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Pathogenicity of Highly Pathogenic Avian Influenza Virus (H5N1) in Different Duck Breeds

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ABSTRACT

Avian influenza viruses (AIVs) pose a global threat, with wild waterfowl serving as key reservoirs for transmission to poultry. The present study investigated the pathogenicity, viral shedding patterns, tissue distribution, and pathological effects of a highly pathogenic avian influenza virus (HPAIV) in two duck breeds, including Muscovy and Sudani ducks. An Egyptian H5N1 strain (A/ibis/Egypt/RLQP-229S/2022), originally isolated from a wild ibis, was used. Forty ducks (20 Muscovy and 20 Sudani) were divided into infected and control groups (10 per group per breed). At four weeks of age (Average weight of 1.2 ± 0.1 kg), each infected duck received a single intranasal dose of 10^6 EID_{50}. Cloacal and oropharyngeal swabs were collected at 3, 5, 7, and 10 days post-infection (DPI) to monitor viral shedding, while clinical signs were recorded daily. Mortality was higher in Muscovy ducks, which exhibited higher mortality (70%) than Sudani ducks (50%), with both breeds showing neurological signs and lethargy. Viral load analysis of cloacal swabs via RT-PCR (Targeting the AIV *M* gene), exceeded oropharyngeal shedding, peaking by five DPI and persisting longer in Muscovy ducks (Seven DPI compared to five DPI in Sudani ducks), suggesting that fecal-oral transmission is the primary route of spread and that viral replication is more active in the intestinal tract. Tissue distribution analysis revealed broader viral dissemination in Muscovy ducks, particularly in the brain, lung, kidney, and spleen. These findings demonstrated differential susceptibility between breeds, with Muscovy ducks posing a higher transmission risk due to prolonged viral shedding and tissue tropism. The virus used in the present study carried pathogenicity markers across several proteins, including hemagglutinin (HA), neuraminidase (NA), polymerase basic 1 (PB1), polymerase basic 2 (PB2), nucleoprotein (NP), non-structural protein 1 (NS1), and polymerase acidic (PA) protein. Overall, while both duck breeds are vulnerable to the circulating H5N1 HPAI strain, their susceptibility and clinical outcomes differ. These findings demonstrated that both Muscovy and Sudani ducks are susceptible to H5N1 HPAIV infection, Muscovy ducks showing higher mortality and more extensive viral shedding and histopathological alterations. However, both duck breeds are variable in their susceptibility to H5N1 infection.

Keywords: Avian influenza viruses, Duck breeds, H5N1, Histopathological changes, Pathogenicity, Virus shedding

INTRODUCTION

The emergence of pandemic influenza outbreaks is frequently associated with the ability of influenza A viruses (IAV) to overcome species barriers and establish infection in novel host populations through substantial antigenic evolution (Horimoto and Kawaoka, 2001). Waterfowl serve as a natural reservoir for different avian influenza viruses (AIV) subtypes, playing a crucial role in maintaining viral diversity and facilitating transmission to domestic poultry through asymptomatic viral shedding (Blagodatski et al., 2021).

The highly pathogenic avian influenza virus (HPAIV), first detected in poultry in Guangdong, China in 1996, has since evolved and spread globally, affecting domestic birds, wild avian species, and even humans. This lineage, known as Goose/Guangdong/1/96 (GS/GD), was able to cross species barriers and disseminate across Europe, Asia, Africa, and North America, primarily via migratory birds (Cui et al., 2022; Engelsma et al., 2022; Sagong et al., 2022).

Egypt reported its first H5N1 outbreak in 2005. Over the following years, multiple clades emerged and spread extensively in domestic poultry, with significant economic and public health impacts. The H5N1 strains isolated in Egypt have been classified into clades, such as 2.2, 2.2.1, 2.2.1.1, 2.2.1.2, and 2.3.4.4b (Arafa et al., 2015; El-Shesheny et al., 2021; Mosaad et al., 2023). The ongoing detection of these variants highlights the virus's persistence and adaptive capacity in the region.

Several HPAI H5N1 genotypes within clade 2.3.4.4b appeared in wild birds during late 2020 and were found in several African, Asian, European, and North American nations (Engelsma et al., 2022; Sagong et al., 2022). The AIV H5N1 causes significant morbidity and mortality in poultry and has been reported to cause human infection (Horimoto and Kawaoka 2001). Because domestic ducks can have close contact with wild birds and land poultry simultaneously, they represent a significant source of AIV transmission from wild waterfowl to terrestrial poultry (Kwon et al., 2019). The AIV is categorized into two groups based on pathogenicity in chickens, including HPAIVs, which cause high mortality (Up to 100%) and severe systemic disease; and low pathogenic avian influenza viruses (LPAIVs), which usually result in mild respiratory or enteric symptoms, with significantly lower mortality (Shriner and Root, 2020). Both forms circulate among domestic and wild birds.

Influenza A viruses, which belong to the Orthomyxoviridae family, are further classified into 18 hemagglutinin (H1–H18) and 11 neuraminidase (N1–N11) subtypes based on their surface glycoproteins. The HA glycoprotein represents the primary antigenic determinant of influenza viruses. While antigenic shift in HA can precipitate pandemic emergence through major antigenic changes, antigenic drift enables circulating strains to evade population immunity through gradual accumulation of mutations, as most HA-directed antibodies demonstrate strain-specific neutralization (Wu and Wilson, 2020). These viruses are susceptible to reassortment, particularly in waterfowl, which contributes to the emergence of novel strains with pandemic potential (Taylor et al., 2023). Previously, the H5N1 HPAI viruses of the Asian lineage did not cause significant harm or death in ducks (Perkins and Swayne, 2002). The ability of domestic ducks to harbor H5N1 HPAI viruses raises public health concerns, emphasizing the need to limit their further spread and circulation (Kim et al., 2009).

