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(RESEARCH ARTICLE)

AVIAN INFLUENZA VIRUS INFECTION AMONG WORKERS AND BIRDS IN LIVE BIRD MARKETS IN ACCRA, GHANA

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ABSTRACT

Avian influenza (AI) poses a significant zoonotic risk, particularly in live bird market (LBM) settings where human–poultry interaction is frequent. This cross-sectional study aimed to determine the prevalence of avian influenza virus (AIV) infection among workers and poultry at eight LBMs in Accra, Ghana, and to assess the knowledge, attitudes, and biosecurity practices of market workers. A total of 81 market workers and 396 birds were enrolled. Human nasopharyngeal/throat swabs were tested by real-time reverse transcription PCR (rRT-PCR); serum samples were tested for antibodies to influenza AH1N1pdm09 and B Victoria by haemagglutination inhibition (HAI) assay. Tracheal and cloacal swabs from poultry were similarly tested by rRT-PCR. Influenza A-positive bird samples were sequenced using Oxford Nanopore technology, and phylogenetic trees were constructed using MEGA11.

No human samples tested positive for influenza virus by rRT-PCR (0%). Serological analysis revealed that 32.9% of workers had high-titre antibody responses to AH1N1pdm09 and 11.4% to B Victoria. Among 396 bird samples, 118 (29.8%) tested positive for influenza A H9 and 7 (1.8%) for H5. Phylogenetic analysis placed the H5N1 isolates within clade 2.3.4.4b, clustering with West African strains from 2021–2022. The H9N2 strains formed a well-supported monophyletic clade indicating a recent, localized outbreak. Notably, the H5N1 HA carried the Q218L mutation, associated with enhanced binding to human-type α2-6 receptors, highlighting zoonotic risk. PPE usage was critically low: only 4.9% used gloves and 1.2% used face masks. These findings underscore the need for intensified AIV surveillance, improved biosecurity enforcement, and targeted health education in Ghanaian live bird markets.

Keywords: Avian Influenza; Biosecurity; Live Bird Markets; Prevalence; Zoonosis; Ghana

1 INTRODUCTION

Avian Influenza (AI), commonly known as bird flu, is a highly contagious viral disease affecting numerous avian species, including domestic poultry such as chickens, ducks, turkeys, and geese, as well as wild and pet birds [1]. The causative agents are influenza type A viruses belonging to the family Orthomyxoviridae, classified as highly pathogenic avian

influenza (HPAI) or low pathogenic avian influenza (LPAI) based on their capacity to cause disease in poultry. HPAI viruses, notably H5N1, can cause mortality rates approaching 100% in domestic poultry, while LPAI viruses typically produce milder clinical illness [2]. Globally, HPAI outbreaks, particularly H5N1 have caused catastrophic losses in the poultry sector through deaths and mass culling, severely disrupting food supply chains and causing enormous economic damage [3,4]. Several subtypes, including H5, H7, and H9, are capable of infecting humans, causing severe respiratory disease with case fatality rates of 40–55% for H5N1 [5,6,7].

In Africa, HPAI has been reported across Nigeria, Egypt, South Africa, Uganda, and the Democratic Republic of Congo, involving subtypes including H5N1, H5N8, and H5N2 [8,9]. Wild and migratory birds in Central and West Africa serve as a persistent reservoir for these viruses, amplifying the risk of geographic spread [10]. In Ghana, highly pathogenic H5N1 was first confirmed in poultry in 2015, causing significant losses and triggering outbreak response measures [11,13]. Subsequent surveillance has detected circulating AIV subtypes in commercial and backyard poultry in the Greater Accra Region [4,18,19]. A molecular and serological study in the Ashanti Region also reported evidence of AIV exposure in poultry farmers [20].

Live bird markets (LBMs) represent particularly high-risk interfaces for interspecies transmission of influenza viruses. The cohabitation of multiple avian species with varying susceptibility profiles, high bird turnover, inadequate biosecurity, and direct human–poultry contact in LBMs create conditions favorable for viral reassortment and amplification [16,17]. Poultry workers including traders, slaughterers, and veterinary officers who frequently visit these settings face significant occupational exposure to AIV [14,15]. Despite these risks, there is limited data on the prevalence of AIV at LBMs in Accra, and available information on the biosecurity practices and knowledge of live poultry traders (LPTs) remains sparse [3]. The absence of detailed surveillance data from LBMs, coupled with poor knowledge of circulating subtypes and weak biosecurity enforcement, impedes effective outbreak control [16,17].

Studies from comparable African settings, including Nigeria and Bangladesh, have documented circulating H9N2 in LBMs and seroprevalence to multiple influenza subtypes among market workers [21,22,24]. However, the Ghanaian LBM landscape remains inadequately characterized, limiting the ability of national health authorities to assess zoonotic risk and design targeted interventions. This study was therefore designed to determine the prevalence of AIV among poultry and workers at LBMs in Accra, to characterize any circulating AIV strains by sequencing, and to assess the knowledge, attitudes, and biosecurity practices of market workers.

2 MATERIALS AND METHODS

2.1 Study Design

A cross-sectional study was conducted between [month, year] and [month, year] at selected LBMs in Accra. Structured questionnaires were administered to consenting participants to collect data on demographics, occupational roles, biosecurity practices, and history of exposure to poultry. Biological samples were concurrently collected for laboratory analyses.

2.2 Study Setting

The study was conducted across eight LBMs in the Greater Accra Region of Ghana: Kantamanto, Dansoman, Mallam Atta, Dome, Kaneshie, Mallam, Achimota, and Nungua Markets. These markets were selected on the basis of high poultry transaction volumes and geographic distribution across Accra, ensuring a representative overview of AIV dynamics in the city's major LBM environment.

2.3 Inclusion and Exclusion Criteria

Eligible participants were workers aged 18 years and above who provided written informed consent and were directly involved in poultry-related activities including husbandry, slaughtering, sales, and veterinary services at the selected LBMs. Veterinary workers with frequent and direct contact with poultry in these markets were also included. Individuals not directly engaged in LBM activities (e.g., casual visitors or bystanders), as well as those unwilling or unable to provide informed consent, were excluded.

2.4 Sample Size and Study Population

The target study population comprised of LBM workers and market birds. The minimum sample size for human participants was calculated using the formula $n = Z^2P(1-P)/d^2$, where $Z^2 = 1.96$ (95% confidence level), $P = 0.5$ (conservative estimate), and $d = 0.05$ (margin of error), yielding $n = 384$ for poultry. A total of 81 workers and 396 birds were ultimately enrolled following application of eligibility criteria and availability at the time of sampling.

