EROACTIVITY AND PROTECTIVE EFFICACY OF CLANDESTINELY TRADED AVIAN INFLUENZA VACCINES IN NIGERIA

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VACCINE PRODUCTION AND QUALITY CONTROL

Avian influenza (AI), particularly highly pathogenic avian influenza (HPAI) strains such as H5N1, poses a significant threat to poultry worldwide. While AI vaccination is a crucial control measure in many countries, its use is restricted in Nigeria. However, its clandestine use in Nigeria raises concerns about vaccine quality and efficacy. Therefore, this study evaluated the quality of these imported vaccines. Four different imported commercially available AI vaccine brands were tested for antigen content (antigenicity) via the hemagglutination (HA) test and reverse-transcription polymerase chain reaction (RT–PCR). Additionally, two of these vaccine brands were assessed for immunogenicity and neutralizing capacity. The results revealed low levels of HA antigen and Matrix 1 gene (M1) transcripts, as evidenced by HA titres below 4 log2 and qPCR cycle threshold values of 30 and 32, respectively. Seroconversion studies in vaccinated chickens revealed a delayed and suboptimal antibody response; hence, geometric mean titres (GMTs) ranged from 12-80 which is much lower than the recommended range of GMT 160-320. The vaccines failed to induce sufficient neutralizing antibodies, as evidenced by the inability to neutralize the virus in a chicken embryo model and the persistent viral HA activity in the *in-vitro* micro hemagglutination assay. These findings underscore the risks associated with the unregulated use of AI vaccines in Nigeria, potentially leading to false confidence and lax biosafety measures. This study highlights the necessity of further research to address the challenges of AI control in Nigeria, as well as stricter regulation of vaccine quality, including vaccine antigenicity, immunogenicity, and protective efficacy tests when approved. Additionally, improved biosecurity measures, such as intensive surveillance, testing, and culling on infected farms, including compensation of affected farmers, are strategies that must be fully implemented to reduce the incidence of AI outbreaks.

Key Words: avian influenza, vaccine quality, immunogenicity, Nigeria, poultry health, bio-security

EVALUATION OF THE IMMUNOGENICITY AND SAFETY OF CELL CULTURE ADAPTED FOWL POX VACCINES

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VACCINE PRODUCTION & QUALITY CONTROL

Fowl Pox (FP) is a globally important avian disease caused by fowl pox virus (FPV), and in the absence of standardized treatments, vaccination remains the most effective method for preventing and controlling FP outbreaks. Fowl Pox vaccines in most countries are propagated on the chorioallantoic membrane (CAM), However, the adoption of cell culture-based production methods has led to widespread use and application in modern veterinary vaccinology, yet this approach has not been implemented in Nigeria. The use of cell culture-adapted vaccines for FP is reported to be more economical and productive than the use of CAM-adapted vaccines, whose production is more time-consuming. Furthermore, FP vaccines generated in CAM exhibit the drawback of diminished infectious activity in hens. Consequently, the pursuit of FPV vaccines tailored for a cell culture exhibiting elevated infectious and immunogenic characteristics has become essential. The use of different cell cultures and different strains of viruses by researchers to improve and increase vaccine effectiveness and quantity to meet the demand of the poultry industry has been ongoing in developed countries; thus, there is a need for Nigeria to align with the best global practices. This study evaluated and compared the immunogenicity and safety of vaccines developed using various cell lines. FPV was cultured in Vero cells, chicken embryo fibroblast (CEF), duck embryo fibroblast (DEF), and quail embryo fibroblast (QEF), and was harvested following the achievement of 80-90% cytopathic effect in the cells. Harvested vaccine sterility checks using tryptose soya and thioglycolate media were conducted, and the tissue culture infective dose (TCID50/mL) was calculated via the Spearman-Kärber formula. Pre- and post-vaccination antibody levels were checked and monitored via the agar gel immunodiffusion (AGID) test and enzyme-linked immunosorbent assay (ELISA), respectively. The safety of all the vaccines was assayed in 25 experimental birds vaccinated with 10 times the standard field dose through the wing web stab. Postmortem and histopathological haematoxylin and eosin (H&E) staining was conducted on sacrificed birds for vaccine reactions. This study demonstrated that the fowl pox vaccines produced in both primary and continuous cell lines were safe and immunogenic, with the following titres: CEF = $10^{9.3}$ $TCID_{50}$/mL, QEF = $10^{8.6}$ $TCID_{50}$/mL, DEF = $10^{7.6}$ $TCID_{50}$/mL, and Vero = $10^{7.3}$ $TCID_{50}$/mL all of which were produced in suspension cell culture. “Takes” were observed in birds within 3-5 days post vaccination. By ELISA, birds produced measurable FP-specific antibodies, and vaccines were found to be safe. Further studies should be conducted to determine the longevity of the induced antibodies before booster doses are administered.

