Journal of Agriculture and Environment
Vol. 20 No. 2, 2024: 297-309

Immunoinformatic analysis of conserved immunodominant epitopes derived from Newcastle disease virus strains prevalent in West Africa

M.B. Bello[1, 2] and N. Lawal [1,2]

[1]Center for Advanced Medical Research and Training, Usmanu Danfodiyo University PMB 2346 Sokoto, Nigeria
[2]Department of Veterinary Microbiology, Usmanu Danfodiyo University PMB 2346 Sokoto, Nigeria

ABSTRACT

The purpose of this study was to design an epitope-based vaccine targeting the currently circulating genotypes of Newcastle disease virus (NDV) in West Africa using immunoinformatic approaches. Complete fusion protein sequences from West African NDV isolates were retrieved and aligned using MEGA11 software. Then, conserved immunodominant B cell, T helper and T cytotoxic epitopes were predicted for the construction of the vaccine candidate, NDVaxWA. Subsequently, antigenicity, allergenicity, toxicity and other physicochemical properties of NDVaxWA were evaluated using an array of *in silico* tools. Our findings revealed a total of 11 immuno-conserved epitopes comprising 3 BCL, 2 CTL and 6 HTL epitopes used for NDVaxWA construction. A molecular docking of Toll-like receptor-7 with the NDVaxWA indicated successful binding. An in silico immune simulation revealed robust humoral and cell mediated responses following vaccination with NDVaxWA. Taken together, NDVaxWA is a potential vaccine candidate for improved protective efficacy against the currently circulating NDV strains in West Africa.

Keywords*:* Immunoinformatic; Epitopes; Newcastle disease virus; vaccine

INTRODUCTION

Newcastle disease is one of the most economically significant avian diseases that constitute huge threats to the poultry subsector (Alexander *et al*., 2012) . The disease affects a wide variety of birds with diverse clinical outcomes ranging from asymptomatic enteric to severe systemic infection causing up to 100% mortality among unprotected chickens (Awan *et al*. 1994). Given its huge economic impact, ND has been included among the list A diseases that should immediately be reported to the World Organization of Animal Health (OIE) (Ganar *et al*. 2014a). The disease is caused by the Newcastle disease virus (NDV), an avian orthoavulavirus-1 which belongs to the genus orthoavula virus in the family paramyxoviridae. In general, the virus is classified into viscerotropic velogenic strains associated with high mortality and severe enterohemorrhagic lesions, neutrophic velogenic strains that predominantly cause neurological and respiratory lesions, mesogenic strains associated with moderate respiratory and neurological symptoms, lentogenic strains that lead

correspondence: bbtambuwal@gmail.com
https://dx.doi.org/10.4314/jagrenv.v20i2.26

to mild respiratory disease the as well as avirulent strains that cause asymptomatic infection despite their replication in the intestinal tissues (Desingu *et al.,* 2021.). This pathotypic classification is based on OIE-recommended tests such as intracerebral pathogenicity index (ICPI) in 1-day-old chicks, mean death time of chicken embryonated eggs (MDT) and intra venous pathogenicity index in 6 weeks old chickens. Noteworthy, most of the NDV isolates associated with the currently ongoing fifth ND enzootic are either velogenic or mesogenic (Absalón *et al*. 2019).

Although NDV has an enormously high phylogenetic diversity with currently more than 20 distinct genotypes (Dimitrov *et al*. 2019), all isolates of the virus are serologically grouped into a single serotype. Thus, despite the genetic diversity, all the vaccine strains currently available for the control of NDV are either members of genotype 1 or 2 (Bello *et al.,* 2018) , which are phylogenetically divergent from currently prevailing genotypes in different parts of the world (Izquierdo-Lara *et al*. 2019). Hence the call for the development of the so-called genotype-matched vaccines which are believed to prevent clinical disease and substantially reduce the duration and load of virus shedding following viral challenge. However, in a subregion such as West Africa where several genotypes are currently prevalent (Snoeck *et al*. 2013), developing genotype-matched vaccines based on a single prevalent NDV strain may not provide a lasting solution to the menace of NDV in the poultry industry. A more robust vaccine design is therefore needed to address the shortcomings of the current vaccines in inducing strong neutralizing immunity against the prevailing strains.