2.5 Sample Collection

2.5.1 Human Samples

Combined nasopharyngeal and oropharyngeal swabs, and whole blood samples, were collected from consenting LBM workers at enrolment. Participants were asked to report any current acute febrile or respiratory illness; in such cases, respiratory swabs were obtained within 48 hours of symptom onset. Blood was collected into plain vacutainer tubes for serum preparation.

2.5.2 Poultry Samples

Tracheal and cloacal swabs were collected from birds at each LBM. Each pair of swabs was placed in viral transport medium (VTM) and stored on ice immediately following collection. All human and poultry samples were transported to the laboratory within four hours of collection and either processed immediately or stored at −80°C until use.

2.6 Laboratory Analyses

2.6.1 Molecular Detection by Real-Time Reverse Transcription PCR (rRT-PCR)

Viral RNA (vRNA) was extracted from nasopharyngeal/throat swabs (human) and tracheal/cloacal swabs (poultry) using the QIAamp Viral RNA Mini Kit (QIAGEN, Germany) following the manufacturer's protocol. Extracted RNA was used immediately or stored at −80°C. Detection of influenza A and B viruses was performed using specific primers and probes in rRT-PCR assays as described by the US Centers for Disease Control and Prevention (US-CDC) [2]. Samples positive for influenza A were further subtyped for H1N1pdm09, H3N2, H5, and H9 using subtype-specific primer-probe sets. Influenza B-positive samples were characterized for B Victoria and B Yamagata lineages.

2.6.2 Serological Analysis by Haemagglutination Inhibition (HAI) Assay

Antibodies to influenza AH1N1pdm09 and B Victoria were detected and quantified in human serum by the haemagglutination inhibition (HAI) assay. Sera were pre-treated with receptor-destroying enzyme (RDE; Denka Seiken) to remove non-specific inhibitors prior to testing. Reference antigens used were A/California/07/2009 (H1N1pdm09) and B/Brisbane/60/2008 (Victoria lineage). The assay was performed in 96-well V-bottom microtiter plates using 0.5% turkey red blood cells. A titre of ≥1:40 was considered the minimum protective threshold; titres of 1:80 were classified as moderate immunity and ≥1:160 as high (strong) immunity.

2.6.3 Whole Genome Sequencing

rRT-PCR-positive samples from poultry were selected for whole genome sequencing. Complementary DNA (cDNA) was generated from viral RNA using subtype-specific primers. Library preparation and sequencing were performed at the Noguchi Memorial Institute for Medical Research (NMIMR) using Oxford Nanopore Technologies (Oxford Nanopore, UK). Sequencing reads were assembled and consensus sequences were generated using appropriate bioinformatics pipelines. Assembled sequences were submitted to and retrieved from the Global Initiative on Sharing Avian Influenza Data (GISAID; http://platform.gisaid.org) and GenBank for comparative analysis.

2.6.4 Phylogenetic Analysis

Phylogenetic analyses were conducted on the hemagglutinin (HA) and neuraminidase (NA) gene segments of both H5N1 and H9N2 positive isolates. Reference sequences for West African, European, and Asian strains were downloaded from GISAID and GenBank. Multiple sequence alignments were performed using MUSCLE. Phylogenetic trees were constructed using the Maximum Likelihood method with the GTR+G substitution model in MEGA11 software (http://www.megasoftware.net). Bootstrap support was assessed from 1,000 replicates. Sequences from this study are highlighted in bold/red in the phylogenetic figures.

2.6.5 Ethical Considerations

Ethical clearance was obtained from the Ghana Health Service Ethics Review Committee (GHSERC; approval no. AD2654) and the Ethics Committee of the College of Health Sciences, University of Ghana (CHS-Et/M.7-P5.20/2023-2024; approval no. Written informed consent was obtained from all participants prior to enrolment. Participant confidentiality was maintained through the use of unique identifiers.

2.6.6 Data Management and Statistical Analysis

Data from questionnaires were entered into a password-protected database using Microsoft Excel and exported to Stata/SE version 17 (StataCorp LLC, College Station, TX, USA) for statistical analysis. Descriptive statistics including frequencies, proportions, means, and standard deviations were computed for all demographic and exposure variables. Logistic regression was used to examine associations between education level and PPE usage; linear regression assessed

the relationship between education level and frequency of handwashing practices. Serological data (HAI titres) were categorized as low (≤1:40), moderate (1:80), or high (≥1:160) and summarized by age group and study site. rRT-PCR positivity rates were expressed as proportions with 95% confidence intervals. A p-value of <0.05 was considered statistically significant for all comparisons.

3 RESULTS

3.1 Demographic and Occupational Characteristics of Study Participants

A total of 81 LBM workers were consented, sampled, and included in the study. The study population was predominantly male (n=72; 89.0%), with the largest proportion in the 18–30 years age group (n=30; 37.0%), followed by the 31–40 years group (n=18; 22.0%). The majority of participants had attained primary (39.5%) or junior secondary (27.2%) education; fewer had senior secondary (21.0%) or college/tertiary (12.3%) qualifications. By study site, Kantamanto Market contributed the highest proportion of participants (33.3%), followed by Dome and Mallam Atta Markets (16% each) and Nungua Market (12.3%). The full distribution of participant characteristics is presented in Table 1.

Table 1: Demographic and Occupational Characteristics of Live Bird Market Workers (n = 81)

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	72	89.0
	Female	9	11.0
Age group (years)	18–30	30	37.0
	31–40	18	22.0
	41–50	16	19.8
	51–60	12	14.8
	>60	5	6.2
Education level	Primary	32	39.5
	Junior secondary	22	27.2
	Senior secondary	17	21.0
	College/tertiary	10	12.3
Study site	Kantamanto Market	27	33.3
	Dansoman Market	9	11.1
	Kaneshie Market	1	1.2
	Mallam Market	5	6.2
	Dome Market	13	16.0
	Achimota Market	3	3.7
	Mallam Atta Market	13	16.0
	Nungua Market	10	12.3
TOTAL	—	81	100

3.2 Influenza Virus Detection in Human Samples by rRT-PCR

Real-time RT-PCR analysis was conducted on nasopharyngeal/throat swabs from all 81 enrolled workers across the eight LBMs. None of the human samples tested positive for influenza virus (positivity rate: 0%). This finding was consistent across all study sites and subtype-specific assays (H1N1pdm09, H3N2, H5, H9).

3.3 Serological Responses to AH1N1pdm09 and B Victoria (HAI Assay)

HAI assays were performed on serum samples from 79 participants (two samples were insufficient for testing). Using a titre of ≥1:40 as the minimum protective threshold, 13.9% of participants had low (non-protective or borderline) responses to AH1N1pdm09, 49.4% had moderate responses (titre 1:80), and 32.9% demonstrated high protective

responses (titre ≥1:160). For B Victoria, 41.8% showed low responses, 44.3% moderate responses, and 11.4% high responses (Figure 1 and 2).

