



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating: 4.61

VET 2025; 10(3): 367-371

© 2025 VET

www.veterinarypaper.com

Received: 08-01-2025

Accepted: 12-02-2025

Courage Chandipwisa

Vaccine Production and Quality Control Program, Pan African University Life and Earth Sciences Institute Including (Health and Agriculture), Paulesi, University of Ibadan, Oyo State, Nigeria

Dauda Garba Bwala

Viral Vaccine Production Division, National Veterinary Research Institute, Vom Plateau State, Nigeria

Victoria Olusola Adetunji

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

Mada Alesa

Viral Vaccine Production Division, National Veterinary Research Institute, Vom Plateau State, Nigeria

Uchenna Somtochukwu Okafor

Vaccine Production and Quality Control Program, Pan African University Life and Earth Sciences Institute Including (Health and Agriculture), Paulesi, University of Ibadan, Oyo State, Nigeria

Foulématou Soumah

Vaccine Production and Quality Control Program, Pan African University Life and Earth Sciences Institute Including (Health and Agriculture), Paulesi, University of Ibadan, Oyo State, Nigeria

Stephen Israel

Viral Vaccine Production Division, National Veterinary Research Institute, Vom Plateau State, Nigeria

Corresponding Author:

Courage Chandipwisa

Vaccine Production and Quality Control Program, Pan African University Life and Earth Sciences Institute Including (Health and Agriculture), Paulesi, University of Ibadan, Oyo State, Nigeria

Evaluation of the safety of cell culture-adapted fowl pox vaccines

Courage Chandipwisa, Dauda Garba Bwala, Victoria Olusola Adetunji, Mada Alesa, Uchenna Somtochukwu Okafor, Foulématou Soumah and Stephen Israel

DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i3f.2161>

Abstract

Fowl pox virus infections cause significant economic losses in poultry farming, particularly in endemic regions. Despite the widespread use of chorioallantoic membrane (CAM)-propagated vaccines in Nigeria, outbreaks persist. This study aimed to assess the safety of cell culture-adapted fowl pox virus vaccines in chickens. A total of 25 specific-antibody-negative, five-week-old chickens were vaccinated via the wing web route with ten standard field doses (≈ 0.12 mL per dose) of each cell culture-adapted vaccine. The birds were monitored for 7–21 days for vaccine “takes,” adverse effects, disease, or mortality. Two birds per group were euthanized on day 21 for histological examination using Haematoxylin and Eosin staining. All vaccinated chickens (100%) developed vaccine “takes” without adverse reactions, disease, or mortality. No vaccine-associated disease or virulence reversal was observed (0%), as histological examination showed no “Bollinger bodies.” However, mild histopathological changes were noted, including renal tubular necrosis, hepatocyte necrosis, hemorrhage, and vascular congestion in various tissues. Polymorphonuclear cell clusters were also found in lung and liver tissues. These findings indicate that cell culture-adapted fowl pox vaccines are safe and effective under laboratory conditions. Additionally, they offer advantages over CAM-propagated vaccines, such as ease of production, cost-effectiveness, and higher vaccine yields. Further studies are needed to validate these results, assess vaccine persistence in host tissues, and evaluate the potential for genetic mutations.

Keywords: Safety, fowl pox, vaccines, cell-culture, histopathology, genetic mutations

1. Introduction

Fowl pox (FP) is a viral disease of both domesticated and wild birds ^[1]. The disease poses a significant challenge to the poultry industry that often leads to severe consequences like blindness, low egg production in laying hens, and increased mortality ^[2, 3]. The Fowl pox virus is a cytoplasmic DNA virus categorized in the family *Poxviridae*, subfamily *Chordopoxvirinae*, and genus *Avipoxviruses* (APV) ^[4]. The virus causing FP in turkeys and chickens is predominantly transmitted to poultry via aerosols, biting insects, contaminated food and water ^[4]. Following viral penetration and systemic infection, two forms manifest in birds: cutaneous (dry form), and diphtheritic (wet form) ^[5]. The cutaneous variant is associated with lesions characterized by the presence of grey-white nodules located close to the eyes, skin, beak, and cloaca ^[1] whilst the wet form appears in the upper digestive and respiratory tracts, thus complicating ingestion and inhibits hydration, ultimately resulting in starvation, dehydration and mortality ^[6]. A simultaneous manifestation of a mixed cutaneous and systemic variant of FP infection was observed in juvenile chickens ^[7]. Control measures for FP primarily rely on stringent biosecurity practices and vaccination with either attenuated FPV strains or antigenically similar Pigeon pox virus vaccine ^[5]. However most commercially available live FP vaccines, particularly in Nigeria, are produced using chorioallantoic membrane (CAM) of embryonated eggs ^[8, 9].

