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Farm biosecurity practices affecting avian influenza virus circulation in commercial chicken farms in Bangladesh.

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ABSTRACT

Avian influenza virus (AIV) is of major concern to livestock, wildlife, and human health. In many countries in the world, including Bangladesh, AIV is endemic in poultry, requiring improving biosecurity. In Bangladesh, we investigated how variation in biosecurity practices in commercial chicken farms affected their AIV infection status to help guide AIV mitigation strategies. We collected pooled fecal swabs from 225 farms and tested the samples for the AIV matrix gene followed by H5, H7, and H9 subtyping using rRT-PCR. We found that 39.6% of chicken farms were AIV positive, with 13% and 14% being positive for subtypes H5 and H9, respectively. Using a generalized linear mixed effects model, we identified as many as 12 significant AIV risk factors. Two major factors promoting AIV risk that cannot be easily addressed in the short term were farm size and the proximity of the farm to a live bird market. However, the other ten significant determinants of AIV risk can be more readily addressed, of which the most important ones were limiting access by visitors (reducing predicted AIV risk from 42 to 6%), isolation and treatment of sick birds (42 to 7%), prohibiting access of vehicles to poultry sheds (38 to 8%), improving hand hygiene (from 42 to 9%), not sharing farm workers across farms (37 to 8%), and limiting access by wild birds to poultry sheds (37 to 8%). Our findings can be applied to developing practical and cost-effective measures that significantly decrease the prevalence of AIV in chicken farms. Notably, in settings with limited resources, such as Bangladesh, these measures can help governments strengthen biosecurity practices in their poultry industry to limit and possibly prevent the spread of AIV.

1. Introduction

On a global scale, the socio-economic importance of the poultry industry is large and growing, having resulted in poultry currently forming 70% of the global avian biomass [1] and the industry providing 40% of all animal protein to the global population [2]. Unfortunately, the industry is marred with problems associated with avian influenza virus (AIV). From around the change of the century, high pathogenicity avian influenza virus (HPAI) H5N1 evolved into a widespread and highly virulent poultry virus with increasingly more frequent spillovers in wild birds, contributing to its spread [3,4]. The current panzootic HPAI H5N1 of clade 2.3.4.4b is causing unprecedented mortality in poultry and wild birds across all continents of the globe except Antarctica and Australia [5,6]. HPAI is concerned not only to birds but also to humans, as it poses a potential pandemic risk and has caused >800 human cases of H5N1 in the past five years [7,8]. Also, in Bangladesh, poultry farming is one of the most significant livestock industry sectors, contributing to the country's economic growth by creating employment opportunities and income for both rural and semi-urban populations [9–11]. The industry grew 2.5-fold between 1995 and 2017 [12].

Bangladesh has two principal poultry production systems: commercial poultry production, where birds are housed in absolute confinement, and small-scale backyard poultry production, in predominantly rural areas [13–15]. Commercial chicken production is the primary source of animal protein, contributing to 20% of protein consumption and 37% of the country's total meat supply [16–18]. Since 2007, HPAI

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has also been a problem for poultry production in Bangladesh; during the two waves of H5N1 in 2007 and 2008, around 547 commercial chicken farms were impacted and culled, involving 1.7 million birds, resulting in an estimated economic loss of US$746 million [19–21]. Among poultry species, chickens are most susceptible to HPAI H5N1, causing high morbidity and mortality [22]. The number of commercial chicken farms in Bangladesh was halved between 2007 and 2013 due to the detrimental effects of H5N1 [23]; the industry faced annual waves of AIV outbreaks from 2007 until 2012. In 2012, vaccination against the virus was introduced [24], and the number of outbreaks dropped significantly [25]. Thus far, >580 HPAI outbreaks in 54 of the 64 districts in Bangladesh have been reported [26–28], the vast majority of which in commercial poultry farms [25,29]. Not only HPAI H5N1 but several other subtypes of HPAI and low pathogenicity avian influenza (LPAI) have been detected in Bangladeshi farms, with H9N2 and H5N1 being the most prevalent [30–32]. Aside from chickens and other birds, infections with AIV in humans who had direct contact with poultry have also been reported in Bangladesh [33,34]. Since the first detection of its kind in 2008 [35], a total of 11 human AIV cases have been reported, where eight were caused by HPAI H5N1 [36], and three were LPAI H9N2 [37]. The co-circulation of different subtypes within the country raises concern about the possible emergence of novel, reassortant viruses with more devastating effects on birds but also with an increasing capability to infect and transmit among humans and other mammals [30,34,38].