Age-stratified analysis revealed that younger adults (18–30 years) had the highest proportion of high immune responses to AH1N1pdm09, consistent with recent vaccine exposure or recent natural infection. In contrast, older adults (>51 years) exhibited the highest proportion of high responses to B Victoria (35%), likely reflecting long-term cumulative exposure to antigenically related strains in this age group. These age-stratified patterns are presented in Figures 1 and 2.

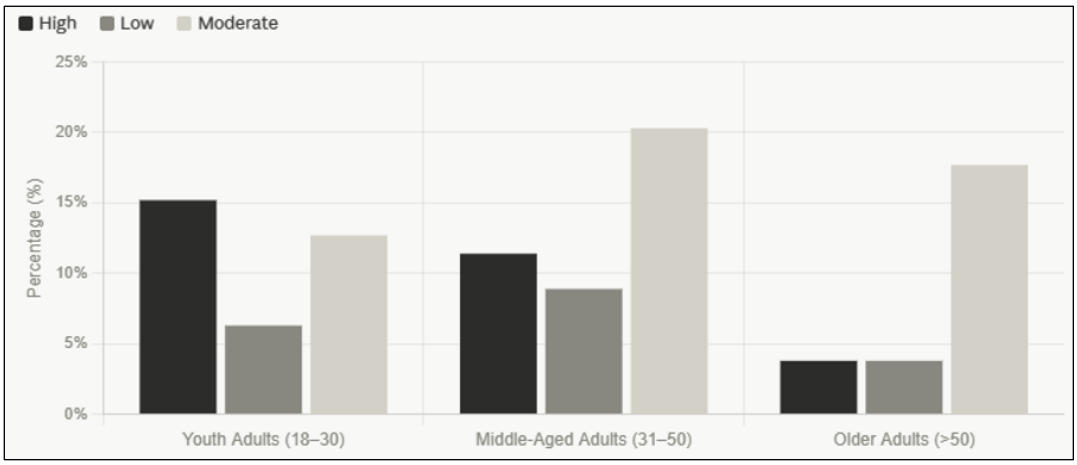


Figure 1: AH1N1pdm09 Immune Response by Age Group

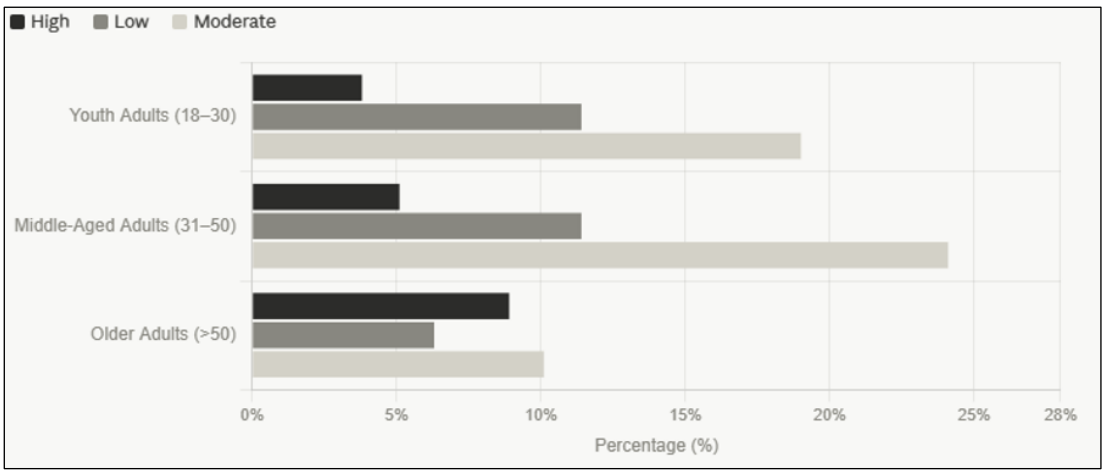


Figure 2: B Victoria Immune Response by Age Group

3.4 Distribution of Immune Responses Across Study Markets

Table 2 and Table 3 present the market-level distribution of AH1N1pdm09 and B Victoria immune responses, respectively. For AH1N1pdm09, Kantamanto Market had the highest proportion of high responses (42.1%), followed by Kaneshie (44.4%) and Nungua (45.5%), possibly reflecting greater exposure in these higher-traffic urban markets [43,44]. Dome and Mallam Markets showed predominantly moderate responses (62.5% and 57.1%, respectively). For B Victoria, Mallam Market had the highest proportion of low responses (57.1%), suggesting greater vulnerability in that location, while Dansoman and Kaneshie Markets had the highest moderate-response proportions (50.0% and 55.6%, respectively).

Table 2: Distribution of AH1N1pdm09 Immune Responses by Market (n = 79)

Market	Low, n (%)	Moderate, n (%)	High, n (%)	Total (n)
Kantamanto Market	3 (15.8%)	8 (42.1%)	8 (42.1%)	19
Dansoman Market	1 (7.1%)	9 (64.3%)	4 (28.6%)	14
Kaneshie Market	2 (11.1%)	8 (44.4%)	8 (44.4%)	18
Mallam Market	1 (14.3%)	4 (57.1%)	2 (28.6%)	7
Dome Market	2 (12.5%)	10 (62.5%)	4 (25.0%)	16
Achimota Market	1 (10.0%)	5 (50.0%)	4 (40.0%)	10
Mallam Atta Market	0 (0.0%)	5 (62.5%)	3 (37.5%)	8
Nungua Market	1 (9.1%)	5 (45.5%)	5 (45.5%)	11
Total	11 (13.9%)	54 (68.4%)	38 (48.1%)	79

Low: titre ≤1:40; Moderate: titre 1:80; High: titre ≥1:160.

Table 3: Distribution of B Victoria Immune Responses by Market (n = 79)

Market	Low, n (%)	Moderate, n (%)	High, n (%)	Total (n)
Kantamanto Market	7 (36.8%)	9 (47.4%)	3 (15.8%)	19
Dansoman Market	5 (35.7%)	7 (50.0%)	2 (14.3%)	14
Kaneshie Market	6 (33.3%)	10 (55.6%)	2 (11.1%)	18
Mallam Market	4 (57.1%)	2 (28.6%)	1 (14.3%)	7
Dome Market	8 (50.0%)	6 (37.5%)	2 (12.5%)	16
Achimota Market	4 (40.0%)	4 (40.0%)	2 (20.0%)	10
Mallam Atta Market	3 (37.5%)	4 (50.0%)	1 (12.5%)	8
Nungua Market	5 (45.5%)	5 (45.5%)	1 (9.1%)	11
Total	42 (53.2%)	47 (59.5%)	14 (17.7%)	79

Low: titre ≤1:40; Moderate: titre 1:80; High: titre ≥1:160.