While CAM-based vaccines have been widely used, the present several drawbacks, including low titres, inability to illicit immune responses, high production costs, and labor-intensive manufacturing processes, ultimately limiting vaccine availability [9, 10]. These limitations have resulted in an inadequate supply of FP vaccines to meet the growing demands of the poultry industry. In view of the above shortcomings of CAM vaccines, the search for fowl pox virus vaccine adapted to a cell culture with high infectious and immunogenic properties becomes critical [9]. Given these challenges, there is an urgent need to develop FP vaccines using cell culture systems, which offer several advantages over CAM-based production. Cell culture-adapted vaccines can enhance viral replication efficiency, improve immunogenicity, produce vaccines with high titres, and streamline large-scale production, making them more reliable and cost-effective alternative [9]. Furthermore, cell culture technology has become a cornerstone of modern veterinary vaccinology, facilitating the production of safer and more effective vaccines [11]. In response to these needs, this study evaluated the safety of various locally produced cell culture-adapted live FPV vaccines in chickens, with the goal of optimizing vaccine efficacy and addressing the supply gap in

FP prevention.

2. Methods

Authorization for this research involving the use of experimental animals (Specific pathogen free chickens), including eggs, was secured from the National Veterinary Research Institute, Vom Animal Use and Care Committee, under approval number: NVRI/AE/02/164/24.

Five birds free of maternal antibodies randomly allocated into four groups after statistically calculated via Federer's formula were vaccinated with 10 times the standard field dose (≈ 0.12 mL per dose) of the four FP cell culture adapted vaccines propagated in four cell lines namely: chicken embryo fibroblast (CEF), duck embryo fibroblast (DEF), quail embryo fibroblast (QEF), and Vero cells with titres: CEF= $10^{9.3}$ TCID₅₀/mL, QEF= $10^{8.6}$ TCID₅₀/mL, DEF= $10^{7.6}$ TCID₅₀/mL, and Vero cells= $10^{7.3}$ TCID₅₀/mL. One group of five birds were kept as unvaccinated control group. The birds were vaccinated by pricking the wing web with a specialized applicator containing the vaccine, at five weeks of age and kept under the deep litter management system, while being feed and watered *ad libitum*. The experimental plan and treatment groups were as outlined in Table 1.

Table 1: Experimental design for Fowl pox vaccine study showing the different groupings, and treatments received

Group (N=5Birds)	WEEK		
	Pre-vaccination	Vaccination	Post-vaccination (PV)
	Week 4	Week 5	Week 8 (21 DPV)
B	Stabilise	CEF Vaccine – 10x Std field dose	Terminate Exp., Euthanize 2 birds
D	Stabilise	DEF Vaccine – 10x Std field dose	Terminate Exp., Euthanize 2 birds
F	Stabilise	Vero Vaccine – 10x Std field dose	Terminate Exp., Euthanize 2 birds
H	Stabilise	QEF Vaccine – 10x Std field dose	Terminate Exp., Euthanize 2 birds
K (Control)	Stabilise	No Vaccination	Terminate Exp., Euthanize 2 birds

KEY: CEF-Chicken Embryo Fibroblast, DEF-Duck Embryo Fibroblast, QEF-Quail Embryo Fibroblast, Std-Standard, DPV-Days Post-Vaccination; Exp-Experiment

The birds were then monitored daily for 7-10 days for evidence of 'takes', which is defined, "as a skin oedema or the development of a scab at the vaccination site". The birds were also monitored for the absence of adverse effects/reactions or disease, and/or mortality attributable to the vaccines [8, 10]. On day 21 post vaccination at the termination of the experiment, two birds from each group were randomly selected and humanely euthanized. The euthanized chickens were necropsied and several tissues namely: kidney, liver, lungs, and heart were aseptically sampled and preserved in 10% buffered formalin, The fixed tissues were routinely processed for haematology-and-eosin

staining (H&E) examination under a light microscope (Carl Zeiss Axio Imager A1 binocular) for histopathological changes [12].