Biosecurity is the application of measures that reduce the risk of disease agent introduction and dissemination. The prime means to combat HPAI is by improving biosecurity within the industry [34,39]. Good biosecurity generally consists of three elements: isolation, traffic control, and sanitation [40,41]. Key elements that FAO has used to make a series of biosecurity guidelines to prevent and control HPAI in poultry farms globally [42]. Also, in Bangladesh, to mitigate the HPAI problems, the government introduced biosecurity guidelines based on the FAO recommendation for commercial poultry [43]. Unfortunately, it has been frequently observed that biosecurity practices on commercial poultry farms in Bangladesh are insufficient. For example, visitors can often access commercial poultry farms without disinfecting their shoes, clothing, or equipment. In addition, wild birds can often enter poultry sheds, while wild and domestic animals frequently roam farm premises, and poultry waste is commonly left in the open [44,45]. Several biosecurity-related risk factors were identified in three studies conducted in Bangladesh that compared outbreaks with randomly selected non-outbreak commercial poultry farms. These risk factors included the exchange of egg trays with market vendors, the number of farm employees and farm workers trading chickens, and the presence of free-roaming chickens, feral animals, and wild animals on the premises [46–48]. Thus, there are ample indications that failing biosecurity is part of the HPAI problem in Bangladesh's commercial poultry farming. And Bangladesh is not alone in this. In a study conducted in Pakistan, H9N2 infection in commercial poultry farms was associated with the direct sale of eggs and birds to live bird markets (LBMs) [49]. In Nepal, significant risk factors for H9 outbreaks on commercial poultry farms were found to include inadequate disinfection of footwear upon entrance and disinfection of poultry sheds through fumigation, as well as access by unauthorized visitors [50]. Similar problems also occur in developed countries. For instance, inadequate hygienic measures for farm visitors and the sharing of farm equipment among farms in Japan [51], improper disposal of dead birds, and the presence of mammalian wildlife on commercial farms in the United States [52], and a high number of contacts between farms through reuse of cardboard egg trays in the Netherlands [53] were significant risk factors for AIV infections on commercial chicken farms.

Most of the commercial poultry farms in Bangladesh are small-scale farms having a poultry population of ≤4000, accounting for 81% of the total commercial poultry farms in Bangladesh [44]. For many of the farm holders, financial constraints and impracticalities may be important impediments to implementing and maintaining Department of Livestock Services (DLS) biosecurity guidelines, which consist of as many as 135 different recommendations [43]. To develop an effective and affordable avian influenza prevention and control strategy for Bangladesh, a robust understanding of the biosecurity risk factors associated with AIV circulation in commercial poultry farming is critical. This would allow prioritization of the 135 recommendations and guide communication and enforcement strategies, improving biosecurity and reducing HPAI risk. Here, we investigated how variation in biosecurity practices across commercial poultry farms affects their AIV infection status, allowing for a quantitative assessment of how AIV mitigation strategies in poultry production affect AIV risks to those production systems.

2. Materials and methods

2.1. Ethics approval

The research protocol was reviewed and approved by both the Animal Experimentation Ethics Committee and the Ethics Committee at the Chattogram Veterinary and Animal Sciences University (Protocol: CVASU/Dir(R&E) EC/2019/126(1)).

2.2. Study system

Commercial chicken farming systems in Bangladesh are primarily classified into layers, broilers, and Sonali chicken farms. Broiler and Sonali (crossbred of cock Rhode Island Red × hen Fayoumi) farming raises chickens for meat, whereas layer farming raises hens for egg production, though layer birds are ultimately also sold for meat once their egg production drops [54]. From January 2017 until July 2019, we performed a cross-sectional study in 225 commercial chicken farms in 6 different Bangladeshi districts: Dhaka, Narsingdi, Gazipur, Mymensingh, Pabna, and Rajshahi (Fig. 1). Within this period of two and a half years, we organized a total of 18 sampling campaigns, and each campaign was limited to a restricted area within Bangladesh, lasting a maximum of 4 days. Farms were selected randomly. Out of the total of 225, 59 were broiler farms (typically housing 1000 to 2500 chickens), 65 were Sonali farms (typically housing 300 to 2000 chickens), and 101 were layer farms (typically housing 1000 to 4000 chickens).

2.3. Biological samples and data collection

We collected an average of 3 pooled fecal swabs ranging from 1 to 5 pooled samples from each farm; each pooled fecal sample consisted of 4 swabs (Fisher brand sterile polyester swabs with plastic shaft) depending on chicken farm size and number of sheds in the farm. We collected swabs by swabbing freshly deposited fecal droppings, making sure that swabs were collected evenly spaced within sheds. Occasionally, not only swabs from fresh fecal matter but also cloacal and oropharyngeal swabs from dead or apparently sick birds were included in the pools. We stored the pooled swabs in a 3.6 ml cryovial or 10 ml falcon tube containing 3 ml viral transport media (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) [56]. All vials were placed in a liquid nitrogen container (−196 °C) and later stored at −80 °C in the laboratory until testing. The DLS guidelines provide as many as 135 biosecurity recommendations, with a significant proportion of these recommendations being specific to farms of specific sizes and relating to specific production systems. Hence, we collected data from each farm relating to the biosecurity checklist and consisting of 20 categorical variables, with two to three levels each, evaluating the extent to which each investigated farm was constructed and operated in accordance with the DLS biosecurity guidelines.

These 20 variables include live bird market (LBM) present within

Fig. 1. Map showing districts of Bangladesh. The hued regions represent the districts where commercial chicken farms were studied. The map was created with ArcGIS v10.4.1 (ESRI, Redlands, CA, USA) using a shapefile from DIVA-GIS (https://www.diva-gis.org/) [55].

500 m distance from farm (yes/no), unrestricted visitor access to the farm (yes/no), isolation and treatment of sick birds (yes/no), vehicles having entry into the farm shed (yes/no), hand hygiene (none/water only/water and soap), workers work and visit other farms too (yes/no), wild bird have access to farm premises (yes/no), disinfecting equipment used at the farm (yes/no), transport mode of live birds and eggs (own van/shared van), footbath present at farm entrance (yes/no), rodent access to farm premises (yes/no), use of separate dedicated clothing for farm workers (yes/no), backyard poultry access into farm premises (yes/no), stray dog access to farm (yes/no), sharing equipment with other farms (yes/no), disinfecting vehicles prior to transport (yes/no), poultry drinking water treatment (yes/no), disinfecting poultry housing between batches of new chickens (yes/no), appropriate disposal of dead birds (yes/no; i.e. bury or remove from premises rather than discard in open), appropriate disposal of litter and waste (yes/no).