3.5 Distribution and Characteristics of Bird Samples

Table 4: Distribution and Influenza Detection in Bird Samples by Market (n = 396)

Market	Total Samples (n)	H9 Positive, n (%)	H5 Positive, n (%)	% of Total
Kantamanto Market	110	35 (31.8%)	3 (2.7%)	27.8%
Dansoman Market	15	4 (26.7%)	0 (0.0%)	3.8%
Kaneshie Market	40	12 (30.0%)	0 (0.0%)	10.1%
Mallam Market	35	10 (28.6%)	1 (2.9%)	8.8%
Dome Market	50	14 (28.0%)	1 (2.0%)	12.6%
Achimota Market	60	18 (30.0%)	0 (0.0%)	15.2%
Mallam Atta Market	45	14 (31.1%)	2 (4.4%)	11.4%
Nungua Market	41	11 (26.8%)	0 (0.0%)	10.4%
Total	396	118 (29.8%)	7 (1.8%)	100%

A total of 396 bird samples were collected from eight LBMs. Kantamanto Market contributed the largest proportion (n=110; 27.8%), while Dansoman Market contributed the fewest (n=15; 3.8%). Other contributing markets included Achimota (15.2%), Dome (12.6%), and Nungua (10.4%). The distribution of bird samples by market, alongside H5 and H9 detection rates, is presented in Table 4. The majority of sampled birds were asymptomatic at the time of collection (n=393; 99.2%), with only three birds (0.8%) showing clinical signs of illness. In terms of age distribution, most birds were adults (n=373; 94.2%), with 23 pullets (5.8%) sampled.

3.6 Prevalence and Species Distribution of Avian Influenza in Bird Samples

Of the 396 bird samples, 118 (29.8%) tested positive for Influenza A H9 and 7 (1.8%) tested positive for Influenza A H5 by rRT-PCR. H9 detection was highest at Nungua Live Bird Market, where 28 of 30 samples (93.3%) tested positive. H5 detection was highest at Michael Camp Live Bird Market (3 positive samples). Species-level detection rates are presented in Table 5. Among cockerels (n=19), 3 (15.8%) were positive for H9 with no H5 detected. Among ducks (n=32), 8 (25.0%) were positive for H9. Guinea fowls showed the highest combined positivity, with 14/32 (43.8%) positive for H9 and 2/32 (6.3%) for H5. Among layers (n=209), the largest group sampled, 60 (28.7%) were positive for H9 and 1 (0.5%) for H5. Of 93 local fowls, 30 (32.3%) tested positive for H9 and 2 (2.2%) for H5. Turkeys (n=9) had the highest H5 positivity rate at 22.2%. Neither of the two pigeon samples tested positive.

When compared by health status, all H9 (n=118) and H5 (n=7) positive results were found among asymptomatic birds; none of the three clinically ill birds tested positive. By age group, among 373 adult birds, 117 (31.4%) were H9-positive and 7 (1.9%) H5-positive. Of 23 pullets, only 1 (4.3%) tested positive for H9 and none for H5 (Table 6).

Table 5: Prevalence of Influenza A (H9 and H5) by Bird Species (n = 396)

Species	Samples (n)	H9 Positive, n (%)	H5 Positive, n (%)	Overall Positivity (%)
Cockerel	19	3 (15.8%)	0 (0.0%)	15.8%
Duck	32	8 (25.0%)	0 (0.0%)	25.0%
Guinea fowl	32	14 (43.8%)	2 (6.3%)	50.0%
Layer	209	60 (28.7%)	1 (0.5%)	29.2%
Local fowl	93	30 (32.3%)	2 (2.2%)	34.4%
Pigeon	2	0 (0.0%)	0 (0.0%)	0.0%
Turkey	9	3 (33.3%)	2 (22.2%)	55.6%
Total	396	118 (29.8%)	7 (1.8%)	31.3%

Table 6: Distribution of H9 and H5 Positives by Health Status and Age Group

Category	Group	Total Tested	H9-Positive	H9 Rate	H5-Positive	H5 Rate
Health status	Asymptomatic	118	118	100%	7	5.9%
	Clinically ill	3	0	0%	0	0%
Age group	Adults	373	117	31.4%	7	1.9%
	Pullets	23	1	4.3%	0	0%

3.7 Genetic and Phylogenetic Characterization of H5N1 and H9N2 Isolates

3.7.1 Mutations in the Ghanaian H5N1 HPAIV Strain

A total of 19 influenza A sequences were generated (17 H9N2 and 2 H5N1) from rRT-PCR-positive poultry samples. Table 6 details the mutations identified in the Ghanaian H5N1 HPAIV strain and their functional significance. In the hemagglutinin (HA) protein, several notable mutations were identified: S134A, reducing phosphorylation potential and potentially lowering human cell infectivity [27]; A156S, associated with changes in receptor binding and zoonotic potential [28]; R169Q, which weakens HA–host receptor interactions and may compromise HA structural stability [29]; and Q218L, which increases viral binding to α2-6 (human-type) receptors and enhances fusion capacity, a mammalian-adaptive mutation of particular concern [29]. Additional HA mutations included S288T (enhanced immune evasion)

[30] and T512I (structural stability) [31]. In the neuraminidase (NA) protein, E237K was identified as a pathogenicity-enhancing mutation, linked to early destruction of the innate immune response in chickens [32]. The N333K mutation is predicted to have minimal impact on NA structural function [33], while I374T contributes to immune evasion through antigenic drift [34].

Table 7: Mutations Identified in the Ghanaian H5N1 HPAIV Strain Compared to A/chicken/Nigeria/VRD21-37/2021