3. Results

After vaccination, 100% (20/20) of the birds in the four groups that received 10 times the standard field dose developed "takes" (swelling at the injection site) within 7 days post-vaccination but the non-vaccinated control birds however did not develop "takes". The vaccinated birds that developed "takes" are shown in Figure 1 (a & b) below

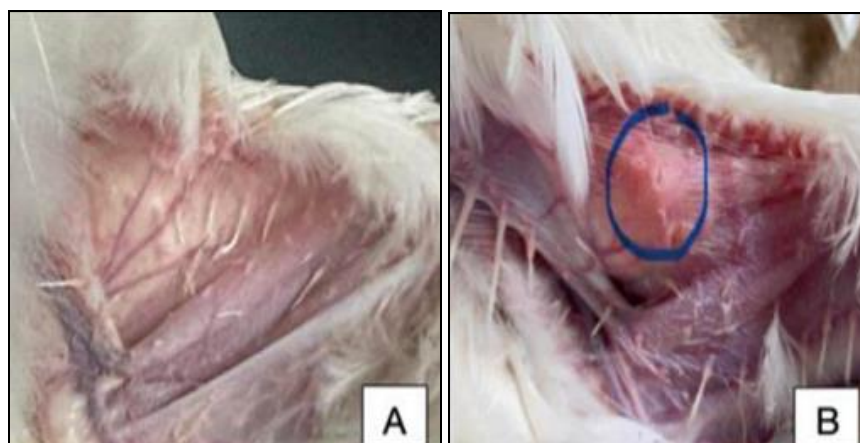


Fig 1: A-An image of the non-vaccinated birds (Control); B-An image showing evidence of "takes" (swelling at the site of vaccination) in vaccinated birds (circled area in blue)

In addition, all the vaccinated birds that received 10 times the standard field dose did not develop any undesirable/adverse symptoms or clinical disease related to the vaccine or vaccination with the 4 different vaccines. However, although these birds did not develop any undesirable/adverse symptoms or clinical disease or mortality related/associated with the vaccine or vaccination with the 4 different vaccines, there were minor histological changes including necrosis of the renal tubules and the renal corpuscles in the kidney tissues of CEF vaccinated birds (Figure 2B), while there were congestion of the renal vessels in QEF vaccinated birds (Figure 2D). The lung tissues on the other hand had congestion/ hemorrhages in blood/pulmonary vessels and

clusters of polymorphonuclear cells in all the vaccinated birds (Figure 3G-J). The cardiac tissues have normal muscle and coronary vessels except the DEF vaccinated birds which had congested coronary vessels (Figure 4M). The hepatic tissues of the all the vaccinated birds had several histological changes including severe necrosis of the hepatocytes (CEF vaccinated birds-Figure 5Q) congestion of the central vein and necrosis of the hepatocytes (Figure 5R=DEF vaccinated group); congestion of the portal vein (Figure 5S=QEF vaccinated group), and aggregation of polymorphonuclear cells and partial congestion of the portal vein (Figure 5T=Vero vaccinated group).

