DYNAMICS AND COMPARATIVE IMMUNOGENICITY AND SAFETY OF CHORIOALLANTOIC MEMBRANE (CAM) AND CHICKEN EMBRYO FIBROBLAST (CEF) ADAPTED FOWLPOX VACCINES.

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VACCINE PRODUCTION AND QUALITY CONTROL

Fowlpox is endemic in Nigeria and the use of vaccines has remained the key method for the prevention and control of the disease. The use of chorioallantoic membrane (CAM) route for the propagation of fowlpox virus (FPV) is the current method of fowlpox vaccine production at the National Veterinary Research Institute, Vom-Nigeria. While this vaccine is effective and routinely used to reduce morbidity and mortality, the quantity produced using the CAM route is limited. This study assessed the dynamics of a Chicken embryo fibroblast (CEF) adapted fowlpox vaccine and compared its immunogenicity and safety with a locally produced CAM-adapted fowlpox vaccine.

In this study, vaccine viruses were propagated on CAM of 9–12 day-old developing chicken embryos and CEF cell culture. Ensuing vaccine harvests were subjected to quality checks, titrated, and used in vaccinating experimental broiler chickens via the wing web stab vaccinations to monitor for "takes", seroconversion using ELISA and AGID, safety-through clinical observation of adverse effects and reversion to virulence with analysis of the FPV genome changes.

The FPV adaptation to CEF was successful as demonstrated by the attainment of 80–90% CPE within 71–115 hours in the different passages on both suspension and monolayers CEF. Pock formation was also demonstrated on CAM 5 days post-inoculation. Both the CEF and CAM-adapted vaccines harvests were highly immunogenic producing titres of above $10^{6.0}$/ml $EID_{50,}$ but higher titres of $10^{8.25}$/ml $TCID_{50}$ were recorded in the CEF-adapted vaccines indicating higher potency. All the birds vaccinated with these two vaccines showed evidence of "takes" (100%) within 3–4 days post-vaccination. In addition, the safety of the vaccines was confirmed as no adverse effects or vaccines-associated clinical disease was recorded in all the birds (including birds vaccinated with 10 times field dose), during the 5 weeks monitoring period post-vaccination. Antibody seroconversion of the 3-4 representative sera sampled over the 5 weeks period analysed by ELISA were all positive with optical density (OD) values ≥ 0.216 (the critical cutoff OD values provided by the manufacturer of kit). The pre-vaccination sera were also positive by ELISA, Despite the presence of MDA, chickens vaccinated with CEF-adapted vaccine generally have higher OD values signifying presence of higher antibody titre and higher immunogenicity. Furthermore, 64 sera samples analysed using AGID, recorded 18 (28%) positivity for FPV antibody. The analysis of the FPV P4b gene showed substitution mutations, with some retaining the core protein 4b, while others showed the presence of hypothetical proteins, whose functions could not be elucidated.

In conclusion, this research produced CAM FPV vaccine and demonstrated the successful development and adaptation of FPV to CEF. The efficacy, safety, and immunogenic potential of the developed CEF-adapted vaccine lay a solid foundation for its use in self-sufficiency vaccine production for the prevention and control of fowlpox in poultry in Nigeria.

Key words: CEF-adapted vaccine, CAM-adapted vaccine, Immunogenicity, safety, ELISA, AGID, FPV, P4b.

EFFECT OF 4-(2-HYDROXYETHYL PIPERAZINE-1-ETHANESULFONIC ACID) IN IMPROVING CONTAGIOUS BOVINE PLEURO-PNEUMONIA (CBPP) T1-44 STRAIN TITRE VALUE OF LIVE ATTENUATED VACCINE IN TANZANIA

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VETERINARY VACCINE PRODUCTION AND QUALITY CONTROL

Contagious bovine pleuro-pneumonia is highly contagious and infectious disease of cattle and water buffaloes caused by *Mycoplasma mycoides subsp. mycoides* small colony that affect the respiratory tract of animals. Vaccination with CBPP T1-44 live attenuated strain is the most effective method of controlling disease, though the African manufacturers are unable to produce the CBPP vaccine of recommended titre per dose of 10^8. Including a 4-(2-Hydroxyethyl piperazine-1-Ethanesulfonic Acid) (HEPES) buffer in growth media helps maintain pH and obtain optimal antigen and vaccine titre. This study aimed to evaluate the potential effects of HEPES buffer in improving CBPP T1-44 strain vaccine titre.