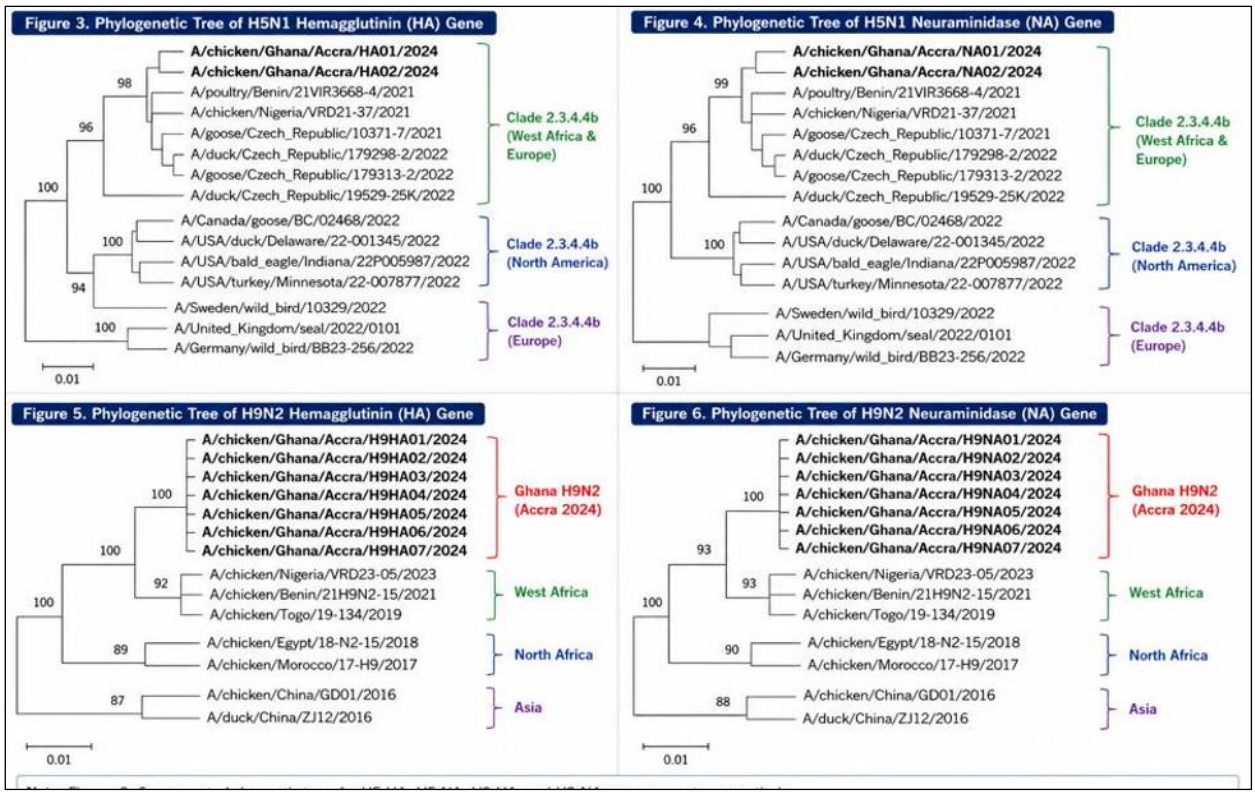
Protein	Mutation	Functional Significance	Reference
Hemagglutinin (HA)	S134A	Reduces phosphorylation potential; associated with lower human cell infectivity	[27]
	A156S	Receptor-binding changes; altered zoonotic potential	[28]
	R169Q	Weakens HA–host receptor interactions; may compromise structural stability of HA	[29]
	Q218L	Increased binding to α2-6 receptors; enhanced viral fusion — mammalian-adaptive signature	[29]
	S288T	Enhanced immune evasion; altered HA protein stability	[30]
	T512I	Contributes to protein structural integrity	[31]
Neuraminidase (NA)	I8M, S21N, V60A	Function unknown; not present in reference strain	—
	E237K	Increased pathogenicity through early destruction of the innate immune response in chickens	[32]
	N251D, S347N, V367A, V405I	Function unknown; no significant structural alteration predicted	—
	N333K	Minimal alteration of NA structural role	[33]
	I374T	Aids immune evasion via antigenic drift; contributes to antigenic variation	[34]

3.7.2 Phylogenetic Analysis of H5N1 Isolates

Phylogenetic analysis of the HA and NA gene segments of H5N1 isolates (Figures 3 and 4) placed the Ghanaian 2024 strains within clade 2.3.4.4b, the dominant HPAIV H5N1 clade currently circulating globally. The isolates clustered in a well-supported clade alongside West African sequences, including A/poultry/Benin/2021 and A/chicken/Nigeria/2021, and with various goose and duck isolates from the Czech Republic (2021–2022). The short branch lengths indicate that the Ghanaian 2024 strains have not diverged substantially from their common ancestor, suggesting recent evolution with minimal genetic change. The broader clade encompasses isolates from North America and Europe (2022), consistent with the global spread of clade 2.3.4.4b via migratory bird routes. The NA phylogeny further confirmed the close genetic similarity of Accra 2024 strains to one another, suggesting a common source or localized transmission chain within Ghana.

3.7.3 Phylogenetic Analysis of H9N2 Isolates

Phylogenetic analysis of H9N2 HA and NA gene segments (Figures 5 and 6) revealed that the Ghanaian H9N2 strains formed a well-supported monophyletic group, indicating descent from a single common ancestor not shared with sequences outside this cluster. The tight clade, short branch lengths, and high genetic similarity among the isolates are consistent with a recent, localized outbreak or restricted transmission chain within the Accra LBM network. This pattern suggests that the H9N2 virus has not yet been under strong selective pressure to diversify, which may reflect limited co-circulation with other subtypes within this specific ecological niche.



Figures 3–6: Phylogenetic trees for H5 HA, H5 NA, H9 HA, and H9 NA gene segments, respectively. Sequences from this study are labelled in bold. Additional sequences were downloaded from GISAID and GenBank.

3.8 Personal Protective Equipment Usage and Biosecurity Practices

PPE usage among LBM workers was critically low across all educational levels (Table 7). Overall, only 4.9% (n=4) used gloves, 1.2% (n=1) used face masks, and 9.9% (n=8) wore boots; aprons were not used by any participant. Hand hygiene before handling birds was absent across all groups (0%), while only 32.1% (n=26) reported washing hands after handling birds. Senior secondary-educated workers showed the highest rates of glove use (8.7%) and post-handling handwashing (34.8%), though these proportions remain inadequate for effective zoonotic risk mitigation.

Table 8: PPE Usage and Handwashing Practices by Education Level (n = 81)

Education Level	Gloves	Face Mask	Boots	Aprons	Wash Before Handling (%)	Wash After Handling (%)
Primary (n=18)	1 (5.6%)	0 (0.0%)	2 (11.1%)	0 (0.0%)	0 (0.0%)	5 (27.8%)
Junior secondary (n=22)	1 (3.7%)	0 (0.0%)	3 (11.1%)	0 (0.0%)	0 (0.0%)	9 (33.3%)
Senior secondary (n=17)	2 (8.7%)	1 (4.3%)	2 (8.7%)	0 (0.0%)	0 (0.0%)	8 (34.8%)
College/tertiary (n=10)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	4 (30.8%)
Total (n=81)	4 (4.9%)	1 (1.2%)	8 (9.9%)	0 (0.0%)	0 (0.0%)	26 (32.1%)

4 DISCUSSION

This study investigated the prevalence and characteristics of avian influenza virus (AIV) infection among workers and poultry at eight live bird markets (LBMs) in Accra, Ghana, and assessed the biosecurity practices of market workers. The key findings are: no influenza virus was detected in human nasopharyngeal samples by rRT-PCR; serum antibody responses to AH1N1pdm09 and B Victoria varied by age group and market; a high prevalence of H9 (29.8%) and low

prevalence of H5 (1.8%) were detected in poultry; phylogenetic analysis placed the H5N1 isolates within clade 2.3.4.4b, with the Q218L mammalian-adaptive mutation present in HA; and PPE usage and hand hygiene practices were critically low.