Key Words: Vaccine, Immunology, Safety, Potency, Sterility

COMPARATIVE ANALYSIS OF STATIC AND AGITATED INCUBATION METHODS IN OPTIMIZING CONTAGIOUS BOVINE PLEUROPNEUMONIA VACCINE PRODUCTION.

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VACCINE PRODUCTION AND QUALITY CONTROL

Contagious bovine pneumonia, caused by Mycoplasma mycoides subsp. mycoides, remains one of the most important cattle diseases in Sub-Saharan Africa due to the high morbidity and mortality rates. The low potency and short duration of protection in the commonly used CBPP vaccines, and persistent issues of production consistency undermine disease control efforts. Such limitations indicate how urgent it is to review and optimize vaccine production processes in order to make the vaccines more effective and reliable. Static incubation techniques were compared with agitated techniques to identify conditions that yield maximum vaccine potency. Some of the key parameters including mycoplasma titre, pH determination, and optical density (OD), were monitored in order to depict growth dynamics and determine the optimal harvesting period After 48 hours, the mycoplasma titre from the static method was higher at 9.92, while the method agitated peaked earlier, between 24 and 36 hours, with a lower titre of 9.61. Though agitation enhanced growth, it resulted in rapid identification of the medium and an earlier fall in titre. The static approach, on the other hand, showed more sustained production despite slower growth. Both methods require ideal harvesting periods to ensure vaccine effectiveness, which necessitate exact monitoring. These results therefore offer practical suggestions for vaccine manufacturing: the agitated method performs better in cases where rapid production cycle is needed, while static methods yield higher vaccination titre but at a slower pace. This supports the broader efforts that are necessary in mitigating this disease burden for cattle health and economic stability, especially in countries where CBPP is endemic, by optimizing vaccine production processes with robust quality controls.

Key Words: Contagious Bovine Pleuropneumonia (CBPP), vaccine production, static incubation, agitated incubation, vaccine optimization

EVALUATION OF VARIOUS FORMULATIONS OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)VACCINE (T1/44) FOR THERMOTOLERANCE AND SHELF LIFE

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VACCINE PRODUCTION AND QUALITY CONTROL