2.4. Laboratory testing

We tested pooled swab samples from each farm to detect the presence of the AIV Matrix (M) gene. Following the manufacturer's instructions, RNA was extracted using a MagMAXTM-96 AI/ND Viral RNA Isolation Kit (Applied BiosystemsTM, San Francisco, CA) in a King-FisherTM Flex 96-well robot (Thermo ScientificTM, Waltham, MA). The extracted RNA from the swab samples was tested for the M gene using real-time reverse transcription PCR (rRT-PCR) with reference primers and probes [57,58]. Positive samples for the M gene were further subtyped for H5, H7, and H9 using hemagglutinin gene-specific primers and probes in a reverse transcription-polymerase chain reaction (rRT-PCR) assay [58,59]. Samples that tested positive for the M gene but negative for the H5, H7, and H9 genes were categorized as AIV HA/untyped. A farm was considered AIV-positive if at least one pooled sample was M-gene positive.

2.5. Statistical analyses

We performed all statistical analysis using R version 4.2.0 within RStudio version 2022.02.2 [60]. Using the ggplot2 package, we presented the prevalence with a 95% confidence interval (CI) of the AIV M gene and its subtypes (H5, H9). Using function pie, we illustrated frequency distributions of the 20 farm biosecurity variables using pie charts. We computed Cramer's V among these 20 categorical explanatory variables to check for collinearity [61]. Variables that had a Cramer's V >0.3 with any of the other 19 explanatory variables were considered for elimination [61,62].

Using the remaining explanatory variables after elimination, we performed a Generalized Linear Mixed Effect Model (GLMM) in R package lme4 to assess the effects of bio-security practices on AIV presence/absence at the investigated farms. In this binomial model, we considered two additional continuous variables: the size of the flock on the farm (divided by 100) and the age of the flock in weeks (minus the average age of all flocks investigated). Finally, we considered three random variables in the model: sampling campaign, number of pooled samples per farm, and farm type (broiler, layer, Sonali). Odds ratios for the model estimates were plotted using the package sjPlot. After GLMM, Partial residual plots for all variables in the model were next generated using the ggemmeans function within the ggeffects package [63].

3. Results

We detected a significantly high percentage of farms positive for AIV (39.6%, 95% CI: 33.1–46.3) (Fig. 2A). Moreover, in about one-third of the AIV-positive cases, or 13% of farms, we found H5. H9-positive farms were found in 14% of the investigated farms. While there were no differences in AIV prevalence across the different production systems, there were significant differences in subtype prevalence for both H5 and H9 subtypes. Sonali chicken farms had the highest prevalence of H5 (21.5%, 95% CI: 12.31–3.49) (Fig. 2B), while broiler farms had the highest H9 prevalence (25.4%, 95% CI: 14.98–38.44).

Among the 225 investigated farms, we found that all categories within 17 out of the 20 farm biosecurity variables were near-uniformly distributed (Fig. 3). Among the three variables that stood out was the practice of isolating and treating sick birds, which was not practiced at 65% of the farms. Also, the use of separate clothes when working with poultry was underrepresented and did not apply to 69% of the farms. Finally, the vast majority of farms (68%) used to treat the drinking water before giving it to poultry.