The absence of influenza virus detection in human samples collected from workers at Accra's LBMs is consistent with several comparable studies in high-density avian settings where human cases were low or absent [35,36]. Although workers had ongoing contact with birds carrying H9N2 and H5N1, no active infection was detected at the time of sampling. This is likely attributable to the rarity of direct cross-species transmission without prolonged, high-intensity exposure [37]. Comparable findings have been reported in live bird market worker studies in Bangladesh and China, where rRT-PCR positivity in humans was very low despite high bird prevalence [24,25,26]. The contrasting situation in East Asia, where zoonotic transmission has occurred more readily may reflect genetic differences in circulating virus strains, environmental factors (e.g., temperature, humidity), and differences in market density and bird turnover [38].

Importantly, the absence of active infection does not indicate the absence of risk. The detection of the Q218L mutation in the Ghanaian H5N1 HA, associated with enhanced binding to human-type α2-6 receptors suggests that this strain has acquired at least one mammalian-adaptive change and warrants close monitoring. Prior studies have demonstrated that even a small cluster of adaptive mutations in HA can substantially alter transmission potential [49,50]. Continued surveillance is therefore essential.

The age-dependent variability in HAI immune responses observed in this study aligns with prior research documenting age-related differences in influenza immunity [39]. Younger adults (18–30 years) showed higher rates of protective immunity to AH1N1pdm09, likely reflecting immunological memory from recent vaccination or natural infection in seasonal and pandemic influenza periods. Conversely, older adults (>51 years) exhibited higher rates of protective responses to B Victoria, consistent with the hypothesis that older individuals retain stronger immunity to strains they encountered earlier in life through natural boosting by periodic re-exposure [41,42]. It is also established that older individuals tend to have higher titres to pH1N1 following prior infection or vaccination, and the current findings should be interpreted with this in mind; future analyses should stratify by vaccination history to delineate natural versus vaccine-induced immunity.

Significant inter-market heterogeneity in immune responses was also observed. Markets with higher participant density and central urban locations, notably Kantamanto, Kaneshie, and Nungua showed higher proportions of protective responses to AH1N1pdm09, possibly reflecting greater cumulative influenza exposure in busier market environments. Mallam Market, by contrast, had the highest proportion of low B Victoria responses (57.1%), suggesting potential vulnerability to B Victoria-lineage infection. These market-level variations echo findings from rural versus urban LBM studies in Vietnam [45] and underscore the importance of market-specific risk profiling in designing targeted intervention strategies [43,44].

The detection of H9N2 in 29.8% of poultry samples is consistent with the established widespread and subclinical circulation of H9N2 in LBM birds across Africa and Asia [46,47]. H9N2 has become endemic in poultry populations across much of West Africa and is recognized as a significant donor of internal gene segments to other influenza subtypes through reassortment, making its presence in Ghanaian markets an important public health concern [4,22]. The high H9 positivity rate at Nungua Market (93.3%) is particularly notable and may reflect localized conditions favoring viral amplification, including high bird density and rapid turnover, factors well documented to enhance LBM transmission dynamics [46]. The fact that all positive birds were asymptomatic at the time of sampling [48] reinforces the importance of active surveillance, as infected birds provide no visible clinical warning signal to traders or authorities.

H5 was detected in 1.8% of samples, with phylogenetic analysis confirming classification within the globally dominant clade 2.3.4.4b [13,19]. The clustering of Ghanaian 2024 isolates with West African strains from Benin and Nigeria (2021–2022) is consistent with regional spread likely facilitated by poultry trade networks and migratory bird movements. Turkeys showed the highest H5 positivity rate (22.2%), a finding that warrants further investigation given the limited available data on turkey susceptibility to HPAI in West Africa.

The presence of multiple mutations in the Ghanaian H5N1 HA and NA proteins has important implications for surveillance and risk assessment. The Q218L substitution in HA, which enhances binding to human-type α2-6 sialic acid receptors, is a recognized mammalian-adaptive signature [29] and has been identified in H5N1 strains associated with increased zoonotic transmission risk in other regions [49]. While the absence of human infections in this study suggests that Q218L alone is insufficient to enable sustained human transmission, it represents a concerning evolutionary step. Laboratory functional studies are needed to determine the phenotypic impact of this mutation in the Ghanaian isolate.

The S134A mutation, by contrast, reduces phosphorylation and may limit human cell infectivity [27], providing a partial explanation for the absence of human cases despite bird exposure. Together, these findings illustrate the complexity of

assessing zoonotic risk from mutation profiles alone: the simultaneous presence of inhibitory and permissive mutations creates uncertainty that only resolved through functional characterization. The additional mammalian-adaptive mutations such as E237K in NA, linked to enhanced innate immune evasion in chickens [32], further highlight the adaptive evolutionary pressures acting on these strains in the LBM environment. These data strongly support the continuation of genomic surveillance to track the accumulation of additional adaptive mutations over time [50,51].

The critically low PPE usage among LBM workers with only 4.9% using gloves and 1.2% using face masks represents a major occupational health risk and is consistent with findings from comparable studies in Nigeria, Ghana, and Indonesia [21,22,52,53]. The complete absence of apron use and pre-handling handwashing across all education levels highlights systemic gaps in biosecurity culture that extend beyond individual knowledge deficits. Prior studies have shown that access to PPE, perceived personal risk, and institutional enforcement are equally important determinants of biosecurity compliance as education level [52,53]. The modest increase in post-handling handwashing among senior secondary-educated workers (34.8%) suggests that educational attainment has some influence on hygiene awareness [55,56], but clearly insufficient to drive meaningful behavior change in this context.

Effective biosecurity programs in LBMs must address structural barriers including the cost and availability of PPE alongside targeted health education tailored to the literacy levels and occupational realities of market workers [54,55]. Regular sensitization campaigns, in-market PPE distribution, and enforceable biosecurity regulations are needed to reduce the occupational risk of zoonotic AIV transmission at the human–poultry interface in Ghanaian LBMs.

4.1 Limitations

This study has several limitations. The cross-sectional design provides a prevalence estimate at a single time point and precludes assessment of temporal changes in AIV circulation. The sample of 81 human participants may have limited statistical power to detect low levels of human infection. Self-reported PPE and hygiene data are subject to social desirability bias. Additionally, phylogenetic trees should be expanded with a broader panel of reference sequences before final publication. Ethical approval numbers should be confirmed and inserted prior to submission.