The continuous evolution of pathogen genomics has given birth to computational biology tools for modern vaccine design and development (Rapin *et al*. 2010). A large collection of protein databases and in silico tools are now available for rational vaccine design. Some of these tools have been applied to precisely predict vaccine candidates against several pathogens including the recently emerged SARSCOV-2 (Ong *et al*. 2021). Indeed, post-prediction analysis such as molecular docking, receptor-ligand interactions, population coverage and immunosimulation can all be achieved using these tools (Kalita *et al*. 2006) . Thus, computational biology is now the cornerstone of modern vaccinology as it helps predicts with absolute precision, the immune profile of any rationally designed vaccine without performing any wet laboratory procedure. The present study aims to explore the use of these state-of-the-art bioinformatics tools to predict the conserved immunodominant epitopes in the fusion protein of all NDV isolates currently prevalent in West Africa as potential vaccine antigens.

MATERIALS AND METHODS

Sequence Retrieval and Alignment

Nucleotide sequences of Newcastle disease virus (NDV) Fusion Protein (FP) from West African countries were retrieved from Genbank and aligned using ClustalW in MEGA11. A consensus sequence of 543 amino acids was generated for further analysis.

B-cell Epitope Prediction

B-cell epitopes were predicted using BepiPred 2.0, and characterized for antigenicity (VaxiJen v2.0), toxicity (ToxinPred), allergenicity (AllerTop v2.0), and conservancy (Epitope Conservancy Analysis) (Doytchinova *et al*. 2007).

T-cell Epitope Prediction

***Cytotoxic T-lymphocytes (CTLs)*:** CD8+ CTL epitopes were predicted using NetMHCpan EL 4.1 for selected HLA alleles, characterized for antigenicity, allergenicity, toxicity, and immunogenicity, and evaluated for conservancy.

***Helper T-lymphocyte (HTL)*:** CD4+ HTL epitopes were predicted using IEDB's MHC-II binding tool and assessed for antigenicity, allergenicity, toxicity, and cytokine-inducing properties (IFN-γ, IL4, IL10). Conservancy analysis was also performed.

3D Structure Generation of Selected Epitopes

PEP-FOLD3 was used for 3D structure prediction of selected CTL and HTL epitopes. PatchDock and FireDock were used for molecular docking with chicken MHC class I (3BEW) and class II (6ZWA) receptors.

Multi-epitope Vaccine Construction

A vaccine construct (NDVaxWA) was developed by linking selected epitopes with appropriate linkers and an adjuvant (β-defensin-3) to enhance immunogenicity.

Antigenicity, Allergenicity, and Physicochemical Properties

NDVaxWA's antigenicity, allergenicity, and physicochemical properties were evaluated using VaxiJen v2.0, AllerTop v2.0, Expasy ProtParam, and SOLpro.

Secondary and Tertiary Structure Prediction & Validation

The vaccine's 2D structure was analyzed using PSIPRED, while 3D modeling was performed using trRosetta, I-TASSER, Robetta, and Phyre2. Validation was done using MolProbity, ERRAT, VERIFY3D, and PROCHECK, with additional refinement via GalaxyRefine.

Protein-Protein Docking

NDVaxWA was docked with human TLR3 (1ZIW) and TLR7 using PatchDock and refined with FireDock to assess immune receptor interaction.

Immune Simulation

C-ImmSim (http://150.146.2.1/C-IMMSIM/index.php) was used to simulate immune responses, evaluating IgG, cytokines, memory cell production, and antigen clearance.

In silico Cloning & Codon Optimization

The vaccine sequence was optimized using JCat for efficient expression in *E. coli* (strain K12). The optimized sequence was inserted into the pET-28a(+) vector using SnapGene.

RESULTS

Epitope Mapping

A consensus sequence from 66 NDV isolates in West Africa was used for BCL, CTL, and HTL epitope mapping. Of the eight BCL epitopes mapped, only two (222GPQITSPALTQL233 and 348TRIVTFPMSP357) met the immunogenic criteria, showing antigenicity above the threshold, non-toxicity, non-allergenicity, and >75%

conservancy (Table 1). Three CTL epitopes (NAANILRLK, RPLAAAGIV, and LLWLGNNTL) exhibited the best characteristics and were selected for vaccine construction (Table 2). Additionally, 16 HTL epitopes were identified based on toxicity and cytokine-inducing properties, with six highly conserved epitopes (>50%) chosen for the final vaccine (Table 3). Molecular docking with chicken B-F alleles showed all T-cell epitopes had good global energies and hydrogen bonding, with CTL epitopes demonstrating stronger binding than HTL epitopes.