Contagious Bovine Pleuropneumonia (CBPP), is a disease caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm), remains a critical livestock disease mainly in sub-Saharan Africa, posing a significant threat to the livelihood of farmers. The widely used T1/44 CBPP vaccines are heat-labile and still depend on cold chain technologies for maintaining their stability. However, their efficacy is hindered by poor thermal stability, especially in tropical regions where high temperatures prevail requiring strict cold chain conditions during storage and transport. This limitation creates to logistical difficulties in preserving the vaccine's potency, especially in regions with inadequate cold chain infrastructure. Hence, there is a need to improve the stability and shelf life of the existing CBPP vaccine to enhance its efficacy for remote distribution in rural regions. This study was therefore designed to examine the thermotolerance and shelf-life of various CBPP T1/44 vaccine formulations containing different stabilizing agents. Seven thermo-stabilizer formulations were tested: (1) 80% lactalbumin + 13% gelatin, (2) 80% casein + 13% gelatin, (3) 16% skimmed milk, (4) 7.5% skimmed milk + 7% maltose, (5) 7.5% skimmed milk + 7% maltose + 13% gelatin, (6) 7.5% skimmed milk + 7% sucrose, and (7) 7.5% skimmed milk + 7% sucrose + 13% gelatin were used for the production of CBPP vaccine (T1/44 strain). Each formulation was freeze-dried following the standard protocols. Accelerated stability testing was conducted to determine thermo-tolerance and shelf life at 40C, 250C, and 370C over 1, 3, 7, 10, and 14 days. For each vaccine, mycoplasma viability was determined in CFU. Significant differences between groups were evaluated using two-way ANOVA, with a confidence level of 5% and $p < 0.05$. The results showed that formulation 2, with 80% Casein and 13% Gelatin, gave the best mycoplasma titers of 4.4×10^7, 1.2×10^6, and 8.0×10^6 CFU, respectively, over the increased temperatures, remaining stable over the 14-day period, outperforming other stabilizers which rapidly declined in titre. This formulation emerged as the most effective thermostabilizer in the study. This confirms the promising candidature of 80% Casein plus 13% Gelatin as an effective thermo-stabilizer to address current storage and distribution challenges. This study not only provides a comprehensive evaluation of stabilizers but also lays the groundwork for more resilient CBPP vaccines which could significantly support disease control measures in resource-limited settings. xvi

Key Words: Stabiliser, Contagious Bovine Pleuropneumonia vaccine, Thermo-stability, viable count, Shelf life, Mycoplasma mycoids

INTRODUCTION OF EMBRYONIC SKIN OF SHEEP CELLS AS AN ALTERNATIVE CELL LINE FOR POTENCY EVALUATION OF LUMPY SKIN DISEASE VACCINES

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VACCINE PRODUCTION AND QUALITY CONTROL

Lumpy skin disease is a highly contagious viral infection that primarily affects cattle and is caused by the Lumpy Skin Disease Virus (LSDV), a member of the Capripoxvirus genus in the *Poxviridae* family. The virus spreads across national borders, exhibiting high morbidity rates of up to 90% but generally low mortality rates. The disease is characterized by fever, skin nodules, and oedema. Animal vaccination is the most effective strategy for preventing the spread of the virus. In situations of outbreaks, timely vaccine approval is a critical stage in containing the virus within a specific region and eradicating it in a further phase. The LSDV potency test is currently assessed using the Madin-Darby Bovine Kidney cell lines. However, the scarcity of MDBK cells has prompted research into alternative cell lines for potency testing. This study investigated the potential use of the Embryonic Skin of Sheep cell lines as a substitute for MDBK cells. Six distinct LSD Vaccines with known titres were used to infect both cell lines. The cells were monitored for the presence of Cytopathic Effects (CPE) and vaccine titre for ten days post-infection. The embryonic skin of sheep cell lines (ESH) showed CPE on the second day post-infection, whereas Madin-Darby Bovine Kidney cell lines showed CPE on the third day. The vaccine titres were calculated using the Spearman–Kärber method. The average titre for ESH cells ranged between 3.833 and 4.367 ($TCID_{50}$/dose) for the sampled vaccines and 4.400 and 5.167 ($TCID_{50}$/dose) for the control vaccines. The Madin-Darby Bovine Kidney cells showed an average titre range of 4.200 and 5.867 ($TCID_{50}$/dose) for the sampled vaccines and 3.867 and 5.767 ($TCID_{50}$/dose) for the control vaccines. One-way analysis of variance and an independent two-tailed t-test were used to estimate the significant differences; the *P*-values revealed no statistical differences between the two types of cells in terms of vaccine titre ($TCID_{50}$/dose). The findings suggest that the Embryonic Skin of Sheep cell lines can serve as a reliable and cost-effective alternative to MDBK cells for conducting LSD vaccine potency testing, which will also contribute to the vaccine quality control protocols and improved disease control measures.

Key Words: CAPV, LSDV, ESH, MDBK, POTENCY TEST.