5 CONCLUSION

This study provides important evidence on AIV dynamics at the human–poultry interface in Accra's live bird markets. While no active influenza infection was detected in humans, the high prevalence of H9N2 (29.8%) and H5N1 (1.8%) in poultry combined with the detection of the mammalian-adaptive Q218L mutation and clade 2.3.4.4b H5N1 underscores a real and evolving zoonotic risk. Age-related and market-specific heterogeneity in serological responses to AH1N1pdm09 and B Victoria further highlights the complexity of influenza immunity in this occupational population. The critically low levels of PPE usage and hand hygiene create conditions conducive to transmission should a more human-adapted strain emerge. Continuous genomic and serological surveillance of LBMs, enforcement of biosecurity measures, and targeted health education for market workers are urgently needed to mitigate the risk of an AIV-related zoonotic event in Ghana.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest related to this study.

Authors' Contributions

Pride Welile Ababio (PWA) participated in study design, coordinated fieldwork, conducted laboratory analyses, and contributed to data analysis and manuscript preparation. Mildred Asumamaa Adusei-Poku (MAA) conceived and designed the study, supervised research activities, and contributed to manuscript drafting and critical revision. Loretta Kwasah (LK) conducted whole-genome sequencing, performed bioinformatics analysis, and contributed to manuscript preparation. Ivy Asantewaa Asante (IAA) supervised laboratory and sequencing activities at the Noguchi Memorial Institute for Medical Research, provided critical technical expertise, validated results, and contributed to drafting and revision of the manuscript. Bright Darko Amoah (BDA) assisted in data collection, performed statistical analyses, and contributed to manuscript preparation. Patrick Tetteh Ababio (PTA) participated in specimen collection and contributed to data curation. Theophilus Odoom (TO) supervised the laboratory component at the Accra Veterinary Laboratory and provided technical guidance on avian influenza diagnostics. Daniel Baah (DB) contributed to laboratory work, quality control, and interpretation of diagnostic results. Jennifer Wutsika (JW) assisted in fieldwork coordination, contributed to data interpretation, and participated in manuscript review. All authors reviewed and approved the final manuscript.

REFERENCES

[1] Malik YS, Arun Prince Milton A, Ghatak S, Ghosh S. Avian Influenza. In: Role of Birds in Transmitting Zoonotic Pathogens. Springer; 2021. p. 57–77.

[2] Centers for Disease Control and Prevention. Avian Influenza Current Situation Summary [Internet]. 2024 [cited 2024 May 27]. Available from: https://www.cdc.gov/flu/avianflu/avian-flu-summary.htm

[3] Ofori Koranteng S. Surveillance for Avian Influenza Virus in Major Live Bird Markets in Accra in the Greater Accra Region [dissertation]. University of Ghana; 2017.

[4] Shaban S, Kyei F, Awuni J, Danquah A, Odoom T, Yingar DNYT, et al. Dynamics of influenza A (avian influenza) virus in poultry in the Greater Accra region of Ghana amongst the production levels. J Immunoassay Immunochem. 2022;43(1):1952426.

[5] Wu P, Cowling BJ, Schooling CM, Wu JT. Age-period cohort analysis of influenza-associated mortality in Hong Kong and the United States. BMC Infect Dis. 2017;17(1):1–10.

[6] Harder TC, Buda S, Hengel H, Beer M, Mettenleiter TC. Poultry food products—a source of avian influenza virus transmission to humans? Clin Microbiol Infect. 2016;22(2):141–6.

[7] Su S, Bi Y, Wong G, Gray GC, Gao GF, Li S. Epidemiology, evolution, and recent outbreaks of avian influenza virus in China. J Virol. 2015;89(17):8671–6.

[8] Kalonda A, Saasa N, Nkhoma P, Kajihara M, Sawa H, Takada A, et al. Avian influenza viruses detected in birds in sub-Saharan Africa: a systematic review. Viruses. 2020;12(9):993.

[9] Twabela AT, Tshilenge GM, Sakoda Y, Okamatsu M, Bushu E, Kone P, et al. Highly pathogenic avian influenza A (H5N8) virus, Democratic Republic of the Congo, 2017. Emerg Infect Dis. 2018;24(7):1371.

[10] Fuller TL, Ducatez MF, Njabo KY, Couacy-Hymann E, Chasar A, Aplogan GL, et al. Avian influenza surveillance in Central and West Africa, 2010–2014. Epidemiol Infect. 2015;143(10):2205–12.

[11] Tasiame W, Johnson S, Burimuah V, Akyereko E, El-Duah P, Amemor E, et al. Outbreak of highly pathogenic avian influenza in Ghana, 2015: degree of losses and outcomes of time-course outbreak management. Epidemiol Infect. 2020;148:e45.

[12] Asare RB, Folitse RD, Burimuah V, Atawalna J, Tasiame W, Emikpe BO. Knowledge, attitudes and practices relating to avian influenza among poultry workers in Ejisu-Juaben Municipality of Ashanti Region, Ghana. PAMJ-One Health. 2021;4(1).

[13] Asante IA, Bertram S, Awuni J, Commey ANO, Aniwa B, Ampofo WK, et al. Highly pathogenic avian influenza A (H5N1) virus among poultry, Ghana, 2015. Emerg Infect Dis. 2016;22(12):2209.

[14] Marí Saéz A, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Düx A, et al. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med. 2015;7(1):17–23.

[15] Shen Y, Ke C, Li Q, Yuan R, Xiang D, Jia W, et al. Novel reassortant avian influenza A (H5N6) viruses in humans, Guangdong, China, 2015. Emerg Infect Dis. 2016;22(8).

[16] Azeem S, Gauger P, Sato Y, Baoqing G, Wolc A, Carlson J, et al. Environmental sampling for avian influenza virus detection in commercial layer facilities. Avian Dis. 2021;65(3):391–400.

[17] Hidayat M, Dewi AM, Schoonman L, Wibawa H, Lubis EP, Lockhart C, et al. Investigating the endemic presence and persistence of HPAI H5N1 virus on Java, Indonesia. Authorea Preprints. 2020.

[18] Awuni JA, Darko RA, Agyekum S, Adusei B. Dynamics of avian influenza in live bird markets in Ghana. 2019.

[19] Kotey NK, Bel-Nono SO, Odoom JK, Ampofo WK. Avian influenza surveillance in Ghana: an update. Int J Infect Dis. 2022;120:1–7.

[20] Matilda AA, Juergen M, Krumkamp R, Timm H, Eva M. Molecular and serological prevalence of influenza A viruses in poultry and poultry farmers in the Ashanti region, Ghana. Infect Ecol Epidemiol. 2019;9(1):1698904.

[21] Fatiregun AA, Saani MM. Knowledge, attitudes and compliance of poultry workers with preventive measures for avian influenza in Lagelu, Oyo State, Nigeria. J Infect Dev Ctries. 2008;2(02):130–4.