Ducks are a significant source of influenza viruses that can spread to humans and other birds and mammals. Ducks are a natural reservoir of AIV and can act as a reassortment host (Hassan et al., 2020). Although wild ducks can carry AIV without showing clinical disease (Abtin et al., 2022), newer H5N1 variants have caused more severe disease in domestic duck breeds. The virus can replicate systemically, leading to widespread tissue damage, organ-specific variation in virus titers, and increased mortality (Hulse-Post et al., 2005; Samir et al., 2019). Different viruses from the H5 subtype of clade 2.3.4.4b generated systematic infection and demonstrated efficient direct transmission in ducks (Sun et al., 2016).

The H5N1 virus (Clade 2.2.1.2) experimentally infected Sudani ducks (*Cairina moschata*), resulting in severe lung tissue damage and robust viral replication, but only slight alterations in brain histology and reduced viral replication (Samir et al., 2019). The present study aimed to investigate the pathogenicity, viral shedding, and histopathological effects of Egyptian HPAI H5N1 virus (A/ibis/Egypt/RLQP-229S/2022) on Muscovy and Sudani ducks to evaluate the breed-specific susceptibility and the potential risk for virus transmission.

MATERIALS AND METHODS

Ethical approval

The study protocol received approval from the Ethics Committee of the Animal Health Research Institute in Egypt (Approval No. AHRI-EG-2022-042). The experiment took place at the Experimental Animal House Facility, Animal Health Research Institute, Giza, Egypt, in accordance with institutional animal care guidelines.

Virus

The HPAI H5N1 isolate used in the present study, A/ibis/Egypt/RLQP-229S/2022, was originally isolated from a wild Ibis bird (*Threskiornis aethiopicus*) during active surveillance in 2022. It was confirmed as an HPAIV by sequencing the HA cleavage site (Mosaad et al, 2023). The virus was cultivated in the allantoic cavities of 9–11-day-old specific pathogen-free (SPF) embryonated chicken eggs. The harvested allantoic fluid was then clarified by centrifugation, filtered, and titrated to determine the

median egg infectious dose (10^6 EID_{50}/mL). A 1:10 dilution was used for infection. The virus stock was tested by (Real time- polymerase chain reaction) RT-PCR and confirmed to be free of contaminants, including Newcastle disease virus, infectious bronchitis virus, and other AIV subtypes.

Study design

Housing

A total of 40 four-week-old ducks (20 Muscovy and 20 Native Sudani) were obtained from licensed commercial duck farms in Giza, Egypt. The ducks were divided into three groups, including 10 infected Muscovy ducks (Group 1), 10 infected Sudani ducks (Group 2), and a negative control of 20 non-infective ducks, 10 from each breed in 2 separate cages (Group 3). Duck serum samples were tested before the start of the study using Hemagglutination inhibition to confirm that all ducks were free from antibodies against avian influenza (AI).

All ducks were housed in animal Biosafety Level 3 (BSL-3) facilities at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP). Each group was housed in high-efficiency particulate air (HEPA-filtered) negative-pressure isolation units, with 0.5 m^2 space per duck, a 16:8 hours light-dark cycle, an ambient temperature of 25 ± 2°C, with relative humidity of 65%, *ad libitum* access to drinking water, and commercial pellet feed (Formulated for ducks, 22% protein) provided twice daily.

Inoculation and sampling

Ducks in infected groups received 0.1 mL intranasal inoculation of 10^6 EID_{50} of virus diluted 1:10. Oropharyngeal and cloacal swabs were collected on 3, 5, 7, and 10 days post-infection (DPI). Euthanasia was conducted using carbon dioxide inhalation as per ethical protocols. Tissue samples from lungs, brain, kidneys, and spleen were collected at each time point from 2 ducks per group. Ducks found dead or euthanized were immediately necropsied.

Internal organ viral shedding and replication

Tracheal and cloacal swabs were collected at 3, 5, 7, and 10 DPI from the inoculated and control groups with 10^6 EID_{50} virus to evaluate viral shedding from live ducks. To investigate viral replication in tissues, two ducks from each group were euthanized at 3, 5, 7, and 10 DPI. Lung, brain, spleen, and kidney were collected and processed for viral analysis.

Real-time reverse transcriptase-PCR

Swabs and tissue samples after grinding were processed for RNA extraction using Qiagen Viral RNA Mini Kit (Qiagen, Germany). For inactivation, 100 µL of each sample was mixed with 300 µL lysis buffer (Qiagen, Germany) supplemented with β-mercaptoethanol (100:1 v/v) and 3 mg RNA carrier (Qiagen, Germany). The mixture was incubated for 15 minutes at 22-25°C.

Quantitative RT-PCR targeting the conserved region of the *M* gene was carried out using the Qiagen OneStep RT-PCR kit (Qiagen, Germany) as described by Spackman et al. (2002). The amplification was carried out using the 7500 RT-PCR system (Applied Biosystems, USA) under the following cycling parameters. Reverse transcription (RT) at 50°C for 30 minutes, initial denaturation at 95°C for 15 minutes, followed by 40 PCR cycles consisting of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. Each RNA sample was tested in duplicate, and the assay was considered valid only if the cycle threshold (Ct) variations between replicates were less than 1, with a standard slope ranging from -3.2 to -3.7. The Ct values were then converted into equivalent 50% egg infectious dose per milliliter ($eqEID_{50}$/mL) using RNA standards derived from titrated virus.