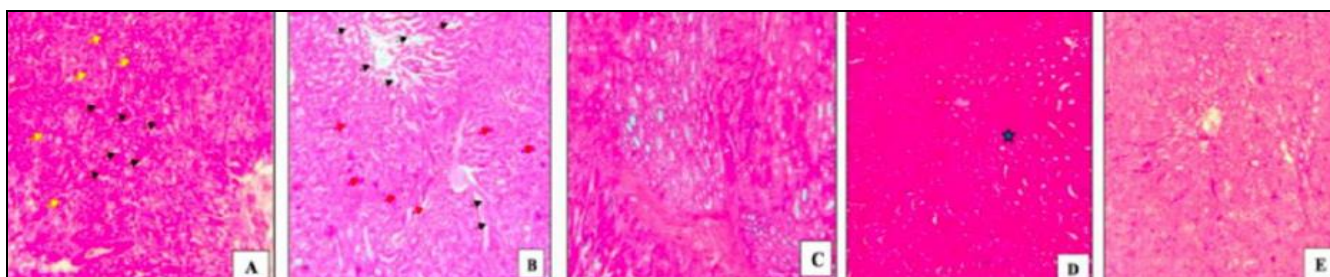


Fig 2(A-E): Histological illustrations depicting various renal lesions

Key: A=Unvaccinated Control Group: Kidney revealing normal corpuscles (black arrows), and tubules (yellow arrows) (H&E x100); B=CEF vaccinated group: Necrosis of the renal tubules (black arrows) and the renal corpuscles (red arrows) (H&E x100); C=DEF vaccinated group: Kidney

revealing no pathology (H&E x100); D=QEF vaccinated group: Congestion of the renal vessels (blue stars) (H&E x100); E=Vero vaccinated group: Kidney parenchyma is normal no pathology observed (H&E x100).

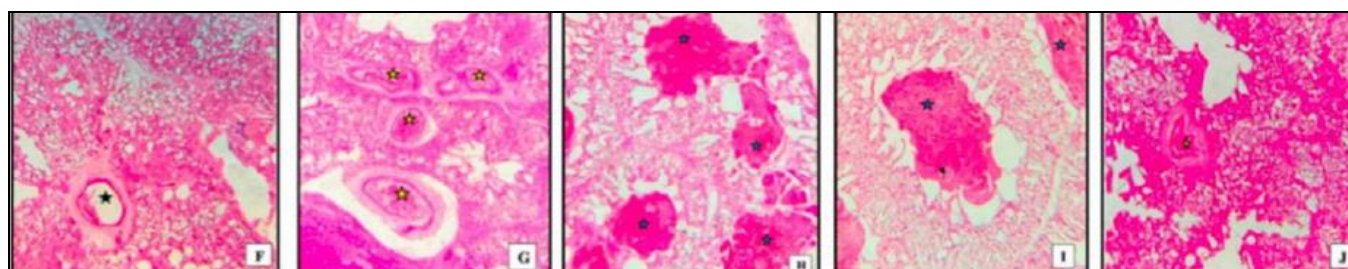


Fig 3(F-J): Histological pictograms showing different pulmonary lesions

Key: F=Unvaccinated Control group: Lungs revealing no pathology. The blood vessels (black stars) are clear of any congestion (H&E x 100); G=CEF vaccinated group: Lung revealing congested blood vessels (yellow stars) (H&E x 100); H=DEF vaccinated group: Lung revealing hemorrhage

(blue stars) (H&E x 100); I=QEF vaccinated group: Lung revealing hemorrhage (blue stars) and congested pulmonary vessels, also present are clusters of polymorphonuclear cells (black arrows) (H&E x 100); J=Vero vaccinated group: Congestion of pulmonary vessel (yellow star) (H&E x 100).

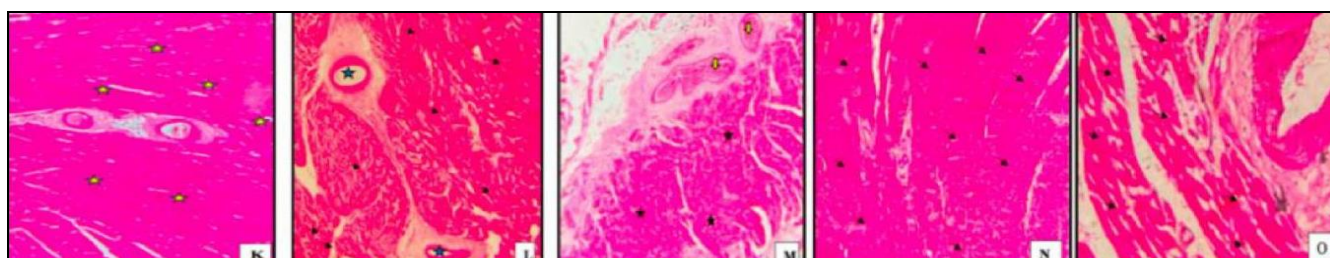


Fig 4(K-O): Histological illustrations showing different cardiac lesions

Key: K=Unvaccinated Control group: Heart revealing normal cardiac muscle (yellow stars) (H&E x 100); L=CEF vaccinated group: Heart revealing normal cardiac muscle (black arrows) and coronary vessels (blue stars) (H&E x 100); M=DEF vaccinated group: Cardiac muscle (black stars)