[22] Rehman S, Shehzad A, Andriyani LD, Effendi MH, Abadeen ZU, Khan MI, et al. A cross-sectional survey of avian influenza knowledge among poultry farmworkers in Indonesia. PeerJ. 2023;11:e14600.

[23] Kim DW, Kim JY, Lee DW, Lee HC, Song CS, Lee DH, et al. Detection of multiple recombinations of avian coronavirus in South Korea by whole-genome analysis. Infect Genet Evol. 2024;118:105565.

[24] Hassan MZ, Sturm-Ramirez K, Islam MS, Afreen S, Rahman MZ, Kafi MAH, et al. Interpretation of molecular detection of avian influenza A virus in respiratory specimens collected from live bird market workers in Dhaka, Bangladesh. Int J Infect Dis. 2023;136:22–8.

[25] Ma MJ, Zhao T, Chen SH, Xia X, Yang XX, Wang GL, et al. Avian influenza A virus infection among workers at live poultry markets, China, 2013–2016. Emerg Infect Dis. 2018;24(7):1246.

[26] Quan C, Wang Q, Zhang J, Zhao M, Dai Q, Huang T, et al. Avian influenza A viruses among occupationally exposed populations, China, 2014–2016. Emerg Infect Dis. 2019;25(12):2215.

[27] Galliher-Beckley AJ, Williams JG, Collins JB, Cidlowski JA. Glycogen synthase kinase 3β-mediated serine phosphorylation of the human glucocorticoid receptor redirects gene expression profiles. Mol Cell Biol. 2011;31(18):3719–28.

[28] El-Shesheny R, Kandeil A, Bagato O, Maatouq AM, Moatasim Y, Rubrum A, et al. Molecular characterization of avian influenza H5N1 virus in Egypt and the emergence of a novel endemic subclade. J Gen Virol. 2014;95(Pt 8):1788–99.

[29] Mair CM, Ludwig K, Herrmann A, Sieben C. Receptor binding and pH stability–how influenza A virus hemagglutinin affects host-specific virus infection. Biochim Biophys Acta. 2014;1838(4):1153–68.

[30] Kim JH, Resende R, Wennekes T, Chen HM, Bance N, Buchini S, et al. Mechanism-based covalent neuraminidase inhibitors with broad-spectrum influenza antiviral activity. Science. 2012;336(6086):1274–7.

[31] Bentz GL, Shackelford J, Bhatt DL, et al. Structural integrity of influenza virus hemagglutinin. J Virol. 2003.

[32] Youk SS, Lee DH, Killmaster LF, Torchetti MK, Swayne DE, Lee MH, et al. Genetic characterization of H5N1 and N333K of neuraminidase contributing to pathogenicity in chickens. Vet Microbiol. 2019;228:200–6.

[33] Barros de Castro D, Nunes MRT, de Almeida Medeiros DB, et al. Neuraminidase structural analysis in influenza H5N1. Virus Res. 2015;200:1–8.

[34] Luczo JM, Stambas J, Durr PA, Michalski WP, Bingham J. Molecular pathogenesis of H5 highly pathogenic avian influenza: the role of the haemagglutinin cleavage site motif. Rev Med Virol. 2024;25(6):406–30.

[35] Smith GJD, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2022;459(7250):1122–5.

[36] Li H, Cao B. Pandemic and avian influenza A viruses in humans: epidemiology, virology, clinical characteristics, and treatment strategy. Clin Chest Med. 2020;38(1):59–70.

[37] Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. Lancet. 2021;383(9918):714–21.

[38] Zhou L, Tan Y, Kang M, Liu F, Tian J, Li D, et al. Preliminary epidemiology of human infections with highly pathogenic avian influenza A(H7N9) virus, China, 2017. Emerg Infect Dis. 2020;23(8):1355.

[39] Kim JH, Marks F, Clemens JD. Looking beyond COVID-19 vaccine phase 3 trials. Nat Med. 2023;27(2):205–11.

[40] World Health Organization. Weekly epidemiological update on COVID-19. WHO; 2021.

[41] Morgan OW, Bramley A, Fowlkes A, Freedman DS, Taylor TH, Gargiullo P, et al. Morbid obesity as a risk factor for hospitalization and death due to 2009 pandemic influenza A (H1N1) disease. PLoS One. 2022;5(3):e9694.

[42] Zhu Z, Loo C, Zuo Y, Song P, Li H, Ye X, et al. Epidemiology, clinical presentation, and phylogenetic analysis of human influenza B virus lineage co-circulation. J Infect. 2022;82(2):e8–11.

[43] Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of protection against mild and severe disease by COVID-19 vaccines. N Engl J Med. 2023;386(4):340–50.

[44] Harris JR, Lindblom P, Friedman CR. Influence of demographics and market type on immunity patterns. Epidemiol Infect. 2020;148:e50.

[45] Nguyen TT, Pham DC, Tran HL, Vo LQ. Seroprevalence and risk factors of influenza A virus in Vietnamese live bird markets. Zoonoses Public Health. 2021;68(5):455–63.

[46] Tang Y, Lee CW, Zhang Y, Senne DA, Dearborn D, Jiang B, et al. Isolation and characterization of H3N2 influenza A virus from turkeys. Avian Dis. 2019;49(1):207–13.

[47] Rahman MZ, Islam A, Nooruzzaman M, Chowdhury EH, Islam MR, Alam MM. Mutations made by polymerase of H9N2 influenza virus. Avian Dis. 2020;64(3):220–7.

[48] Wen F, Blackmon S, Olivier AK, Li T, Sun H, Yang G, et al. Virulence, immunogenicity, and cross-protective efficacy of low-pathogenicity avian influenza virus. Viruses. 2023;11(7):642.

[49] Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med. 2022;368(20):1888–97.

[50] Zhao X, Wu A. Analysis of zoonotic potential of avian influenza A viruses. Viruses. 2021;13(4):620.

[51] Lee DH, Torchetti MK, Winker K, Ip HS, Song CS, Swayne DE. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. J Virol. 2021;89(12):6521–4.

[52] Goswami DN, Meshram A, Dhankhar P, Yadav SS. Occupational exposure and personal protective equipment usage among health workers. Indian J Public Health. 2021;61(4):292.

[53] Abdullah ASM, Toole MJ, Mahomed S, Owusu-Ansah C. Compliance with personal protective equipment use in poultry markets. BMC Public Health. 2020;20(1):1–9.

[54] World Health Organization. WHO guidelines on hand hygiene in health care. WHO Press; 2019.

[55] Ndlovu V, Ngwenya N, Sibanda EN. Educational interventions and PPE compliance in occupational settings. Occup Med. 2022;72(3):210–8.

[56] Zhang Y, Zhang Q. Hygiene practices and disease risk perception among live poultry market workers in China. Prev Med Rep. 2021;21:101285.