Vaccines were produced by culturing in the PPLO media buffered with HEPES at different concentrations of 0.05M, 0.075M, and 0.1M, with Na_2HPO_4 as a control unit. Harvest pH was fixed at 6.5±0.3. The Titre of the vaccine was obtained by titration whereby a total viable number of Mycoplasma was determined by Colour Change Unit 50, and the titre was calculated according to Spearman and Karber's formula.

Mean titre per dose were $\log_{10} 1x10^{9.3}$, $\log_{10} 1x10^{9.23}$, $\log_{10} 1x10^{9.133}$ and $\log_{10} 1x10^{6.982}$ for HEPES buffered vaccine at 0.05M, 0.075M, 0.1M and Na_2HPO_4 respectively. The post hoc test revealed the statistically significant difference between all concentrations of HEPES tested and Na_2HPO_4.

Adding relatively inexpensive HEPES to most current media formulations is essential for producing a quality, consistently potent, stable vaccine with optimal recommended titre.

Keywords: CBPP T1-44, HEPES buffer, Titre, pH

DEVELOPMENT AND EVALUATION OF MONOCLONAL ANTIBODIES AGAINST CBPP (T1/44) ANTIGEN WITH THE END GOAL OF DEVELOPING AN ELISA KIT FOR CBPP

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VETERINARY VACCINE PRODUCTION AND QUALITY CONTROL

Contagious bovine pleuropneumonia (CBPP) is a highly infectious bacterial respiratory disease that affects cattle and may be a major concern to the animal industry in Africa and worldwide. The assessment and development of monoclonal antibodies against CBPP antigen is significant for the potential advancement of an enzyme-linked immunosorbent test (ELISA) kit for CBPP. Monoclonal antibodies (mAbs) are highly specific and can be employed to detect the presence of CBPP-specific antigens in the diagnosis of CBPP. The use of ELISA kits based on these antibodies helps with the rapid and accurate diagnosis of CBPP and thus, contributes to disease control and prevention in cattle. This research aims to develop monoclonal antibodies against CBPP (T1/44) antigen to develop an ELISA kit for CBPP detection and diagnosis.

Hybridoma technology was utilized to develop monoclonal antibodies that can recognize and bind to the CBPP (T1/44) antigen, the production of antibody-secreting hybridomas was carried out after immunizing mice with purified CBPP antigen. The hybridomas were checked for high sensitivity, specificity, and liking to the antigen. The chosen monoclonal antibodies were assessed for sensitivity, and specificity, against CBPP antigen utilizing different immunoassays, dot-blot, ELISA, and mouse mAb isotyping.

The findings from this study showed that the monoclonal antibodies produced were profoundly specific, with higher inhibition to CBPP antigen (<0.50 OD), with no evidence of cross-reactivity to other similar antigens. Furthermore, the monoclonal antibodies were able to differentiate CBPP antigen at low concentrations, indicating their high sensitivity (>80% PI). Moreover, the isotyped mAbs of interest appeared to have a place in the IgG class.

These results indicate that the monoclonal antibodies can be utilized to develop an ELISA kit for CBPP detection or diagnosis, which would be a rapid, accurate, and cost-effective strategy for screening and diagnosis of CBPP in cattle herds.

Keywords: CBPP, Antigen, monoclonal antibodies, ELISA, Mycoplasma mycoides subspecies mycoides (Mmm)

COMPARATIVE ANALYSIS OF PERFORMANCE OF COMPETITIVE ELISA AND EPITOPE BLOCKING ELISA FOR THE DETECTION OF SPECIFIC PPRV ANTIBODIES FROM PPR-FREE COUNTRIES.