revealing congested coronary vessels (yellow arrows) (H&E x 100); N=QEF vaccinated group: Cardiac muscle (black arrows) with no pathology observed (H&E x 100); O=Vero vaccinated group: Cardiac muscle (black arrows) with no pathology observed (H&E x100).

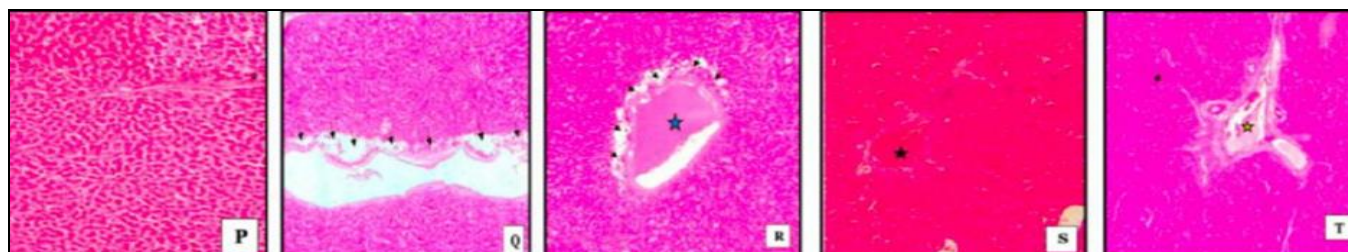


Fig 5(P-T): Histological pictograms depicting various hepatic lesions

Key: P=Unvaccinated Control group: Normal Liver, hepatic lobules with radially hepatocytic beams that radiate towards the central vein (H&E x 100); Q=CEF vaccinated group: Liver revealing severe necrosis of the hepatocytes (black arrows) (H&E x 100); R=DEF vaccinated group: Liver revealing congestion of the central vein (blue star) and necrosis of the hepatocytes (black arrows) (H&E x 100); S=QEF vaccinated group: Congestion of the portal vein (black stars) of the liver is observed (H&E x 100); T=Vero vaccinated group: Aggregation of polymorphonuclear cells (black arrows) and partial congestion of the portal vein (yellow star) (H&E x 100).

CEF=Chicken Embryo Fibroblast Adapted Vaccine; DEF=Duck Embryo Fibroblast Adapted Vaccine; QEF=Quail Embryo Fibroblast Adapted Vaccine; Vero=Vero Cells Adapted Vaccine; H&E=Haematoxylin and Eosin.

4. Discussion

Fowl pox remains a significant challenge in poultry production, leading to economic losses due to high morbidity, poor growth, and reduced egg production [13]. Since there are no standardized treatments, vaccination remains the primary control strategy to control FP. Traditionally, FPV vaccine are produced using the chorioallantoic membrane (CAM) of embryonated chicken eggs. However, this method has several drawbacks, including producing low titre vaccines, high production costs, and labor-intensive processes [8, 9]. These limitations have necessitated the search for alternative vaccine production methods, particularly the adaptation of fowl pox virus to cell culture systems, which offer improved efficiency, high viral titres, and scalability [11]. This study evaluated the safety of Fowl pox vaccines derived from primary cell cultures (CEF, DEF, and QEF), and a secondary cell line (Vero cells). The results of this study demonstrate that cell culture-adapted fowl pox vaccines are both safe and effective. All vaccinated birds developed vaccine “takes,” characterized by localized swelling at the inoculation site, indicating a strong immune response. Additionally, none of the vaccinated birds exhibited clinical disease, adverse reactions, or mortality, reinforcing the safety of these vaccines under laboratory conditions. Histopathological analysis revealed minor tissue changes in vaccinated groups, including renal tubular necrosis in CEF-vaccinated birds, vascular congestion in QEF- and DEF-vaccinated birds, and inflammatory cell infiltration in the lungs and liver across all vaccinated groups. However, these alterations did not translate into clinical illness, suggesting that the vaccines remain safe for use. When compared to findings from previous studies, these results align with earlier reports indicating that cell culture-adapted fowl pox vaccines induce a strong immune response while maintaining a favorable safety profile [14, 15]. The presence of mild histopathological changes is also consistent with existing literature, which suggests that such tissue responses may be transient and not necessarily indicative of long-term adverse effects [16-18]. Importantly, the absence of