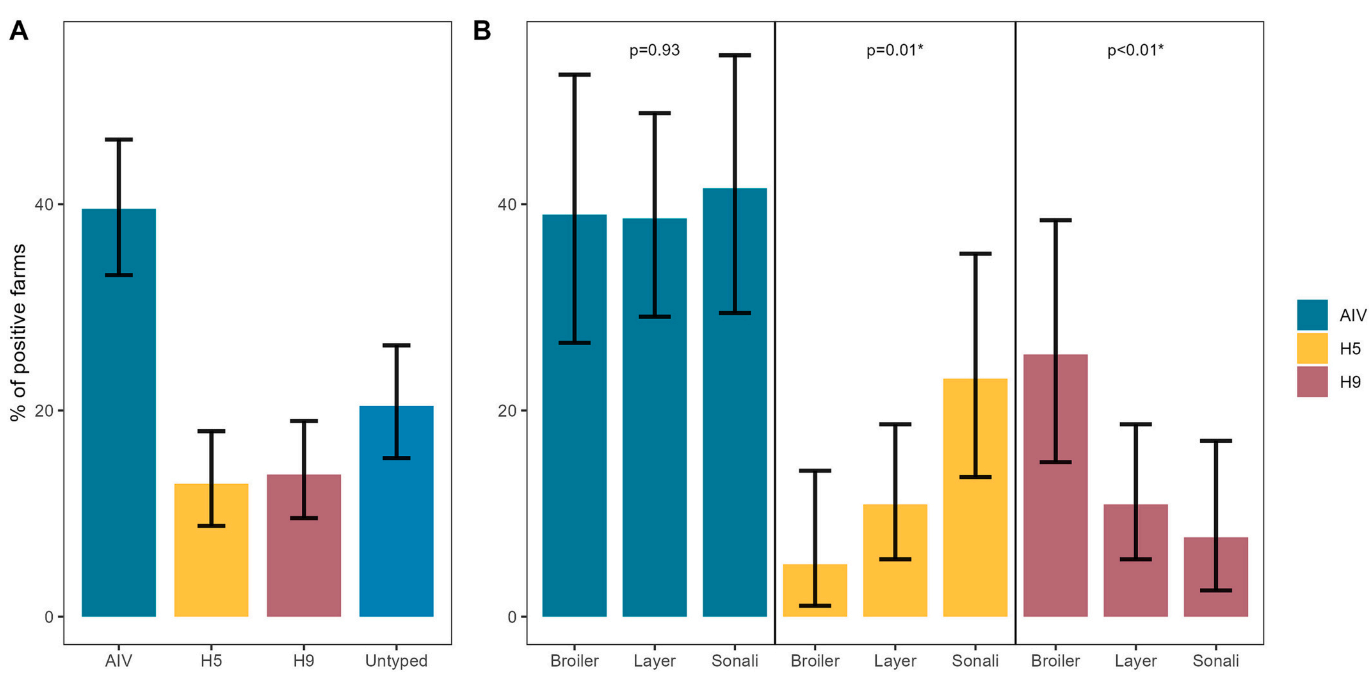

Fig. 2. The prevalence of AIV and its investigated subtypes, along with their 95% CI across all 225 investigated commercial poultry farms in Bangladesh (Panel A) and separated across the three different production systems (Panel B).

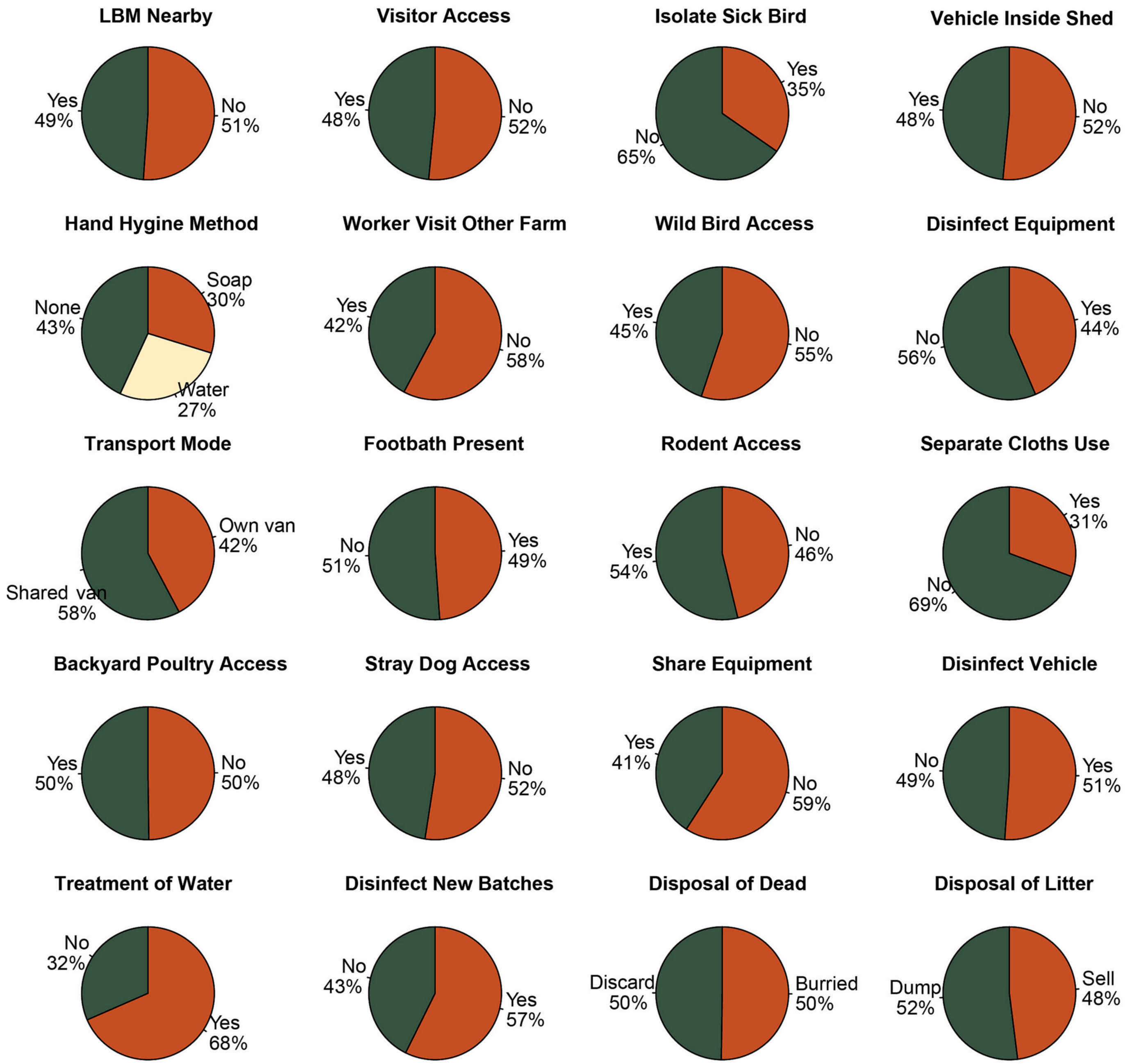

Fig. 3. Frequency distribution of the different categories within the 20 variables describing the biosecurity standards within the 225 studied representative commercial poultry farms in Bangladesh.

Taking all 20 variables together and considering our selection of farms was representative, the picture emerges that, in many cases, DLS's biosecurity recommendations [43] are not being followed and that there is considerable room for biosecurity improvement. Indeed, the near-equivalent representation of good and poor biosecurity standards across all farms is an important prerequisite for a powerful multivariate analysis to help identify the effect size of the various biosecurity practices investigated here.