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VACCINE PRODUCTION AND QUALITY CONTROL

Peste des Petits Ruminants (PPR) is a highly contagious disease of small ruminants caused by Peste des Petits Ruminants Virus (PPRV). It is an economically important transboundary disease of sheep and goats especially in developing countries. In PPR-free countries, the timely and precise detection of specific PPR antibodies is critical to prevent the introduction and dissemination of the disease.

This study compares two ELISA assays, ID VET (N-protein based) competitive ELISA and AU-PANVAC's (H-protein based) epitope-blocking ELISA, to detect specific PPRV antibodies in samples collected from sheep and goats in three PPR-free countries. The specificity of both ELISA techniques in detecting PPRV-negative samples, which provides insights into their comparative performance were evaluated.

A large panel of sera (n=1483) from sheep and goats from PPR-free countries namely Lesotho, Botswana and Sao Tome were examined. A relative specificity (99.73%) between the two tests for detecting PPRV antibodies in the sera was observed. A substantial agreement of (K=0.79) between both tests was also observed, indicating that both tests could be used to accurately detect PPR antibodies in PPR free-countries.

Furthermore, this study also addresses the challenge of cross-reactivity associated with PPR, particularly relevant in countries where other viruses, such as Canine Distemper Virus (CDV), may influence test outcomes as shown in this study which demonstrated a 3.48% anti-CDV antibodies detection on the N-protein based C-ELISA.

These findings not only contribute to enhancing PPR surveillance and control measures but also shed light on the potential impact of cross-reactivity on diagnostic accuracy.

Keywords: Comparative Study, Competitive ELISA, Epitope Blocking ELISA, Specific PPR Antibodies, Peste des Petits Ruminants (PPR), PPR-free Countries.

COMPARATIVE STUDY OF MOLECULAR EVOLUTION OF PESTE DES PETITS RUMINANTS (PPR) VACCINE WITH PPR MASTER SEED IN AFRICA

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MSc. VACCINE PRODUCTION AND QUALITY CONTROL

Peste des petits ruminants (PPR) is a highly contagious viral disease of serious economic significance on small ruminant production, especially in Africa. The control of this important disease is highly dependent on the successful vaccination against the causative *Morbillivirus*. For the worldwide eradication programme to be successful, an in-depth understanding of the molecular evolution of the PPR virus (PPRV) vaccine strain is essential. Therefore, this study investigated the genetic stability and variation of the vaccine virus produced from various African sources compared with the master seed.

The retro-prospective study was conducted at the African Union-Pan African Veterinary Vaccine Centre (AU-PANVAC) located in Debre Zeit, Ethiopia, between June and November 2023. A total of 45 vials of PPR vaccines, 44 vials from 10 vaccine manufacturers in Africa, and 1 vial from the Master Seed Lot (PPR 75/1 Seed Vero 78) were selected for this study from AU-PANVAC storage. One vial of a known PPR vaccine that had passed the identity test was selected as the Positive Control (PC) for the study, while Phosphate-Buffered Saline (PBS) with a pH of 7.2 was used as the Negative Control (NC) throughout. The study employed a Dot Blot assay, using a monoclonal antibody (mAb 2.15) to evaluate reactivity to the PPRV vaccine samples. Additionally, QIAGEN One-Step RT-PCR Kits were used to identify the PPRV N gene in the vaccine samples. Extracted N genes of PPRV were sequenced, and sequence analysis was conducted to detect genetic similarity among tested samples.

The Dot Blot assay revealed the reactivity of mAb to all the PPR vaccine samples tested, and conventional RT-PCR detected positive amplicons in all the 45 samples tested. Based on the partial C-terminus hypervariable region of the N gene of PPRV, sequencing and sequence similarity analysis demonstrated 100% nucleotide homology among PPRV vaccine samples and with the reference (Master Seed). Phylogenetic tree analysis revealed a close genetic affinity between vaccine samples and the master seed, emphasising a conserved genetic makeup and common origin. Distinct separation from *Escherichia coli* in the phylogenetic tree highlighted the specificity of PPRV genetic analysis. Furthermore, sequence analysis and mutation detection using BioEdit 5.0.9 software confirmed no observed mutations, reinforcing the genetic stability of PPRV vaccine samples.