Histopathology

Approximately 1 cm^3 samples of lung, brain, spleen, and kidney were fixed in 10% neutral buffered formalin. Tissues were dehydrated, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E; Bancroft and Gamble, 2008). Slides were evaluated under a light microscope (Olympus BX50, Japan). Lesions were scored semi-quantitatively based on severity of inflammation, necrosis, and hemorrhage using a 0–3 scale in which 0 means no lesion, 1 means mild lesions, 2 means moderate lesions, and 3 means Severe.

Genetic markers for pathogenicity

The H5N1 virus (A/ibis/Egypt/RLQP-229S/2022) amino acid sequences accession numbers were retrieved from the Genbank database for the *HA* gene OP491851, the *NA* gene OP491854, the *PB2* gene OP491860, the *PB1* gene OP491859, the *NS1* gene OP491857, the *PA* gene OP491856, and the *NP* gene OP491855.For comparative analysis, closely related viral sequences were retrieved from the GenBank database. Multiple sequence alignments and pairwise comparisons were generated for each gene segment using the Clustal-V algorithm in *Mega 5* to identify genetic markers and determine their positions within the encoded proteins..

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v20. A two-tailed Student's t-test was used to

compare viral shedding data. Results were considered significant at $p \leq 0.05$.

RESULTS

Clinical signs and lesions

The control groups of Muscovy and Sudani ducks remained healthy throughout the current study, exhibiting no clinical signs or mortality. However, ducks experimentally infected with H5N1 (A/ibis/Egypt/RLQP-229S/2022) displayed neurological signs. Mortality rates differed significantly between species, with Muscovy ducks experiencing higher mortality (70%) compared to Native Sudani ducks (50%). The mean death time (MDT) was shorter in Sudani ducks (4 days) than in Muscovy ducks (5.2 days; Table 1). Postmortem findings revealed no pathological lesions in the control groups. In contrast, infected Muscovy ducks exhibited severe multisystemic involvement, including pericarditis, nephrosis, splenomegaly, pancreatitis, and petechial hemorrhages in the heart and spleen, along with brain congestion. Infected Native Sudani ducks displayed less extensive lesions, primarily pericarditis and nephrosis. Notably, Muscovy ducks showed more pronounced pathological changes compared to Sudani ducks, suggesting higher disease severity in this species (Table 1).

Virus shedding

Statistical analysis revealed no significant difference ($p > 0.05$) in viral shedding levels between Sudani and Muscovy ducks based on swab samples. However, significant temporal variations ($p \leq 0.05$) were observed in viral shedding across different days post-infection. The RT-PCR detection showed prolonged viral shedding in Muscovy ducks, which persisted until 10 DPI, while Sudani ducks cleared the virus earlier, with shedding detectable only until 7 DPI (Figure 1While both breeds showed early infection (3 DPI), only Sudani ducks excreted the virus through the oropharynx and cloaca on the 3, 5, and 7 DPI (Figure 1).

Table 1. Clinical signs and mortality of Muscovy and Sudani ducks at the end of the study

Breed	Mortality	MDT	Clinical signs	Post mortem
Muscovy	7/10 (70%)	5.2	Nervous manifestation	Pericarditis, petechial hemorrhage on the heart, brain hemolysis, pancreatitis, splenomegaly
Sudani	5/10 (50%)	4	Nervous manifestation	Pericarditis, nephrosis

MDT: Mean death time (days)