In checking for collinearity between these 20 explanatory variables, we detected 17 pairs of variables with a Cramer's V >0.3 (Supplementary Fig. 1). To mitigate this problem, we eliminated eight variables from the analyses: the presence of backyard poultry on the premises, disinfecting shed(s) between new batches of chickens arriving, disinfection of transport vehicles, proper disposal of the litter, proper disposal of dead animals, the sharing of equipment across farms, treatment of drinking water for poultry, and the access of stray dogs" variables from the model because of collinearity.

Of the 14 remaining explanatory variables investigated (i.e., 12 categorical and 2 continuous variables), as many as 11 showed significant effects on the presence/absence of AIV in the investigated farms (Table 1, Fig. 4). For the continuous variables, a one-unit rise in flock age (in weeks) resulted in a predicted 5% decrease in AIV risk, while a one-unit rise in flock size (per 100) resulted in a 16% increase in AIV risk in chickens.

While apparently small effects, considering the potential full range of these variables, the predicted AIV prevalence could vary by as much as 50% by age (Fig. 5A) and 80% by flock size (Fig. 5B), respectively. Obviously, the age of the flock sampled at the respective farms is not a biosecurity-related factor, but flock size in larger production facilities is evidently more at risk of being AIV-contaminated. We present the

Table 1
Estimates with standard error (SE) and *p*-value of generalized linear mixed effect model of presence/absence of AIV on commercial poultry farms in Bangladesh as a function of farm characteristics, including biosecurity practices. Explanatory variable (reference category)

	Category	AIV			
		Estimate	Std. error	Statistic	p-value
Age of flock (in weeks)		−0.05	0.02	−2.32	0.02
Flock size (in hundred)		0.14	0.05	2.63	0.01
Nearby LBM present at <500 m (Yes)	No	−2.99	0.65	−4.60	<0.01
Visitor access to inside the farm (Yes)	No	−2.36	0.63	−3.75	<0.01
Isolate and treat sick birds (No)	Yes	−2.34	0.68	−3.43	<0.01
Vehicles entry into the farm shed (Yes)	No	−2.00	0.60	−3.33	<0.01
Method of hand hygiene (No use of water)	Water	−1.46	0.75	−1.95	0.06
	Water and Soap	−1.99	0.70	−2.86	<0.01
Workers from this farm visit other farms (Yes)	No	−1.88	0.63	−2.97	<0.01
Wild bird access around farm premises (Yes)	No	−1.91	0.60	−3.20	<0.01
Cleaning equipment used in farm (No)	Yes	−1.54	0.62	−2.47	0.01
Live bird and egg transport mode (Shared van)	Own van	−1.30	0.59	−2.22	0.03
Footbath present at farm entrance (No)	Yes	−0.96	0.59	−1.63	0.10
Rodents present in farm environment (Yes)	No	−0.78	0.57	−1.36	0.18
Use of separate clothes inside the farm (No)	Yes	0.12	0.65	0.19	0.85

categorical variables in order of their effect on the presence/absence of AIV on farms. The presence of an LBM within 500 m of a commercial poultry farm was identified as a highly significant risk factor, with the presence of a nearby LBM being associated with an AIV prevalence of as much as 50%.

In comparison, the absence of an LBM reduced that risk to a mere 5% (Fig. 5C). Visitors' access to the farm had a predicted AIV risk of 42% versus only 6% if visitor access was restricted (Fig. 5D). Isolation and treatment of sick birds also had a major effect, reducing the risk of AIV on a farm by 90%, with no isolation and treatment of sick birds being associated with an AIV prevalence of 42% versus only 7% if sick birds received special treatment (Fig. 5E).

When poultry transport vehicles had direct access to poultry sheds, the predicted AIV prevalence was 38%, while it was only 8% if they had not (Fig. 5F). If workers used both water and soap prior to and after handling poultry rather than none at all, the odds of AIV presence were reduced by 86%. The predicted AIV prevalence among farms was thus only 9% when using water and soap, increasing to 14% if only water was used to a whopping 42% if neither was used (Fig. 5G). Farms where workers also worked at other farms had a predicted risk of AIV presence of 37% compared to 8% if they worked on one farm exclusively (Fig. 5H). The data also revealed that the absence of wild birds reduced the odds of AIV by 85% compared, resulting in a predicted AIV prevalence of 37% if wild birds were not excluded compared to 8% if they had no access (Fig. 5I).

Although still significant, the next two factors did not result in large discrepancies in predicted AIV prevalence. Cleaning and disinfection of equipment reduced the AIV odds by 79%, with the predicted prevalence for farms where equipment was not cleaned being 33% versus 9% where they were (Fig. 5J). Finally, the use of a shared poultry transport vehicle versus the use of an own vehicle was associated with predicted AIV prevalence of 30% and 11%, respectively (Fig. 5K). Footbath at the entrance, rodent access, and use of dedicated farm-working clothing had no significant effect on the presence of AIV at commercial poultry farms.

4. Discussion

We conducted a comprehensive assessment of biosecurity practices across a random selection of commercial poultry farms in Bangladesh and evaluated the impact of these practices on AIV presence in these farms. We found that a large proportion of farms did not follow DLS's biosecurity recommendations. Our study revealed an alarmingly high percentage of AIV-infected premises, amounting to 40%, with 13% of farms testing positive for HPAI H5 and 14% for H9. AIV infection rate among commercial poultry farms appeared particularly determined by (in order of impact) the flock size of poultry in the farm, distance to LBM, access of visitors, treatment of sick birds, access of transport vehicles, hand hygiene, sharing of workers across farms, wild bird access, disinfection of farm equipment and the sharing of vehicles to transport poultry.