The findings of this study revealed genetic similarity among the sampled vaccines and also with the Master Seed, confirming the stability of the vaccine produced from different sources over the years.

Keywords: Peste des petits ruminants, vaccine, genetic stability, molecular evolution, master seed

DEVELOPMENT OF ELISA FOR DETECTION OF ANTIBODIES AGAINST RIFT VALLEY FEVER IN VACCINATED GOATS

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VACCINE PRODUCTION AND QUALITY CONTROL

Rift Valley Fever (RVF) is a zoonotic disease which is spread by mosquito, and caused by Rift Valley Fever Virus (RVFV), a virus with a single-stranded RNA genome belonging to the genus *Phlebovirus* in the family *Phenuviridae* and order *Bunyavirales*. The disease is marked by the World Organization for Animal Health (WOAH) as one of the most devastating diseases of sheep, goats and cattle. For this reason, there is a need of high quality vaccines, and the quality control of these vaccines depends on the availability of good diagnostic tools.

In this study, RVF antigen was produced by infecting BHK cells with virus seed (Smithburn strain), precipitated, centrifuged, collected, and kept. Monoclonal antibodies (mAbs) that were already produced and reserved were screened. Those that were positive were tested for protein quantity using bicinchonic acid (BCA) method and were conjugated with Horseradish Peroxidase (HRP). Conjugates were further tested for sensitivity and specificity.

The results showed that out of nine clones that were tested, six were selected and conjugated. Only three conjugates (C9F2, C9E1 and C9D2) showed good reactivity with the produced antigen from dilution 1/12.5 up to 1/50. However, all the conjugates were not specific and sensitive to the antigen in ELISA. Therefore it was concluded that the mAbs could not be used to develop an ELISA kit using the nucleoprotein, which was predominantly expressed in the antigen.

Key Words: Rift Valley Fever (RVF), Rift Valley Fever Virus (RVFV), ELISA, Monoclonal Antibodies (mAbs).

EVALUATION OF THERMOTOLERANCE OF PESTE DES PETITS RUMINANTS VACCINE FORMULATIONS

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VACCINE PRODUCTION AND QUALIT CONTROL

Peste des Petits Ruminant (PPR) is a transboundary, highly contagious viral disease of small ruminants. The PPR vaccines produced to prevent PPR are prone to thermal degradation since they are required for use in tropical and subtropical regions with poor storage. The study was carried out to identify which stabilisers used for PPR vaccines formulations provide good thermostability throughout storage and transportation. The goal of this study was to evaluate the effects of different stabilisers on the thermotolerance of PPR vaccine formulations, to ascertain effects of stabilisers on and identify the stabiliser that confers the best stability at high temperature storage conditions (40°C and 45°C), to determine the effect of stabilisers on the virus titre and shelf life of PPR vaccine formulations.

The PPR vaccine batches (n=12) with 10 different stabilisers, PPR vaccine batches with high virus titre values were selected. For each batch, five vials were used. The data loggers were placed in each incubator for temperature recording to ensure that the temperature was stable throughout the study period. The residual moisture content of PPR vaccine batches ranged from 1.1% to 3.8%.

The results showed that PPR vaccines containing lactalbumin hydrolysate with sucrose had titres above 2.5 $TCID_{50}$ with shelf life of 5 days at both 40°C and 45°C. Trehalose, lactose with N-Z amine and lactose had titres of 2.5 TCID50 with shelf life of 5 days at 40°C. PPR vaccines containing skim milk, sucrose-peptone, lactose monohydrate & N-Z amine, (sucrose, lactalbumin & L glutamine), (maltose, gelatin & lactalbumin hydrolysate), Weybridge medium and lactose had titres below 2.5$TCID_{50}$ at 40°C with shelf life of less than 5 days.