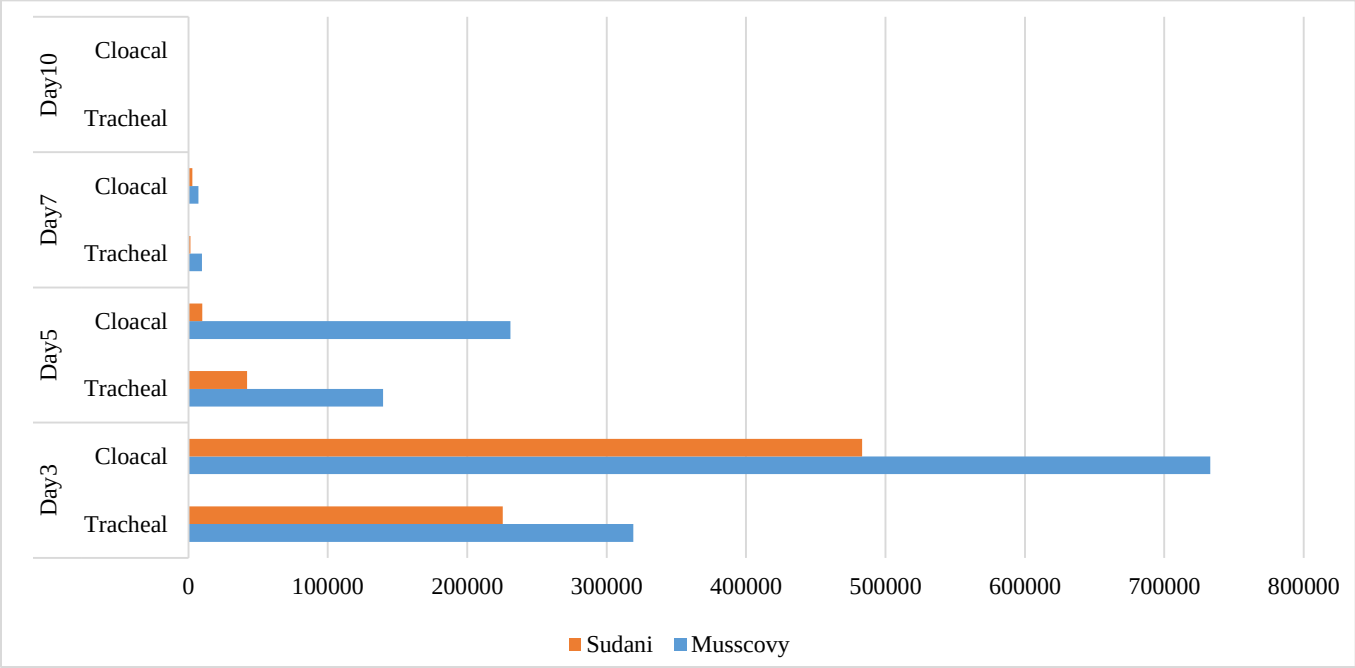


Figure 1. Virus shedding from swabs of both duck breeds at 3-, 5-, 7-, and 10-days post-infection. There was no significant difference between Sudani and Muscovy breeds in shedding results of H5 from tracheal swabs, $p > 0.05$, but there was a significant difference between both breeds in cloacal swabs, $p < 0.05$. Higher significant differences exist in shedding results on different days post-infection at $p \leq 0.05$. There was a gradual decrease in shedding from the 3rd to the 10th DPI.

Virus distribution in tissues

Widespread systemic infection was observed in Muscovy ducks, with viral detection in all examined tissues (Figure 2), and there was no statistically significant variation in viral distribution across different organs ($p > 0.05$); Both breeds demonstrated a highly significant difference in their shedding from tissues ($p \leq 0.05$). Additionally, the virus load was much higher in the brains and lungs than in other organs in both breeds (Table 2).

Histopathology

The histopathological changes in Muscovy ducks infected with H5N1 were observed in the brain tissue, featuring thickening of cerebral blood vessels, neuronal degeneration, and gliosis. In contrast, Sudani ducks exhibited perivascular lymphocytic cuffing in the brain along with gliosis, neuronal degeneration, and neuronophagia. The lungs of Muscovy ducks infected with H5N1 illustrated interstitial edema and infiltration of mononuclear inflammatory cells, accompanied by severe congestion of interstitial blood vessels. Interstitial capillary congestion was present in Muscovy ducks, and the bronchioles displayed hyperplasia of their lining epithelium. Comparatively, the lungs of Sudani ducks infected with H5N1 revealed diffuse interstitial edema with infiltration of mononuclear inflammatory cells, as well as hyperplasia of goblet cells lining the bronchial wall, along with bronchial and bronchiolar hyperplasia. The kidneys of Muscovy ducks infected with H5N1 exhibited vacuolar degeneration of the lining epithelium in some renal tubules, while others showed renal tubular necrosis. Interstitial capillary congestion and severe interstitial hemorrhage were noted in Muscovy ducks. Meanwhile, the kidneys of Sudani ducks infected with H5N1 demonstrated severe vacuolar degeneration and necrosis of the lining epithelium of renal tubules, alongside severe interstitial vascular congestion and interstitial hemorrhage. The spleen of Muscovy ducks infected with H5N1 indicated severe lymphoid depletion in the white pulp with splenic hemorrhage, whereas Sudani ducks infected with H5N1 presented with lymphoid depletion and diffuse hemorrhage in the spleen.

Molecular markers of pathogenicity

The pathogenicity markers of the challenge H5N1 virus used in the present study were compared to other related viruses from ducks in the same country but with different years (2012, 2016 and 2021) and different subtypes within the same or different clades (H5N1 and H5N8 of clade 2.3.4.4b and 2.2.1.2) as shown in Table 3.

The HA protein of the virus used in the present study has a cleavage site, *EKRRKR*, that is common in the same clade, indicating a highly pathogenic nature and its ability to replicate in different tissues in birds. The NA protein is expressed in different lengths among viruses. The shorter length protein is generally found in domestic ducks due to an amino acid deletion in the region from 49-68, which is found in the parent virus A/bar-headed goose/Qinghai/3/2005(H5N1). However, other H5N8 viruses had full-length NA.

The pathogenicity markers in the *PB1* protein are *V3* and *G622*; the pathogenicity of the used virus, A/ibis/Egypt/RLQP-229S/2022 (H5N1), is assumed to be varied compared to other H5N1 or H5N8 viruses found in ducks, as it contains *R3* and *A622*. At the same time, it shares with other viruses the same pathogenicity marker *E627* of the *PB2* protein. *PA* and *NP* proteins indicated the presence of pathogenicity markers *D383*, *V105,* and *K184*, respectively, while *NS1* has *S42* and *A149* markers.