While many of these risk factors have previously been found to be important [46–48], we here uniquely quantified their independent effects, allowing prioritization of inexpensive and easy-to-implement biosecurity practices that will have a great impact on reducing AIV prevalence in poultry farms in Bangladesh and similar settings elsewhere. The prevalence of AIV among commercial poultry farms was higher than findings in earlier studies [59,64], including the prevalence of H5 and H9 [65,66], which have been circulating in poultry since 2007 [67]. These findings indicate the inadequacy of the vaccination of commercial poultry in Bangladesh against H5N1, which was implemented in commercial poultry in Bangladesh in 2012 [68], and against H9N2 [21] in 2019 to irradicate these problematic viruses from Bangladesh. While since its introduction in Bangladesh, vaccination has resulted in a significant drop in outbreaks [69], vaccination in its current form in Bangladesh apparently suppresses signs of illness but has limitations in decreasing infection risk, leading to silent spread [70]. Introducing vaccination against AIV in the absence of monitoring bears the risk of vaccination-built herd immunity imposing a selection pressure promoting viral antigenic drift and thus enhancing virus evolution [38]. This, in turn, promotes the spreading of drift variants into poultry [21,71]. Also, in Egypt and Mexico, the H5N1 virus has been reported in vaccinated poultry in many antigenically different subclades and has been enzootic despite the introduction of the HPAI H5N1 vaccination [72,73].

Of the two continuous explanatory variables, ***flock size*** had the largest effect across the full range of flock sizes encountered, with the smallest and largest farms having a predicted AIV infection chance of near 0% and 80%, respectively. Also, other studies, both in Bangladesh [59] and elsewhere in Southeast Asia [50], found an effect of flock size on farm infection rates. While reducing flock size to lower the risk of AIV infection has a considerable impact on farm management and viability and may be challenging to implement, our finding suggests that larger farms should be particularly motivated to improve biosecurity standards on their premise. ***Flock age*** was the other significant continuous explanatory variable, with young poultry being more at risk of AIV infection. This is probably due to young birds being generally more vulnerable to infection and disease [74,75]. Moreover, older birds may have less viral shedding because of previous infection or due to the effect of vaccination developing with age, AIV antibodies increasing over time [64]. Furthermore, although the age of the flock cannot be altered, like for flock size, the finding highlights the significance of maintaining high biosecurity standards throughout this stage of the poultry production cycle.

Below, we discuss the 11 significant categorical explanatory variables in our model in order of their effect size on the presence or absence of AIV in poultry farms. ***Presence of an LBM within 500 m*** of the commercial poultry farm had a dramatic effect on predicted AIV risk, increasing it from 5 to 50%. In Bangladesh, commercial poultry is generally reared and sold close to LBMs [9]; our data indicate that as

