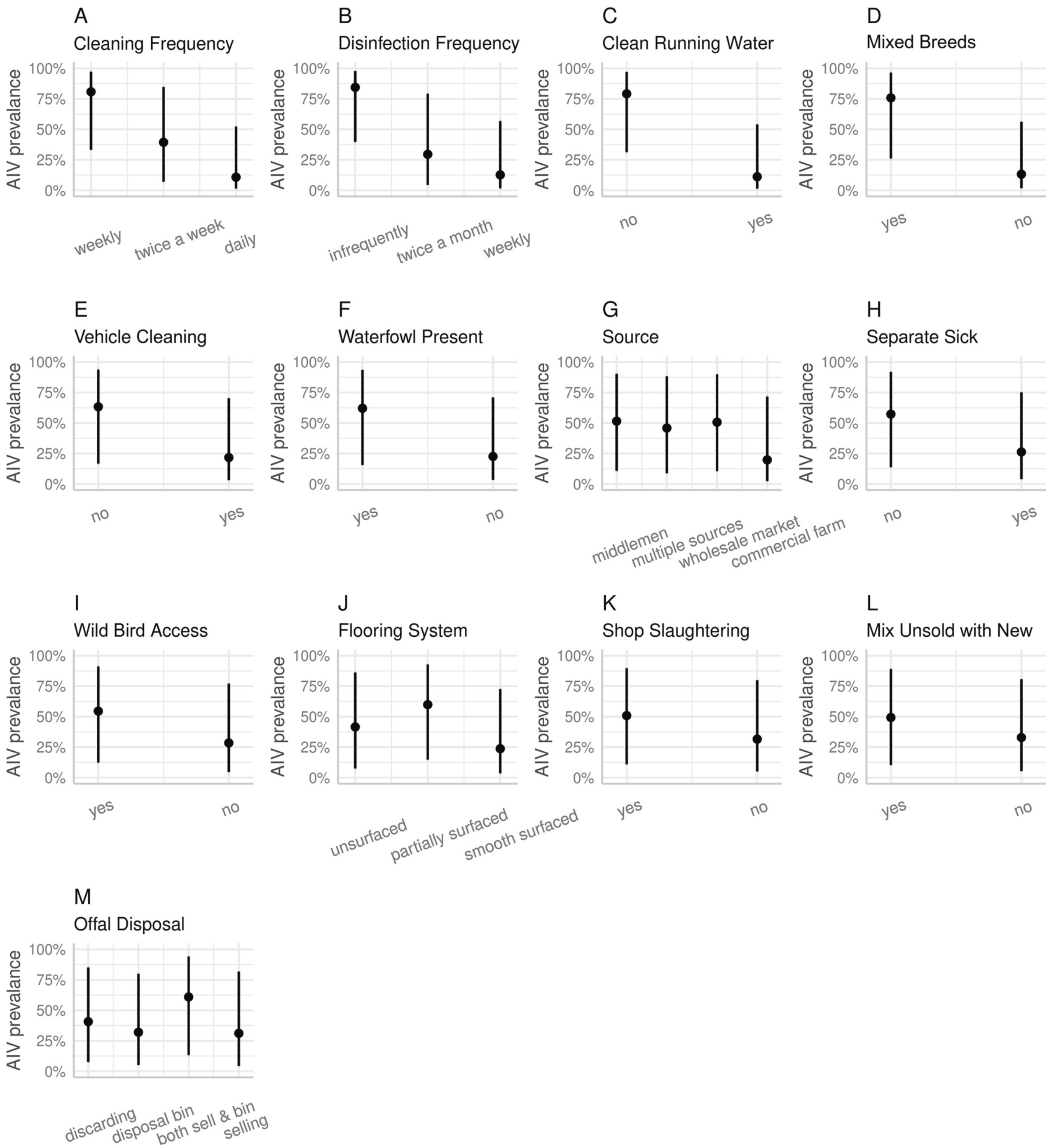

Fig. 4. Marginal mean plots depicting the isolated effects of all 13 explanatory variables (i.e., risk factors) in explaining whether samples taken from stalls are either positive (1) or negative (0) (i.e., effects after correcting for all other effects in the ultimate model (i.e., where all other explanatory values are set to their average values)). Average probability estimates with their 95% confidence limits are provided.

In a setting with limited resources, such as in Bangladesh and many other countries in south and south-east Asia, improvements in biosecurity should come at no or only minimal costs. This holds not only for the costs of the desired improvements in infrastructure, the cost of executing the new practice, and possible associated loss of income but also for the administrative costs of implementation and compliance checking. In Guangzhou, China, implementing a weekly, one-day closure for enhancing disinfection and cleaning successfully reduced AIV infection in LBMs [62]. Also, during a zoonotic outbreak of H7N9 in China in 2013, closing LBMs was demonstrated to be surprisingly effective in reducing the risk of human infection by up to 99% [62]. However, such closures, even only if one day a week, are obviously associated with a loss of income and will also not be popular amongst consumers.

Moreover, our study suggests that drastic reductions in AIV prevalence in LBMs can be achieved without closures and by means of more widely adopting specific practices that are already common practice for many vendors, e.g., daily cleaning and weekly disinfecting stalls. Yet, aside from cleaning and disinfecting frequently, we have identified a host of other practices and infrastructure characteristics that have dramatic impacts on reducing AIV prevalence at LBMs and are commonly practiced. For poultry vendors to embrace these practices for the benefit of not only poultry and wildlife but also human health will require concerted efforts from a variety of authorities, including state veterinarians, public health officers, and market authorities.

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CRediT authorship contribution statement

Ariful Islam: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Software, Writing – original draft. **Mohammed Ziaur Rahman**: Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. **Mohammad Mahmudul Hassan**: Data curation, Methodology, Resources, Supervision, Writing – review & editing. **Jonathan H. Epstein:** Funding acuisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Marcel Klaassen:** Conceptualization, Formal analysis, Resources, Software, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100643.

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