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Short Communication

Evaluation of H5 Inactivated Vaccine Efficacy in Ostrich Populations: A Serological Study

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Abstract

Background and Aims: Ostriches (Struthio camelus) exhibit marked susceptibility to avian influenza viruses (AIV), particularly H5 subtypes, posing substantial economic risks to global ostrich farming operations. This study evaluated the immunogenicity of an inactivated H5 influenza vaccine (Re-6+Re-8) in Iranian ostriches using hemagglutination inhibition (HI) assays.

Material and methods: We conducted a comparative serological analysis of 30 vaccinated (three-dose regimen) and 30 unvaccinated adult ostriches across two major farming provinces.

Results: Vaccinated birds demonstrated significantly higher HI titers (3.1 ± 0.8 $\log_2$) compared to unvaccinated controls (2.3 ± 0.74 $\log_2$; $p < 0.05$), indicating vaccine-induced seroconversion. However, the modest antibody response fell below protective thresholds established for Galliformes, revealing suboptimal immunogenicity and considerable inter-individual variability.

Conclusions: These findings highlight fundamental differences in immune responses between ostriches and conventional poultry species, emphasizing the urgent need for optimized vaccination protocols and enhanced biosecurity measures in ostrich farming systems.

Keywords: Ostrich, H5 Avian Influenza, Vaccine Immunogenicity, Hemagglutination Inhibition

Introduction

Ostriches (Struthio camelus) show particular susceptibility to avian influenza virus (AIV) infection, with numerous documented cases of highly pathogenic avian influenza (HPAI) H5Nx strain isolations from naturally infected birds across multiple continents (1,2,3). While infected adult ostriches typically display mild clinical signs such as anorexia and depression, juvenile birds often develop severe disease manifestations including neurological symptoms, with characteristic post-mortem findings of splenomegaly and hepatic congestion (2,4,5). A major cause of concern is the ability of infected ostriches to shed high viral loads while exhibiting minimal clinical signs, which creates significant challenges for disease detection and effective control (3).

The global ostrich industry, including Iran's expanding production sector, faces considerable economic threats from HPAI outbreaks. Historical epidemics such as the H5N2 outbreaks in South Africa (2004-2011) resulted in direct economic losses exceeding USD 7.4 million and required culling of approximately 10% of the national flock (6,7). Of heightened concern is the demonstrated potential for AIV to acquire mammalian-adaptive mutations (notably

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PB2 E627K) during replication in ostriches, thereby increasing zoonotic transmission risks (8, 9). These factors collectively underscore the critical need for effective, species-specific control measures in ostrich populations. Avian influenza control in ostrich farms primarily relies on surveillance programs employing hemagglutination inhibition (HI) and RT-PCR testing (10, 11), complemented by strict movement controls. However, recent seroprevalence studies in Iran have detected high rates of H9N2 exposure (24.5% in individual birds, 47.5% at farm level) among unvaccinated ostriches, demonstrating significant natural virus circulation and highlighting population vulnerability (12). The recent emergence of H5N8 in neighboring regions has further elevated outbreak risks (13). This study systematically evaluates the efficacy of H5 vaccination (Re-6+Re-8) in Iranian ostriches through comprehensive serological HI testing, providing critical data for improving disease management in ratite populations.

Methods and Materials

Study Area

The serological epidemiological study was conducted in two provinces of Iran, Khorasan Razavi and Khorasan Jonoubi, which are well-known for ostrich farming. The study target was 30 ostriches without H5 Avian influenza vaccination and 30 adult ostriches that had already been vaccinated (3 times) with the H5 influenza vaccine (Re-6+Re-8) (QYH, Beijing, China). Re-6+Re-8 are inactivated, oil-adjuvanted reassortant vaccines containing H5N1 viruses from different genetic clades of avian influenza. The H5-Re6 strain is based on A/duck/Guangdong/S1322/2010 (clade 2.3.2), used for controlling clade 2.3.2 viruses, while the H5-Re8 strain is from A/chicken/Guizhou/4/2013 (clade 2.3.4.4g), targeting clade 2.3.4.4 viruses. These "Re" (reassortant) vaccines combine surface genes from circulating H5 viruses with internal genes from a traditional A/Puerto Rico/8/1934 (H1N1) strain to create a broad-spectrum or specific-clade vaccine.

Sample Collection

Blood samples were collected from the adult ostriches, and one month after each injection of the inactivated H5 influenza vaccine at the recommended intervals. Blood was collected from the jugular vein through sterile syringes and transferred into serum separator tubes. The samples were allowed to clot at room temperature and then centrifuged at 3,000 rpm for 10 minutes to obtain clear serum. The serum samples were stored at -20°C until further analysis. Blood sampling was carried out following human handling in order to minimize stress on the ostriches.