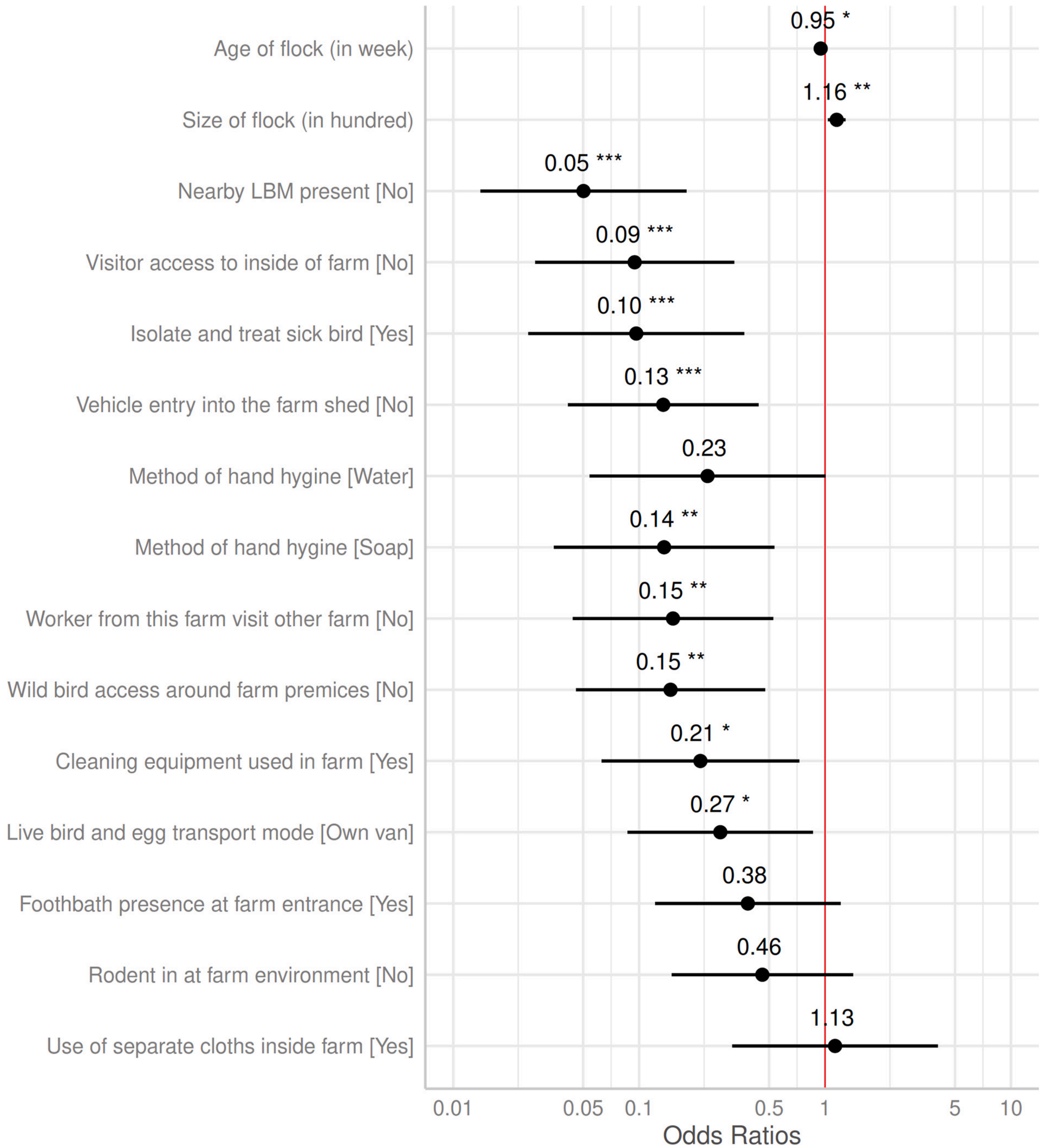

Fig. 4. Odds ratio with 95% confidence interval of the variables of the binomial mixed effect model relating farm characteristics, including biosecurity practices to the AIV presence/absence on commercial poultry farms in Bangladesh (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The top two variables are continuous variables followed by twelve categorical variables ranked by their impact on AIV prevalence.

many as 49% of all farms are within 500 m of an LBM. Like for flock size and age, changing the position of poultry farms relative to LBMs is problematic; farms in the proximity of LBMs should follow biosecurity procedures meticulously. Moreover, the establishment of new farms close to LBMs should be discouraged. Unrestrained ***access of visitors*** increased the predicted risk of AIV from 6 to 42%. That visitors having unrestricted access promotes AIV contamination was earlier reported prior to vaccination in Bangladesh Osmani, Thornton [46] and also proved critical in a study in Nepal [50]. Restricting access of poultry farms to poultry workers, veterinarians, and technical personnel required for the good operation of the farm, which also strictly follows decontamination protocols, thus appears to be of prime importance in reducing AIV risk. Moreover, this is also a straightforward and generally unproblematic farm policy to implement.

We should mention that unrestricted access of visitors was correlated with the ***presence of backyard poultry*** (Cramer's V = 0.33) and ***inadequate disposal of litter*** in the open (0.37). These factors could thus also be of importance and partly responsible for the 'visitor effect' identified in our model, warranting attention for these farm management aspects, too. ***Isolation and treatment of sick birds*** were the next most crucial farm practices, reducing predicted AIV risk from 42% to 7%. This finding is in line with observations by [44] and, again, a practice that may be relatively unproblematic to implement as a standard farm practice. Also, for this variable, a high Cramer's V of 0.35 was found with ***disinfection of sheds before new birds arriving***, which is thus another practice that should importantly be considered when aiming to