ASSESSMENT OF QUALITY AND IMMUNOGENICITY OF COMMERCIALLY AVAILABLE LIVE-ATTENUATED INFECTIOUS BURSAL DISEASE VACCINE IN NIGERIA

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VACCINE PRODUCTION AND QUALITY CONTROL

Commercially available live attenuated Infectious Bursal Disease vaccines are widely used to prevent and manage the risks of infectious bursal diseases in poultry. A comprehensive understanding of vaccine failure mechanisms is essential for establishing quality standards regarding vaccine immunogenicity in poultry. The aim of this study was to assess the quality and immunogenicity of six commercially available live attenuated IBD vaccines in Nigeria. The sterility, vacuum, potency, identity, immunogenicity, and RT−PCR for extraneous avian leukosis viruses (ALV) (subgroups A-E) and chicken infectious anaemia viruses (CIAV) were tested and carried out according to established protocols. The vaccines used in this study revealed the absence of microbial contaminants and the presence of vacuum integrity in vaccine vials. The infectious titre of the vaccine was determined using a simplified median tissue culture infectious dose (TCID50) assay. The viral content assay revealed only two vaccines with minimum viral titre $>10^{2.5}$ TCID50/dose. There is an absence of contaminants agents such as CIAV or ALV(A-E) in the live attenuated IBD vaccines used. Five of the six vaccines were positive for the specific VP2 gene. Vaccine C contained an internal gene, but lacked the VP2 gene which is crucial for immunogenicity. ELISA results demonstrated that only chickens in group C developed detectable IBD-specific antibodies at 14 days post vaccination (dpv) following the first immunization. By 28dpv (14 days after the booster dose) all the vaccinated chickens elicited sufficient protective titres significantly higher than antibody titres in the control group. However, by 42dpv, the antibody levels declined in group A. This result underscores the importance of routine antibody profiling in evaluating vaccine efficacy and quality in ensuring optimal protection poultry population.

Key Words: live attenuated IBD vaccine, sterility, potency, chicken, immunogenicity.

ASSESSMENT OF BOVINE VIRAL DIARRHEA (BVD) CONTAMINATION IN VACCINES: VIRUS VIABILITY EVALUATION THROUGH CELL CULTURE PASSAGING AND REAL-TIME POLYMERASE CHAIN REACTION

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VACCINE PRODUCTION AND QUALITY CONTROL

Bovine viral diarrhea virus (BVDV) is a pestivirus that primarily generally affects cattle and other ruminants. The virus causes a range of symptoms, including birth defects and abortions in the offspring. Bovine-derived products are frequently used in modified live vaccines (MLV), which increases the risk of BVDV contamination. Contamination vaccines not only compromise immunization outcomes but also facilitate the spread of new infections, potentially triggering serious BVD outbreaks, disseminating new infections, and provoking serious outbreaks of BVD. Since detecting BVDV RNA alone is insufficient for a comprehensive risk assessment of BVDV contamination in biological products. It is essential to differentiate between infectious and non-infectious viral particles. Thus, this study evaluated virus viability in BVDV-contaminated vaccines using Madin- Darby Bovine Kidney (MCBK) cell passaging and real-time PCR to monitor changes in e-Ct values across the passages. In this study, six vaccine batches submitted to AU-PANVAC for quality control between January 2022 and June 2024 and identified as BVDV contaminated through real-time PCR were selected. Each batch was reconstituted with phosphate-buffered saline (PBS) and passaged three times in MDBK cells. Samples were collected on days 0 and 5 of each passage, followed by nucleic acid extraction using the QIAGEN RNeasy Mini Kit. Real-time PCR testing was performed at each passage using an ADIAVET kit to determine Ct values. In four of the examined lots, a decrease in the Ct value across passages indicated active viral replication, confirming the presence of viable BVDV particles. However, the remaining two batches showed positive Ct values during passage one and two but became undetectable by passage three suggesting viral inactivation or an extremely low viral load. In conclusion, this study highlights the critical role of routine testing and quality screening in detecting and accessing BVDV contamination in veterinary vaccines. We strongly recommend that vaccine manufacturers implement rigorous BVDV viability screening protocols and employ effective inactivation measures to mitigate contamination risks.