Table 1: The predicted BCL epitopes and their characteristic properties

Epitopes	Antigenicity	Allergenicity	Toxicity	Conservancy
MPKDKEACAKAPLEAYNR	0.306		NT	63.46% (33/52)
QRIQRSVSTSGGRRQ	0.277		NT	1.92% (1/52)
GPQITSPALTQL	0.463	NA	NT	75.00% (39/52)
LTKLGVGNSQ	0.912	NA	NT	46.15% (24/52)
SVIEELDTSYCIE	0.567	A	NT	98.08% (51/52)
TRIVTFPMSP	0.462	NA	NT	80.77% (42/52)
DATYQKNISILDSQVIVTGNLDIS	0.929	A	NT	11.54% (6/52)
KAQQKTLLWLGN	0.628	A	NT	67.31% (35/52)

NA: Non Allergenic, NT: Non Toxic

Table 2: The Selected CTL epitopes with their characteristics

Epitopes	Antigenicity	Immunogenicity	Toxicity	Allergenicity	Conservancy
NTSACMYSK	0.615	NI	NT		96.15% (50/52)
ETLSVSTTK	0.892	NI	NT		38.46% (20/52)
LTTPYMALK	0.511	NI	NT		57.69% (30/52)
ILACYLMYK	0.691	NI	NT		7.69% (4/52)
NAANILRLK	0.689	0.163	NT	NA	100.00% (52/52)
RPLAAAGIV	0.48	0.217	NT	NA	96.15% (50/52)
LLWLGNNTL	0.406	0.105	NT	NA	69.23% (36/52)
LSLDGVTLR	0.814	0.117	NT	NA	1.92% (1/52)
SPALTQLTI	0.9	NI	NT		75.00% (39/52)
SVGNLNNMR	0.842	NI	NT		98.08% (51/52)

NA: Non Allergenic, NT: Non Toxic

Characteristics of the Multiepitope Vaccine Construct

The final vaccine construct was designed by fusing Beta defensin-3 adjuvant with the selected BCL, CTL, and HTL epitopes using appropriate linkers. The construct comprises 266 amino acids, with a molecular weight of 27.17 kDa and an isoelectric point (pI) of 10.18, indicating its basic nature (Table 4).

Molecular Docking

Molecular docking with TLR-7 yielded a binding energy of -5.14 kcal/mol. Analysis using PDBsum revealed the formation of three hydrogen bonds: Leu840-Glu174 and Asn839-Lys241 (twice), indicating strong receptor interaction.

Immune Simulation

The NDVaxWA vaccine triggered a robust immune response, with initial IgM titers rising above 2000 and subsequent induction of IgM + IgG and IgG1 + IgG2 responses. A booster at day 28 resulted in IgM titers peaking at ~60,000 and IgM + IgG at ~70,000, maintaining significant levels even after 350 days. B memory cells persisted post-vaccination. T-helper (Th) and cytotoxic T-cell (Tc) populations expanded significantly, with increased macrophage activity. High interferon-gamma levels and low IL-10 levels suggested a strong immune activation. Other cytokines, including IL-4 and IL-12, were also elevated (Figure 1).

Molecular Dynamic Simulation

The deformability graph showed minimal distortions, with "hinges" around 1 deformability value. The B-factor graph indicated stable atomic fluctuations, supporting structural integrity. NDVaxWA-TLR-3 docking generated an eigenvalue of 6.660619e-06, reflecting the energy required for deformation. The covariance matrix showed a mix of correlated and anti-correlated motions among residues, demonstrating structural stability (Figure 2).