clinical disease, mortality, or vaccine-associated complications in this study supports the growing body of evidence that cell culture-adapted vaccines are a viable alternative to traditional CAM-based vaccines. One of the primary advantages of cell culture-based vaccines is their efficiency in production. Unlike CAM-based vaccines, which are labor-intensive and yield relatively low virus titres, cell culture systems enable higher virus yields, improved scalability, and a more standardized manufacturing process [11]. This makes them a more practical and cost-effective alternative, particularly in regions with high demand for fowl pox vaccines. Furthermore, the ability to propagate the fowl pox virus in both avian and mammalian cell lines expands the potential for optimizing vaccine formulations with enhanced immunogenicity and safety [9]. Despite these promising findings, additional research is needed to fully validate the safety and efficacy of these vaccines in field conditions. Future studies should focus on molecular characterization of the vaccine strains to determine whether genetic mutations occur during cell culture adaptation. Advanced molecular techniques, such as quantitative PCR and immunohistochemistry, could provide further insights into vaccine virus distribution and persistence within host tissues. Additionally, large-scale field trials in commercial poultry farms would be essential in assessing the real-world efficacy and safety of these vaccines. Immunological studies examining cytokine responses and antibody production could further clarify the mechanisms of immune protection conferred by these vaccines.

5. Conclusion

This study provides strong evidence that cell culture-adapted Fowl pox vaccines are both safe and effective under laboratory conditions. Although minor histopathological changes were observed, they did not result in clinical disease or mortality, reinforcing the potential of these vaccines as viable alternatives to conventional CAM-based vaccines. Future research focusing on viral persistence, long-term safety, and immunogenicity will be critical in further validating these findings and optimizing vaccine formulations for widespread use in poultry production.

6. Acknowledgement

I acknowledge the African Union Commission (AUC) for the funding opportunity I had in carrying out this research and the staff members at Pan African University Life and Earth Sciences Institute (PAULESI).

Conflict of Interest

Not available

Financial Support

Funding for this research was provided by the African Union Commission through the Pan African University Life and Earth Sciences Institute (including Health and Agriculture), Ibadan, Nigeria.