Key Words: BVDV, FBS, Vaccine Contamination, Cell Culture, Viability Testing, Real-Time PCR

ASSESSMENT OF IMMUNOGENICITY OF LOCALLY PRODUCED ANTHRAX SPORE VACCINES AND SEROCONVERSION PATTERN IN VACCINATED RUMINANT SPECIES IN GHANA

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VACCINE PRODUCTION AND QUALITY CONTROL

Anthrax is endemic in Ghana and many countries across the five regions of Africa. Routine annual vaccination with an Anthrax spore vaccine has reduced the disease incidence in Ghana. However, it remains endemic with sporadic human exposure and fatalities. There is a dearth of information on the immunogenicity of anthrax spore vaccine produced in Ghana. This study aimed to evaluate the seroconversion patterns in ruminants, notably cattle, and goats following vaccination with locally produced anthrax spore vaccines in Ghana, and estimate the duration and magnitude of elicited antibody response over a 3-month period. An experimental study was conducted at the livestock breeding station in Pong-Tamale, Ghana. A total of 24 apparently healthy cattle and goats of both sexes and of appropriate vaccinable age were enrolled in the study. Baseline health assessments including physical examination complete blood count, and haemoparasite screening were performed prior to vaccination on day 0. The animals were subsequently monitored by collecting blood samples on days post inoculation (dpi) 7, 14, 21, 28 and 90 to evaluate antibody titres against anthrax, using an Indirect ELISA to measure serum IgG levels. The mean Optical density (OD) value before vaccination was 0.172 ± 0.063 for cattle and 0.207±0.076 for goats. In cattle, post-vaccinal titres for dpi 7, 14,21, 28 and 90 were 0.184±0.066, 0.188±0.072, 0.172±0.066, 0.117±0.032, 0.101±0.026 and 0.095±0.028. In goats, titre recorded for the same sampling points dpi were 0.184±0.078, 0.192±0.072, 0.167±0.045, 0.215±0.086, 0.198±0.083 and 0.177±0.060. Optical density values greater than 0.7, indicative of positivity and antibody production were not observed in either cattle or goats throughout the period of the study. This study demonstrated that cattle and goats vaccinated with the anthrax spore vaccine did not elicit sufficient antibody response after 3 months follow up. This calls for further investigation into the vaccine quality control to ensure immunogenicity of the vaccine in the target species.

Key Words: *Bacillus anthracis***,** Anthrax, Anthrax spore vaccine, Seroconversion, Ruminants, Ghana

THE 3Rs RULE APPLIED IN THE CONTROL OF THE RABIES VACCINE PRODUCED IN ALGERIA

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VACCINE PRODUCTION AND QUALITY CONTROL

Rabies is an acute, progressive, and incurable encephalomyelitis caused by neurotropic viruses of the Lyssavirus genus, for which various mammalian species act as reservoirs and vectors. Rabies prophylaxis in Algeria is based on the production and use of a tissue-based rabies vaccine. This study was aimed at applying the rule of 3Rs (reduce, refine, and replace) in rabies vaccine production. It evaluated the rabies vaccine through several quality control tests, including abnormal toxicity and residual virulence assessment, bacterial and fungal contamination tests, physicochemical analysis, the National Institutes of Health (NIH) test, and Pasteur's Rapid Fluorescent Focus Inhibition (RFFIT) test. The results of this experimental study show that the vaccine is not toxic, the rabies virus is well- inactivated, and no bacterial or fungal contamination is found. The level of thiomersal does not exceed 50 µg/ml. Its dissolution time is less than 60 seconds, ensuring the potency of the rabies vaccine by the World Health Organization (WHO). The RFFIT ensures that we can evaluate the effectiveness of the vaccine by measuring rabies virus-neutralizing antibodies without using live animals. These findings affirm the quality and safety of rabies vaccination in Algeria, illustrating that vaccine testing may adhere to stringent criteria while reducing animal use through 3Rs approaches.

Key Words: Rabies vaccine, 3Rs Rule, Nerve tissue-derived vaccine, Quality Control of Rabies vaccine.