Hemagglutination Inhibition (HI)

The HI test evaluated the antibody response to the H5 influenza vaccine. The test was conducted according to the recommendations of the World Organisation for Animal Health (WOAH, previously OIE). The procedure was as follows: The sera were heat-inactivated at 56°C for 30 minutes to remove non-specific inhibitors. Two-fold serial dilutions of each serum sample were prepared in phosphate-buffered saline (PBS) in V-bottom 96-well microplates. An equalized 4 hemagglutinating unit (HAU) of the H5N1 virus antigen (chemical inactivated) was added to all wells and incubated at room temperature for 30 minutes. A 1% chicken red blood cell (RBC) suspension was added to all wells and incubated at room temperature for 30 minutes. The highest serum dilution that completely inhibited hemagglutination was recorded as the HI titer.

Statistical Analysis

The HI titers of the different vaccinated ostrich groups were compared to assess the immune response. The antibodies' geometric mean titer (GMT) in each group was calculated. Statistical analysis was performed using Graphpad, and a t-test was employed to assess significant differences between groups. The statistical significance level was $p < 0.05$.

Results

Serological analysis revealed distinct differences in immune responses between vaccinated and unvaccinated ostrich populations. The vac-

cinated group exhibited geometric mean HI titers of 3.1 ± 0.8 log_2, representing a statistically significant increase ($p < 0.05$) compared to the unvaccinated control group (2.3 ± 0.74 log_2). Within the vaccinated cohort, HI titers ranged from 2 to 4 log_2, with the majority (70%) demonstrating titers of 3 or 4 log_2. In contrast, unvaccinated ostriches showed lower titers ranging from 1 to 3 log_2, with most values clustered at 2 log_2 (Figure 1). This pattern suggests that while vaccination induced measurable seroconversion, the magnitude of response remained modest, failing to reach the protective thresholds typically observed in vaccinated Galliformes.

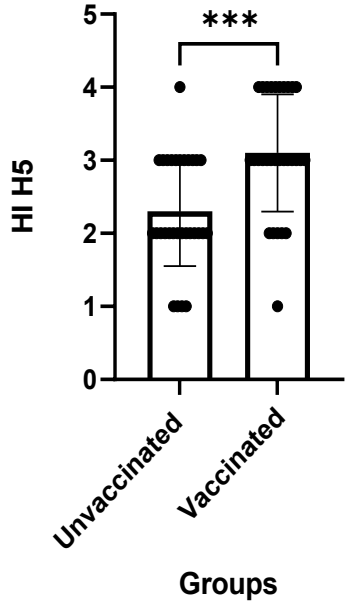

Fig. 1. Bar Graph of Post-Vaccination HI H5 Responses

Discussion

The present findings demonstrate that while inactivated H5 vaccination elicits a statistically significant immune response in ostriches, the achieved antibody levels may be insufficient for robust protection against avian influenza infection. Several factors likely contribute to this suboptimal immunogenicity. First, the physiological differences in immune system function between ratites and Galliformes may account for the attenuated response to conventional vaccine formulations. Second, the antigenic match between the vaccine strains (Re-6+Re-8) and circulating field viruses in the region may be incomplete, potentially limiting cross-protection. The observed HI titers in vaccinated ostriches (3.1 ± 0.8 log_2) fall notably below the ≥4 log_2 threshold associated with protective immunity in chickens, raising concerns about vaccine efficacy in this species. This finding aligns with previous reports of differential immune responses in ratites compared to conventional poultry (1). The presence of low-to-moderate HI titers (1-3 log_2) in unvaccinated controls likely reflects natural exposure to avian influenza viruses in the study region (12), consistent with the known circulation of H5 subtypes in Iranian poultry populations.

Natural infection in ostriches often goes undetected due to minimal clinical signs, despite high viral loads, which complicates surveillance efforts. Clavijo et al. demonstrated extensive AIV replication in multiple tissues and prolonged shedding in experimentally infected ostriches without overt disease, underscoring the limited sensitivity of standard serologic and virologic assays in this species (1). Our modest HI increases align with field serosurveys in South Africa, where only 2.4–3.7% of slaughter and breeder ostriches tested seropositive for H5 despite documented outbreaks, pointing to diagnostic gaps and potential underestimation of exposure (14). The capacity of ostriches to harbor and shed H5Nx strains silently raises zoonotic concerns, particularly given the documented transmission of LPAI H7N1 to abattoir workers and veteranarians during ostrich outbreaks in South Africa, where 10.3% of workers showed seropositivity for H7 and 2.7% for H5 (15). Prolonged viral replication in an asymptomatic host may foster adaptive mutations-such as PB2 E627K-heightening mammalian infectivity. Although our study did not sequence viral isolates, the serological evidence of low-level exposure warrants heightened occupational biosecurity and the use of personal protective equipment among farm and processing personnel.