7. Reference

- Mohamed RI, Elsamadony HA, Alghamdi RA, Shemy ELA, Amer SAM, Safia MA, *et al.* Molecular and pathological screening of the current circulation of fowl pox and pigeon pox virus in backyard birds. *Poultry Science*. 2024;103(12):104249. <https://doi.org/10.1016/j.psj.2024.104249>.
- Minhas SK, Kumar P, Ritu P, Raman MYR, Sweetey K, Gowthaman V. Fowl pox virus: A minireview. *Worlds Poultry Science Journal*, 2023, p. 1-19. <https://doi.org/10.1080/00439339.2023.2273353>.
- Ganapathy K, Parthiban S. Pros and cons on use of live viral vaccines in commercial chicken flocks. *Avian Diseases*. 2024;67(4):410-420. <https://doi.org/10.1637/aviandiseases-D-23-99998>.
- Umar BN, Adamu J, Ahmad MT, Ahmad KH, Sada A, Orakpoghenor O. Fowl pox virus: An overview of its classification, morphology and genome, replication mechanisms, uses as vaccine vector and disease dynamics. *Worlds Poultry Science Journal*. 2021;77(4):929-947. <https://doi.org/10.1080/00439339.2021.1959278>.
- Abd El Hafez MS, Shosha EAE, Ibrahim SM. Isolation and molecular detection of pigeon pox virus in Assiut and New Valley Governorates. *Journal of Virological Methods*. 2021;293:114142. <https://doi.org/10.1016/j.jviromet.2021.114142>.
- Alehegn E, Chanie M, Mengesha D. A systematic review of serological and clinicopathological features and associated risk factors of avian pox. *Br. J. Poult Sci*. 2014;3:78-87. <https://doi.org/10.5829/idosi.bjps.2014.3.3.8553>.
- Akanbi OB, Rimfa AG, Okewole PA. Comparative study on diphtheritic, cutaneous and systemic forms of natural avipoxvirus infection in chickens. *Journal of World's Poultry Research*. 2016;6(3). [Accessed 02 January 2025]. <https://cyberleninka.ru/article/n/comparative-study-on-diphtheritic-cutaneous-and-systemic-forms-of-natural-avipoxvirus-infection-in-chickens>.
- Khalili GM, Khalesi B, Ghaderi M, Taghizadeh M, Shahkarami MK, Karimi RH. Evaluation and optimization of chick embryo fibroblasts for production of a fowl pox vaccine based on cell culture. *Iran J Virol*. 2020;14(2):6-15. <http://journal.isv.org.ir/article-1-394-fa.html>. [Accessed 02 January 2025].
- Yusifova K. The use of cell systems for the manufacture of vaccine against fowl pox. *Fao.org*, 2021. <https://agris.fao.org/search/en/providers/123799/records/6474703e425ec3c088f1a6ea>. [Accessed 02 January 2025].
- World Organization for Animal Health (WOAH). Fowl pox. In: *WOAH Terrestrial Manual*, 2023, Chapter 3.3.10. [Accessed 02 January 2025]. <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>.
- Soccol CR, Soccol VT, Dos SC, Piazenski IN, Candelario JPM. *Introduction to animal cell culture*. Springer, 2024, p. 7-27. https://doi.org/10.1007/978-3-031-55968-6_2.
- Bancroft's theory and practice of histological techniques. Elsevier. 2019. <https://doi.org/10.1016/c2015-0-00143-5>.
- Serbessa AT, Geleta GY, Terfa OI. Review on diseases and health management of poultry and swine. *International Journal of Avian & Wildlife Biology*. 2023;7(1):27-38. <https://doi.org/10.15406/ijawb.2023.07.00187>.
- Gilhare VR, Hirpurkar SD, Kumar A, Naik SK, Sahu T. Pock forming ability of fowl pox virus isolated from layer chicken and its adaptation in chicken embryo fibroblast cell culture. *Veterinary World*. 2015;8(3):245-250. <https://doi.org/10.14202/vetworld.2015.245-250>.
- Sarma G, Kersting BA, Spina G. Field safety and efficacy of a unique live virus vaccine for controlling avian encephalomyelitis and fowl pox in poultry. *Veterinary World*. 2019;12(8):1291-1298. <https://doi.org/10.14202/vetworld.2019.1291-1298>.
- Mockett APA, Deuter A, Southee DJ. Fowl pox vaccination: Routes of inoculation and pathological effects. *Avian Pathology*. 1990;19(4):613-625. <https://doi.org/10.1080/03079459008418717>.
- Verma RK, Gangwar AK. Characterization of fowl pox virus. *Advances in Experimental Medicine and Biology*. 2024;1451:55-74. https://doi.org/10.1007/978-3-031-57165-7_4.
- Shalaby SM, Awadin WF, Hamed MF, Tholoth ELM, Ibrahim I, El AF. Pathological and ultrastructural characteristics of Newcastle and pox diseases in naturally infected pigeons in Egypt. *Advances in Animal and Veterinary Sciences*. 2021;9(11). <https://doi.org/10.17582/journal.aavs/2021/9.11.1995.2004>.

How to Cite This Article

Chandipwisa C, Bwala DG, Adetunji VO, Alesa M, Okafor US, Soumah F, *et al.* Evaluation of the safety of cell culture-adapted fowl pox vaccines. *International Journal of Veterinary Sciences and Animal Husbandry*. 2025;10(3):367-371.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.