Table 3: The final selected HTL and their characteristic properties

Epitopes	Antigenicity	Allergenicity	IFN	IL10	Toxicity	IL4	Conservancy
VNVRLTSTSALITYI	1.0161	NA	I	NI	NT	I	53.85% (28/52)
QQKTLLWLGNNTLDQ	0.4601	NA	I	NI	NT	NI	67.31% (35/52)
AANILRLKESIAATN	0.5839	NA	I	NI	NT	NI	100.00% (52/52)
NAANILRLKESIAAT	0.5676	NA	I	NI	NT	NI	100.00% (52/52)
LGIQVNLPSVGNLNN	0.8119	NA	I	NI	NT	I	96.15% (50/52)
AVIGSVALGVATAAQ	0.9336	NA	I	NI	NT	NI	82.69% (43/52)

NA: Non Allergenic, NT: Non Toxic

Table 4: Antigenicity, allergenicity and physicochemical properties of the NDV FP-based multi-epitope vaccine

Parameters	Results	Remarks
Antigenicity	0.432	Antigenic
Allergenicity	Non-Allergen	
No of Amino acids	266 aa	Suitable
Molecular Weight (Da)	27265.82	Average
Theoretical pI	10.18	Basic
Ext. Coefficient	25815	
Estimated half-life	1 hours (mammalian reticulocytes, in vitro). 30 min (yeast, in vivo). >10 hours (Escherichia coli, in vivo)	Satisfactory
Instability index	32.86	Stable
Aliphatic index	100.30	Thermostable
Grand average of hydropathicity (GRAVY)	0.227	Hydrophobic
Solubility	0.930	Soluble

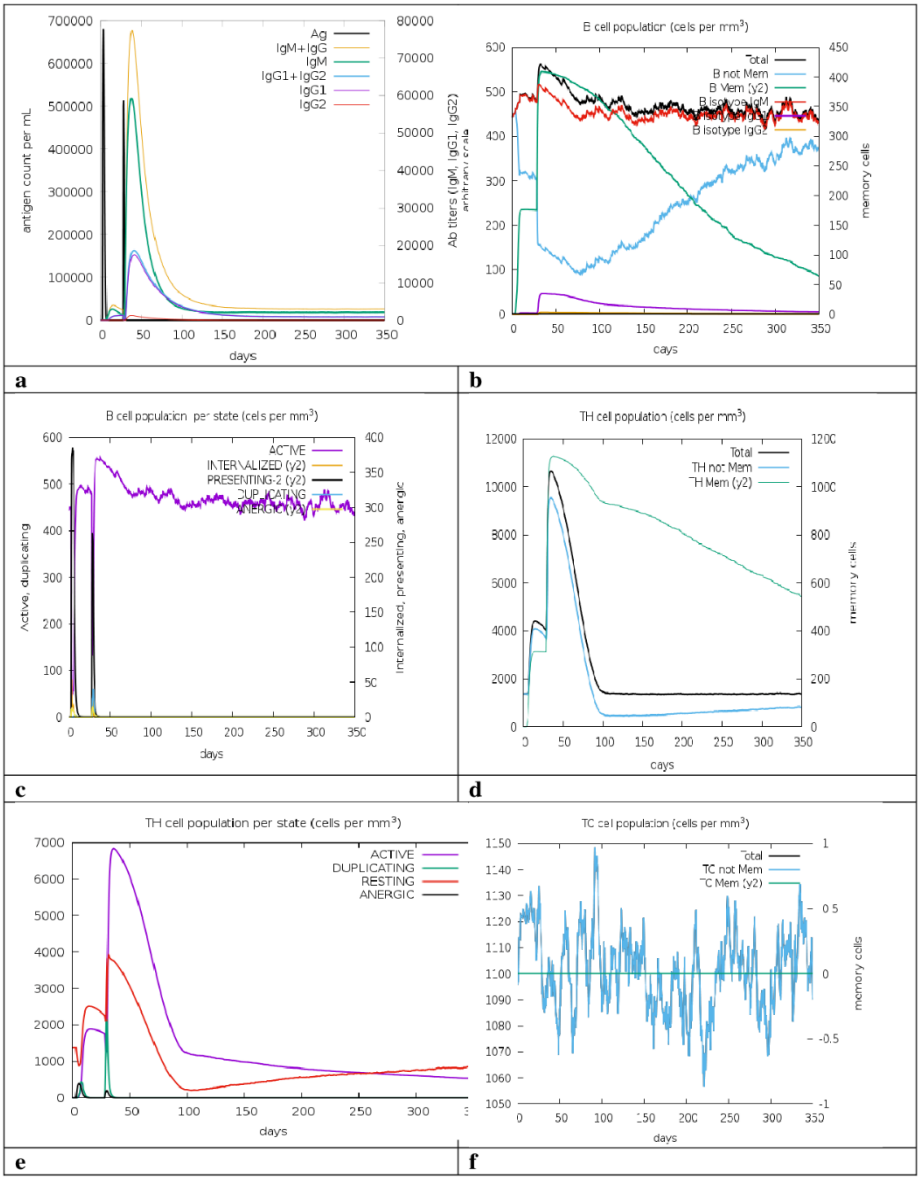

Figure 1: Features immune simulation of NDVaxWA with C-ImmSim
(a) the induction of antibody responses at prime-boost of the vaccine. (b-c) The induction of B-cell population. (d-e) The induction of Th-cells population (f) The induction of T-cells population