Table 2. Virus distribution in tissues of Muscovy and Sudani ducks at 3-, 5-, and 7-days post-infection

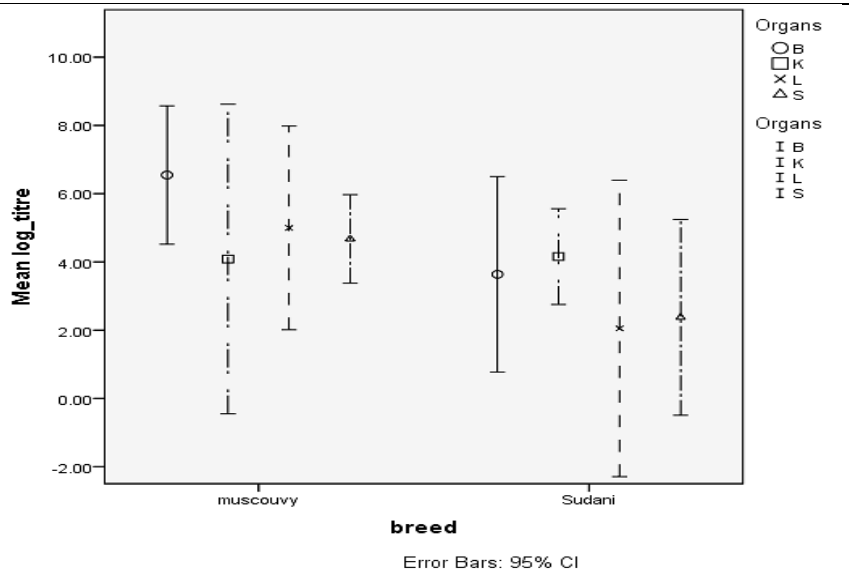
Organs	3rd DPI		5th DPI		7th DPI	
	Muscovy	Sudani	Muscovy	Sudani	Muscovy	Sudani
Brain	4.6×10^4	1.3×10^4	2.3×10^7	2.5×10^6	6.9×10^6	0
Lung	3×10^4	2.8×10^2	4.6×10^6	5.8×10^5	3.7×10^2	0
Kidney	5.9×10^5	1.6×10^4	1.9×10^6	6.6×10^5	1.9×10^4	0
Spleen	6×10^3	1.2×10^5	2.4×10^5	1×10^4	1.5×10^4	0

DPI: Days post-infection; The titers were higher in the brain than in other organs of Muscovy ducks at the 5th and 7th DPI. In Sudani ducks, the titer was higher in the brain than in other organs at the 5th DPI, with no shedding observed at the 7th DPI. The virus distribution in the organs of Muscovy ducks was greater at the 5th DPI than in Sudani ducks, while the virus persisted in the tissues of Muscovy ducks until the 7th DPI, but ceased in Sudani ducks as they recovered from clinical symptoms.

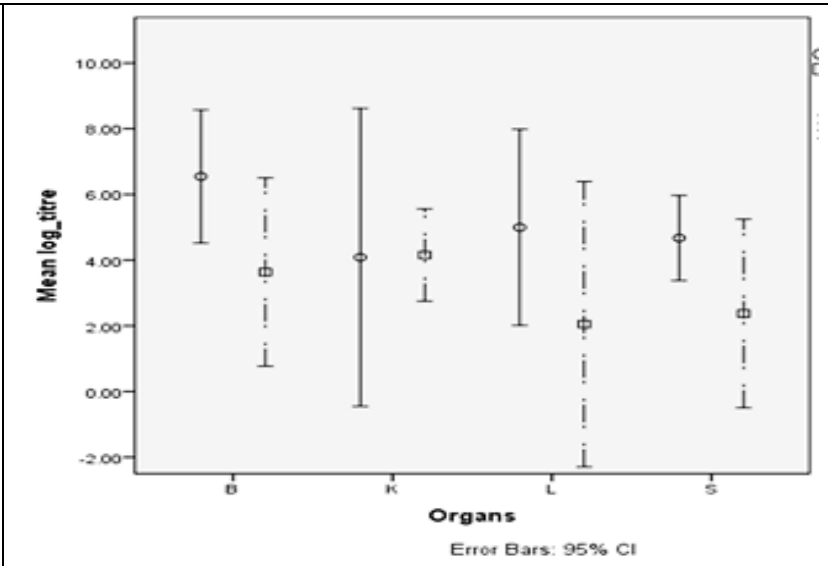
Table 3. Molecular markers of host adaptation and pathogenicity

Virus protein	HA Cleavage site	NA Length	PB1		PB2		PA	NP		NS1	
			G 622	V 3	K 627	D 701	D 383	V 105	K 184	S 42	A 149
A/ibis/Egypt/RLQP-229S/2022 (H5N1)	*EKRRKR*	460 (Δ49-68)	A	R	E	D	D	V	K	S	A
A/bar-headed goose/Qinghai/3/2005(H5N1)	*ERRRKKR*	460 (Δ49-68)	G	V	K	D	D	V	K	D	G
A/duck/Egypt/Q4596B/2012(H5N1)	*EKRRKKR*	449 (Δ49-68)	E	V	K	D	D	V	K	D	G
A/green-winged teal/Egypt/877/2016(H5N8)	*EKRRKR*	470	G	V	E	D	D	M	K	D	A
A/northern shoveler/Egypt/MB-D-817OP/2016(H5N8)	*EKRRKR*	567	G	V	E	D	D	V	K	D	A
A/duck/Egypt/SJCEIRR-BA19903OP/2021(H5N8)	*EKRRKR*	470	G	V	E	D	D	V	K	S	A
A/pintail/Egypt/RA19853OP/2021(H5N1)	*EKRRKR*	469	G	V	E	D	D	M	K	S	A

HA: Hemagglutinin, NA: Neuraminidase, PB1: Polymerase basic 1, PB2: Polymerase basic 2, NP: Nucleoprotein, NS1: Non-structural protein 1, and PA: Polymerase acidic protein. Δ: The complete length of the A/Goose/Guangdong/1/1996 genomic segments is used to number the deletions in NA.