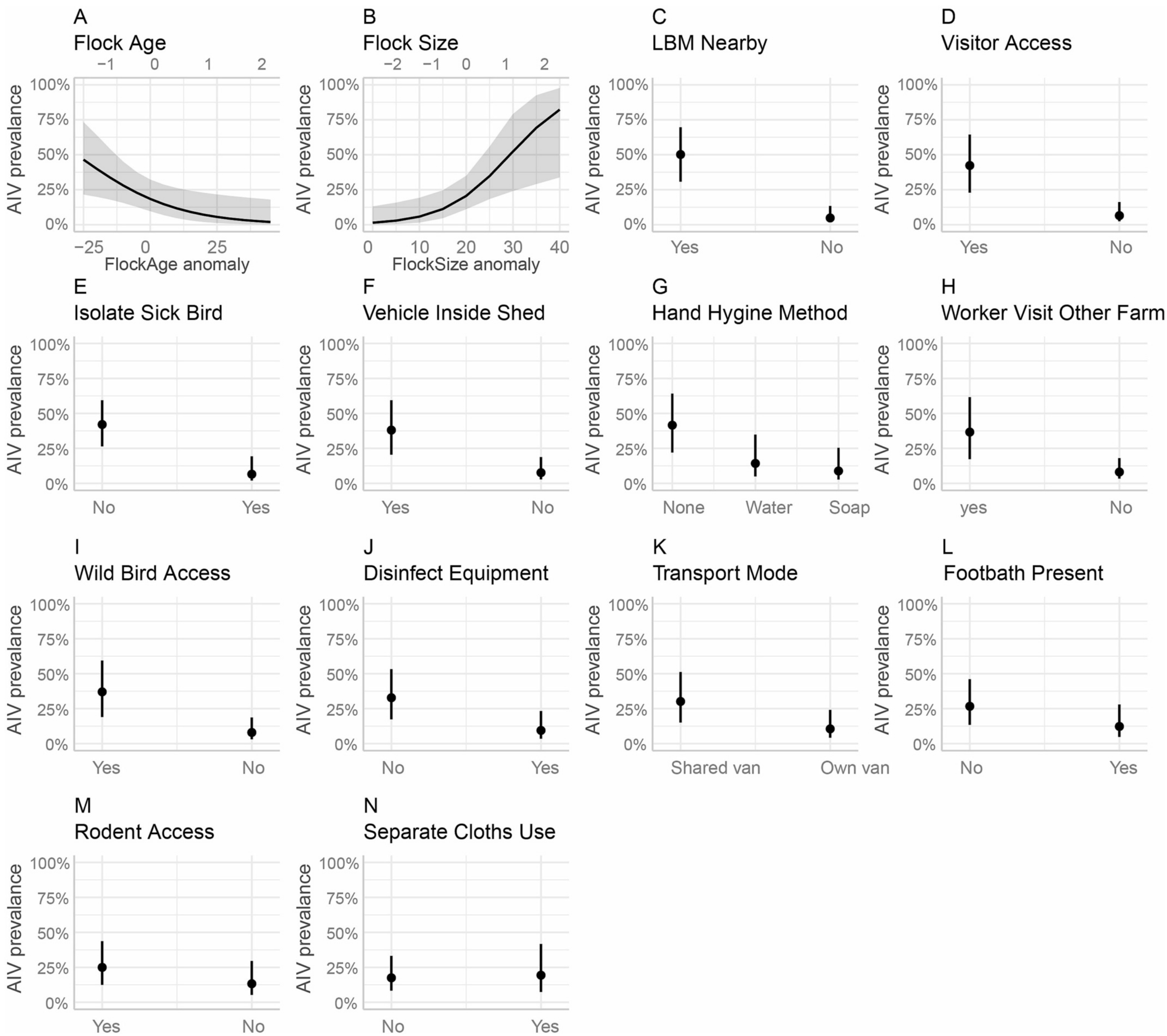

Fig. 5. Estimated marginal means (predicted values adjusted for all other effects) and their 95% confidence intervals for significant explanatory variables. In panels A and B, the top x-axes depict scaled values.

reduce AIV risk on poultry farms. Allowing poultry transport ***vehicles direct entry to farm sheds*** increased the predicted risk of AIV from 8 to 38%. This biosecurity hazard was also previously identified [67]. It increases cross-contamination risk due to vehicles transporting eggs and egg trays [53], chicks, and feed [44] among farms and markets. Although reducing such unrestricted access of transport vehicles to poultry sheds may increase workloads, given its apparent large effect on AIV risk, it requires serious consideration for prioritization. This variable also had high Cramer's Vs with ***disinfection of vehicles*** (0.32), ***the sharing of equipment with other farms*** (0.32), and the ***access of stray dogs*** to the premise (0.31), which are thus also variables that may be of importance and deserve consideration. Predicted AIV risk was reduced from 42% when no ***hand hygiene*** was practiced by farm workers to 14% if hands were washed with water to as low as 9% when hands were washed with water and soap. With farm workers in only 30% of commercial farms practicing hand washing with water and soap, there is an excellent opportunity to dramatically reduce AIV risk by implementing this practice more broadly. Hand hygiene also had high Cramer's Vs. with the ***sharing of equipment*** (0.32) and the ***treatment of water provided to poultry*** (0.43), and these variables thus also warrant attention. ***Sharing of farm workers*** across multiple farms increased predicted AIV infection risk from 8% to 37%. It was earlier found that farms with more workers, with an increasing probability of one or more of these also having duties on other farms, had a higher risk of AIV infection [46]. While bigger farms (with the associated increased AIV risk, see above) need more workers, it should be noted that the presented predictions here are independent of other variables in the model and are thus also not affected by flock size. This finding again highlights the dangers of cross-infection. While not allowing farm workers to work at other farms is likely to have implications for farm management and the workers themselves, our finding suggests that when workers are shared across premises, extreme care should be taken to avoid cross-contamination between farms.

Access by wild birds increased the predicted AIV risk from 8 to 37%. Wild birds are the natural reservoir of AIV, and they can contaminate the environment of the farm with their droppings, contributing to the transmission of AIV [22,76]. Unsurprisingly, many studies have thus found that the presence of wild birds at farms increases AIV risk [48,77,78]. While avoiding all access by wild birds may be problematic to realize, it is mainly ducks and scavengers such as crows that are seemingly implicated in AIV transmission, at least in Bangladesh [79–82], and avoiding their presence on commercial farms should be more feasible. Somewhat less important yet significant factors promoting AIV risk were found to be ***not disinfecting farm equipment*** and the ***transport of birds in vehicles shared with other farms.*** The latter was also found to be of importance in previous studies [47,48,83]. The use of shared vehicles also had high (>0.3) Cramer's Vs with ***sharing of equipment***, ***disinfection of vehicles***, and ***access by stray dogs***. Mitigation for all these factors need not come at great cost and should thus be considered, without maybe the use of own means of transport, which may need investment and possibly come at a prohibitive cost. However, this factor again highlights the risk of cross-contamination, which could be mitigated by better hygiene and disinfection practices, for which costs are limited. The conclusions drawn from our study rely on the data provided by farmers; consequently, we were unable to observe whether they implemented the biosecurity measures they claimed to have taken. While we recognize the possibility that recall bias could influence the responses of farmers; however, we have mitigated this concern by conducting a thorough analysis of the current poultry production cycle.

5. Conclusion

Our research revealed a high prevalence of AIV in all three types of chicken farms, with the HPAI H5 virus being particularly prevalent in Sonali chicken farms. We also found that AIV infection in poultry farms can be effectively mitigated. While some of these may require investments and longer-term planning, our research suggests that improved compliance with existing DLS and FAO recommendations would likely significantly reduce the prevalence of AIV on farms in Bangladesh. The fact that approximately half of the farmers are following the compliance of biosecurity guidelines is proof in case that it can be done. Governments at all levels should take the lead in informing farmers about the benefits of investing in biosecurity compliance and how non-compliance causes high contamination risk with the ever-evolving AIV and providing them with training on practical, low-cost biosecurity measures for which our results provide relevant guidance.

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CRediT authorship contribution statement

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

Declaration of competing interest

We have no financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work.

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.2024.100681.

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