The study's limitations include its relatively small sample size and the absence of challenge experiments to directly correlate HI titers with protective efficacy. Future research should investigate cellular immune responses and evaluate modified vaccination regimens under con-

trolled conditions. Additionally, longitudinal studies tracking antibody persistence and field effectiveness would provide valuable data for refining vaccination strategies in ostrich populations.

Conclusions

These results have important implications for avian influenza control in ostrich farming systems. The limited vaccine-induced immunity suggests that current vaccination protocols may need optimization through strategies such as adjuvant modification, antigen dose adjustment, or alternative delivery routes. Furthermore, the potential for subclinical infection in vaccinated birds underscores the continued importance of comprehensive biosecurity measures and active surveillance programs.

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Disclosure

None

Conflict of Interest

No conflict of interest is declared.

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Ethics Approval

The Animal Ethics (AE) Committee of the University of Tehran approved all animal manipulations with the approval code UT-1202.

Data Availability Statement

All data generated or analyzed during this study are included in this article.

References

1. Clavijo A, Thomas C, Esterhuysen JJ, Brown IH. Pathogenesis and tissue distribution of highly pathogenic avian influenza virus infection in ostriches (Struthio camelus). Avian Dis. 2001;45(3):928–35.
2. Abolnik C, Gerdes GH, Howell PG, et al. Molecular epidemiology of H5N2 avian influenza viruses in South African ostriches (2004–2006). Vet Microbiol. 2012;160 (3-4):423–30.
3. Abolnik C, Gerdes GH, Latif A, Bisschop SP. Serological and virological diagnostics of low and highly pathogenic avian influenza virus infections in ratites. Avian Pathol. 2016;45(1):34–40.
4. Capua I, Marangon S, Terregino C, Cattoli G. Highly pathogenic influenza virus (H7N1) in ostriches: clinical, gross and microscopic findings. Avian Pathol. 2000;29 (3):225–31.
5. Howerth EW, MacLachlan NJ, Brawn JD, Slota KJ. Neurological disease associated with highly pathogenic avian influenza A (H5N2) virus infection in juvenile ostriches. J Wildl Dis. 2012;48(4):995–1005.
6. van Helden PM, Nielsen L, Esterhuysen JJ, van Niekerk JL. Economic impact of H5N2 outbreaks in the South African ostrich industry, 2004–2011. Ostrich. 2016;87(1):52–7.
7. Duminy G. Socio-economic impact of avian influenza in ostriches. In: Murray PD, Cohen J, editors. Emerging Infectious Diseases in Animals. Cambridge: Cambridge University Press; 2016. p. 123–140.
8. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian influenza PB2 E627K mutation and enhanced mammalian adaptation. Nature. 2009;459 (7249):915–9.
9. Long JS, Mistry B, Haslam SM, Barclay WS. Elevated PB2 E627K within mammalian-adaptive influenza A viruses increases polymerase activity and virulence. J Virol. 2016;90(14):6318–26.
10. Swayne DE, Suarez DL. Highly pathogenic avian influenza. Rev Sci Tech. 2000;19(2):463–482.
11. Department of Agriculture, Forestry and Fisheries (DAFF). Avian Influenza Manual. Pretoria: DAFF; 2012.
12. Fallah Mehrabadi MH, A, Mirzaei K, Ghalyanchi Langeroudi A, Ghafouri SA, Tehrani F, Hashemi A. Serological Survey of Avian Influenza (H9N2) in Commercial Ostrich Farms in Iran, 2015. Arch Razi. 2018; 73(4): 325-30
13. Khomenko S, Yershov Y, Kozlov A, et al. Emergence and spread of H5N8 avian influenza in the Middle East. Transbound Emerg Dis. 2018;65(6):91–7.
14. Sinclair M, Brückner GK, Kotze JJ. Avian influenza in ostriches: epidemiological investigation in the Western Cape Province of South Africa. Veterinaria Italiana. 2006;42(2):69–76.
15. Venter M, Treurnicht FK, Buys A, Tempia S, Samudzi R, McAnerney J, et al. Risk of human infections with highly pathogenic avian influenza H5N2 and low-pathogenic H7N1 during outbreaks in ostriches in South Africa. J Infect Dis. 2017;216(Suppl 4):S512–9.