Muscovy, Sudani
B: Brain, K: Kidney, L: Lung, S: Spleen



Muscovy, Sudani
B: Brain, K: Kidney, L: Lung, S: Spleen

Figure 2. Virus distribution in organs of two duck breeds at day 5 post-infection. There was a highly significant difference between Sudani and Muscovy breeds in shedding the H5N1 virus from tissues ($p \leq 0.05$). There was no significant difference between different organs in the shedding results from the tissues ($p > 0.05$).

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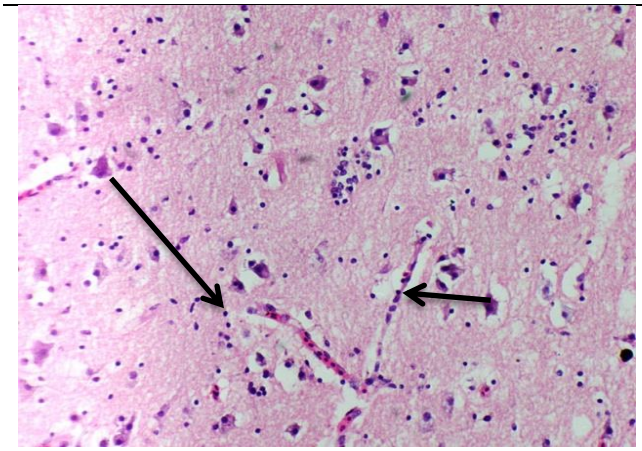
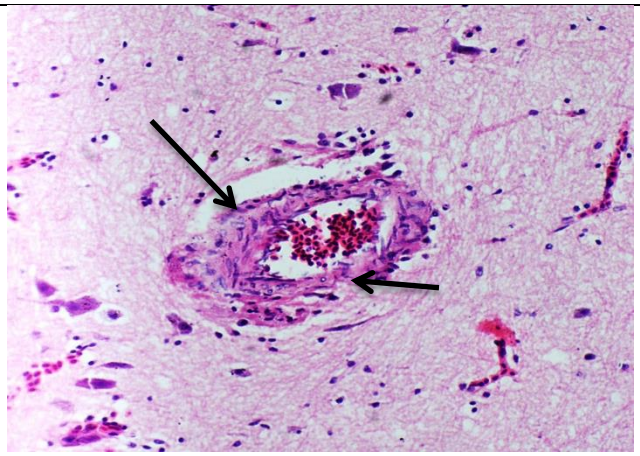
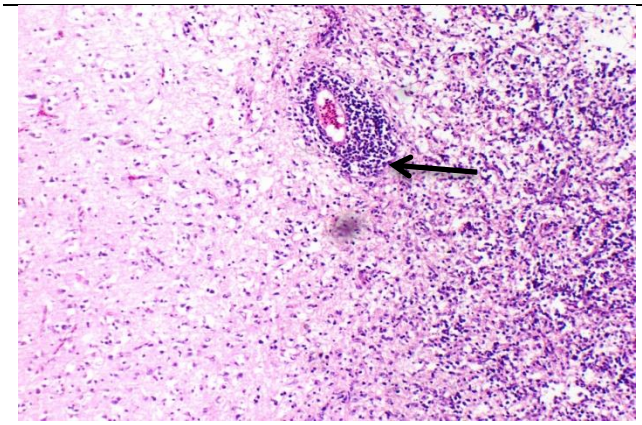
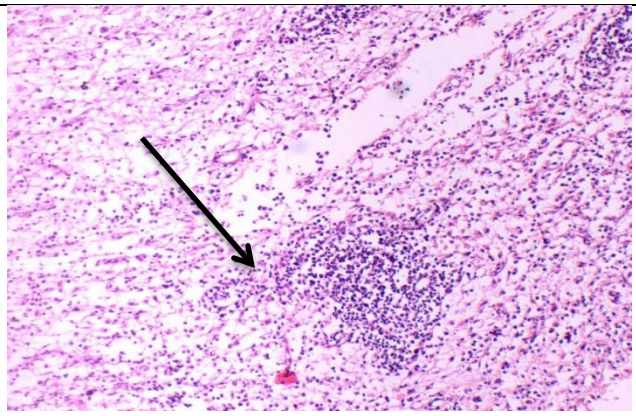
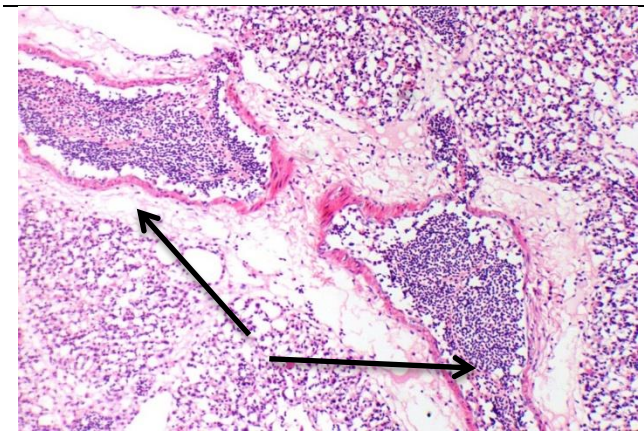
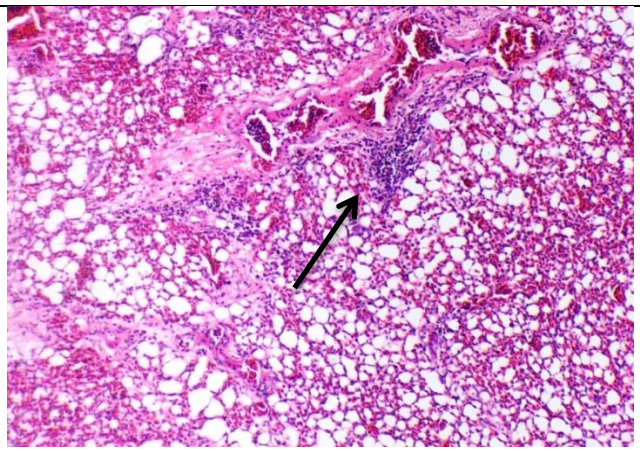
	