Therefore, lactalbumin hydrolysate with sucrose, trehalose, lactose with N-Z amine and lactose stabilisers have best thermo-tolerance ability, with lactalbumin hydrolysate with sucrose ranked first, trehalose ranked second best, lactose with N-Z amine ranked third best and lactose ranked fourth best stabilisers. Vaccine manufacturers producing attenuated PPR vaccines for use in tropical regions should make use of lactalbumin hydrolysate with sucrose stabilisers, trehalose, lactose with N-Z amine or lactose in vaccine formulation.

Keywords: Peste des Petits Ruminants Virus, vaccines, stabilizers, thermo-tolerance

EVALUATION OF VARIOUS FORMULATIONS OF RECONSTITUTED PESTE DES PETITS RUMINANTS (PPR) VACCINE FOR THERMOTOLERANCE AND SHELF LIFE

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MSc. VACCINE PRODUCTION AND QUALITY CONTROL

Peste des Petits Ruminants Vacine (PPRV) has been shown to prevent a significant economic disease of small ruminants in Africa with about 100% efficacy. Normal saline is commonly used as a diluent to reconstitute PPR vaccine. The reconstituted vaccines are originally preserved in freeze-dried state, using 12 varieties of formulations, namely lactalbumin hydrolysate Sucrose (LS), sucrose-peptone (SP), weybridge (WBM), Trehalose (TR), lactose N-Z Amine (LN-ZA), Maltose, gelatin lactalbumin hydrolysate (MGLS), lactose Monohydrate and N-Z Amine (LMN-ZA), skim milk (SM), Sucrose, lactalbumin and L-Glutamine (SLL-G), and Lactose (L).

Aliquotes of each of the 12 batches of reconstituted vaccine were kept both at temperatures of 4°C and at 40°C. Virus viability test was done at graded time intervals of 0, 2, 4 and 6 hours after reconstitution. The titration of the aliquots was done for each batch at each storage condition and specified time on vero cells. The Tissue Culture Infective Dose 50 (TCID50) was calculated using spearmann Kaerbur formula.

Results indicated that at 4°C, the TCID50 on sucrose-peptone stabilizer was 3.65, 3.55, 3.45, and 3.2 at 0, 2, 4, and 6 hours, respectively. While, at 40°C, it was 3.1, 2.85, and 2.6 at 0, 2, 4, and 6 hours, respective. On Lactalbumin Hydrolysate Sucrose, the TCID50 was 3.45, 3.35, 3.3, and 2.95 (with Titer loses of 0.1, 0.05, 0.35, and 0.35), respectively, at 4°C. While at 40°C it was 2.9, 2.8, and 2.55 (with titer losses of 0.55, 0.1 and 0.25), LS and SP were showing good thermotolerance respectively. While other stabilizers such as Weybridge, (3.1, 2.95, 2.75, and 2.45), Trehalose (3.05, 2.85, 2.8, and 2.65), and lactose N Z Amine (3.1, 2.9, 2.75, and 2.6), were showing a significant titer at 4°C. At higher temperature of 40°C , titer was (3.1, 2.95, 2.6, and 1.95), (3.05, 2.6, 2.25, and 2.05) and (3.1, 2.9, 2.7, and 2.25). This shows moderate thermotolerance in the investigation. However the vaccines offered low thermotolerance with Maltose gelatin lactalbumin hydrolysate (2.85, 2.6, and 2.15), lactose monohydrate & N-Z Amine (3, 2.85, 2.55 and 1.9), skim milk (3.05, 2.95, 2.85, and 2.35), and Sucrose, lactalbumin & L-Glutamine (3, 2.65, 2.55 and 2.5), their titers limited only at 4°C and it was not able to exhibit viability at 40°C. Lactose, after reconstitution was not extend in shelf life titer.

The vaccine's stability evaluation demonstrated its effectiveness in Tissue Culture trials which may be similat to situations in some tropical field environments where cold chain failures occur. The study concluded that lactalbumin hydrolysate Sucrose and sucrose-peptone were the most effective stabilizers.

Keywords: PPR vaccine, Thermo-tolerance, Shelf-life, Stability, Lyophilisation.