The Muscovy duck's brain, showing neuronal degeneration (long arrow) and gliosis (short arrow, H&E ×200)	The Muscovy duck's brain, showing thickening of cerebral blood vessel (black arrow, H&E ×200)
	
The Sudani duck's brain, showing perivascular lymphocytic cuffing and vascular congestion (black arrow, H&E ×200)	The Sudani duck's brain, showing focal gliosis (black arrow, H&E ×200)
	
The Muscovy duck's lung, showing severe congestion of interstitial blood vessels (black arrows, H&E ×100)	The Sudani duck's lung, showing vascular congestion with peri-vascular mononuclear infiltration (black arrow, H&E ×100)

Figure 3. Histopathological view of Sudani and Muscovy ducks infected with A/H5N1 from different organs at day 5 post-infection.

DISCUSSION

The adaptability of H5N1 viruses to wild ducks plays a critical role in their widespread dissemination across continents. Asymptomatic shedding by wild waterfowl facilitates the reassortment and transmission of these viruses to domestic poultry, creating opportunities for the emergence of new and potentially more dangerous strains. Given the rapid evolutionary capacity of H5N1, implementing robust surveillance programs in poultry populations is critical for early detection and outbreak mitigation. Wild waterfowl, especially ducks, act as key natural reservoirs for AIV, frequently harboring and transmitting these pathogens without showing clinical symptoms (Blagodatski et al., 2021).

The present findings confirmed that both Muscovy and Native Sudani ducks are highly susceptible to infection with HPAI H5N1, as evidenced by virus shedding and histopathological changes. Muscovy and Native Sudani ducks showed marked viral replication and systemic dissemination. The current findings revealed a significant difference in the response of Muscovy and Native Sudani ducks to infection with a recent H5N1 AIV isolate. Muscovy ducks exhibited higher mortality rates and more severe clinical signs compared to their Sudani counterparts, suggesting a greater susceptibility to the H5N1 strain.

Duck species and breeds exhibit varying levels of susceptibility to H5N1 infection. While the immunological profiles of waterfowl prior to the emergence of highly pathogenic H5N1 strains remain poorly characterized, ducks are known to experience seasonal infections with LPAIV. These endemic LPAIV infections typically peak during autumn migration periods (Diskin et al., 2020; Kent et al., 2022). It is observed that the current H5N1 HPAIV has spread across continents largely due to its high adaptability to wild ducks, including migratory species (PAHO, 2023). In previous studies, H5N1 viruses found in wild waterfowl in Hong Kong induced severe neurological signs and resulted in mortality of ducks in both natural and controlled environments (Sturm-Ramirez et al., 2004; Beerens et al., 2021).

These differences in susceptibility could be attributed to several factors. Genetic variations between the two breeds may influence their immune response to the virus. It is possible that differences in behavior or physiology contribute to varying levels of viral replication and spread. For instance, Muscovy ducks might have behavioral patterns that lead to increased viral exposure or more efficient transmission. Specifically, the present study observed that Muscovy ducks shed more virus through their cloaca, suggesting a greater role for the fecal-oral route in transmission within this breed. In both breeds, the highest viral loads were found in the brain and lungs, suggesting a tendency for the virus to target these tissues (Szeredi et al., 2010). Interestingly, the H5N1 virus was detected in all tested organs of Muscovy ducks, with significantly higher viral loads compared to Sudani ducks.

The observed variations in pathogenicity in the present study aligned with previous studies, highlighting the diverse responses of different duck species to AIV. Some studies have shown that Muscovy ducks tend to experience more severe disease compared to other breeds, such as Pekin ducks (Pantin-Jackwood et al., 2013). In addition, Muscovy ducks were relatively shown to be susceptible to infection with H9N2 AIVs (Wang et al., 2019). This highlighted the importance of considering breed-specific susceptibilities in surveillance and control strategies.

In the present study, the MDT was four days in Sudani ducks, shorter than in Muscovy ducks (5.2 days). The H5N1 isolates exhibited variability in the MDT, ranging from 3.3 to 8.7 following intranasal injection, while other more recent isolates demonstrated an MDT between 1.7 and 4 after the same method of inoculation (Chen et al., 2004).

The mean cloacal titers were greater than the oropharyngeal titers in both breeds. These findings suggest that the virus primarily spreads via the fecal-oral route, with preferential replication occurring in the digestive tract. These current observations indicate that Muscovy and Native Sudani ducks faced a significant challenge in controlling HPAIV transmission, as they appear asymptomatic while shedding the virus for 7 days after infection. There was no significant difference between the Sudani and Muscovy breeds in the shedding results of H5 from swabs. More significant differences were observed in shedding results on different challenge days. Shedding gradually decreased from 3 to 10 DPI.

Comparison with earlier studies in domestic ducks supported the present findings. The duration and intensity of viral shedding through both cloacal and oropharyngeal routes aligned with reports on mule ducks or quails infected with HPAI H5N1 strains (Filaire et al., 2024; Bertran et al., 2013). However, the predominance of cloacal shedding in the current study underscored the fecal-oral route as a critical transmission pathway, especially in settings with a high risk of water

contamination. The current observed shedding patterns aligned with known variations in H5N1 virulence across duck species, including mallards, Pekin ducks, and Muscovy ducks (Zhao et al., 2013; Uchida et al., 2019). These differences underscore the critical role of gastrointestinal replication in viral persistence within waterfowl populations and environmental transmission dynamics. While many H5N1 strains demonstrate high virulence in chickens attributed to their polybasic HA cleavage sites, mallards typically exhibit milder infections, reflecting significant host-specific differences in disease severity (Tang et al., 2009; Sun et al., 2016). Systemic viral spread to different organs reflected the pathophysiology and significant mortalities in both duck breeds. As previously demonstrated, the H5N1 clade 2.3.4.4b/2021 virus is highly infectious and transmissible in anseriformes but relatively poorly adapted to Galliformes (James et al., 2023).

The virulence of H5N1 in ducks is modulated by molecular features such as the HA cleavage site and polymerase complex proteins (Sonnberg et al., 2013). The presence of multiple basic amino acids at the HA cleavage site enhances host protease recognition, enabling systemic spread of the virus (Suguitan et al., 2012). The observed differences in tissue tropism and disease severity among duck breeds may be attributed to genetic variations in the HA cleavage sites of circulating H5N1 strains. The current isolate contained a polybasic motif consistent with the Gs/Gd/1/96 lineage, known for high virulence in poultry (Sonnberg et al., 2013).

The neuraminidase (NA) stalk length, another genetic factor, plays a role in host adaptation. Shortened NA stalks are often linked to adaptation from wild birds to domestic poultry, potentially enhancing viral fitness in terrestrial hosts (Li et al., 2011). The isolate used in the present study exhibited a shortened stalk, which may partially explain its efficient replication in both duck breeds.

In addition, several internal gene mutations have been implicated in host adaptation and pathogenicity. Viral virulence in poultry can be influenced by several polymerase protein alterations (Tada et al., 2011). One well-known mutation in the polymerase complex proteins is the substitution of lysine for glutamate at position 627 of the *PB2* protein (E627K), which is associated with a higher level of viral pathogenicity (Nilsson et al., 2017). The *E627K* mutation in the *PB2* protein enhances replication efficiency in mammalian cells by increasing polymerase activity at lower temperatures (Bogs et al., 2011). This mutation *E627K* raises viral polymerase activity for several AIV subtypes in mammalian cell lines and boosts the pathogenicity of H5N1 viruses (Bogs et al., 2011; Suttie et al., 2019). Combining this mutation with *D701N* of the *PB2* can increase viral polymerase activity, replication, and virulence (Tada et al., 2011; Taft et al, 2015).

The *NP* proteins indicated the presence of pathogenicity markers *V105* and *K184* (Tada et al., 2011). In previous studies, the *K184* mutation enhances viral replication and increases AIV virulence in chickens as well as improves viral replication in mammalian cells by enhancing the interaction between NP and importin-α isoforms (Wasilenko et al, 2009). The *V105* mutation appears to facilitate cross-species transmission from ducks to chickens by selectively enhancing viral replication efficiency in chicken embryonic fibroblasts, while maintaining unchanged replication capacity in duck cells (Tada et al., 2011). The *PA-N383D* mutation, found in some HPAI H5N1 isolates, has been associated with increased polymerase activity and enhanced replication in both mammalian and avian cell lines (Song et al., 2011; Lee et al., 2017). Similarly, *NS1-S42* and *A149* substitutions may suppress host interferon responses, facilitating immune evasion and higher virulence (Li et al., 2006; Hale et al., 2008).

These findings reveal the complex relationship between pathogen genetics and host biology in determining clinical outcomes. A deeper understanding of these molecular interactions enables better forecasting of viral spread and more precise intervention approaches in avian populations.

CONCLUSION

The present study confirmed that both Muscovy and Native Sudani ducks are highly susceptible to infection with HPAI H5N1, with significant viral shedding occurring via the cloacal route, pointing to a gastrointestinal tropism and fecal-oral transmission. The increased mortality observed in Muscovy ducks, despite comparable viral shedding levels, indicated the need for deeper investigation into breed-specific immune responses and neurotropism of H5N1 viruses. The present study underscored the complexity of H5N1 avian influenza and the importance of understanding breed-specific differences in susceptibility. Characterization of these differential susceptibilities enables optimized surveillance strategies and targeted control measures, enhancing protection for both poultry populations and public health.

Future studies should focus on comparative immunopathology among duck breeds, particularly

evaluating cytokine responses and tissue-specific viral replication. Additionally, molecular surveillance of circulating HPAI strains in different avian hosts will be crucial for identifying key mutations associated with host adaptation and virulence. This information is critical for updating biosecurity, vaccination strategies, and outbreak response plans in both commercial and backyard poultry sectors.

DECLARATIONS

Authors' contribution

Hussein Ali Hussein designed the study. Riham I. El Tantawy collected the data. Riham I. El Tantawy and Abdelsatar Arafa conducted the data analysis and wrote the first draft of the manuscript, with Hussein Ali Hussein, Ayman El Deeb, and Basem Ahmed providing co-supervision. All authors confirmed the final edition of the study before submitting to the journal.

Availability of data and materials

The datasets created and analyzed during the current study are accessible from the corresponding author upon reasonable request.

Competing interests

The authors disclose no conflicts of interest.

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Ethical considerations

All authors acknowledge their adherence to ethical standards, including plagiarism, consent for publishing, research misconduct, data fabrication, duplicate publication, and redundancy.

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