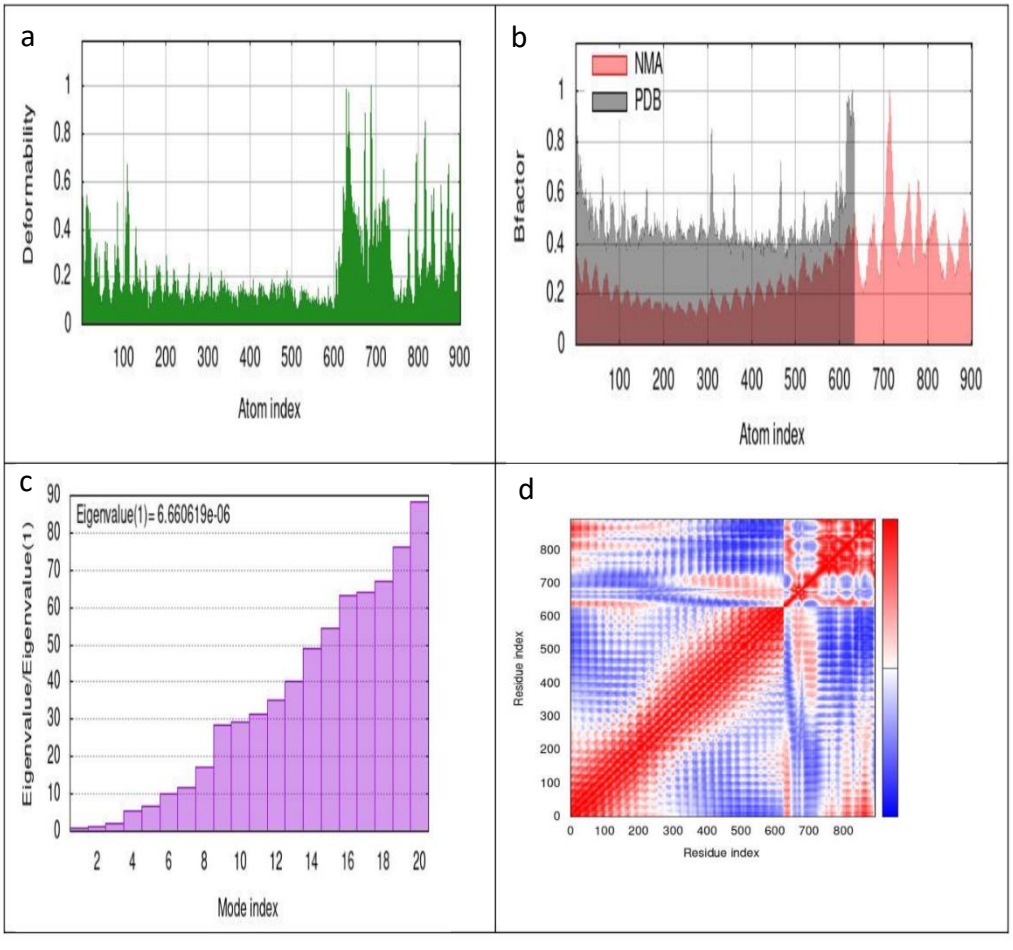

Figure 2: Features the Molecular dynamics with iMODS server
(a) Deformability plot (b) B factor plot (c) The eigenvalue of the NDVaxWA-TLR7 complex (d) The covariance matrix between pairs of residues where red, white, and blue represent correlated, uncorrelated, and anti-correlated motion, respectively.

Abbreviations:
NDV: Newcastle disease virus
BCL: B cell lymphocyte
CTL: Cytotoxic T lymphocyte
HTL: Leper T lymphocyte
MEGA: Molecular Evolutionary Genetics Analysis
TLR-Toll like receptor
IL-Interleukin
IFN- Interferon

DISCUSSION

Although ND vaccines developed more than 6 decades ago are still effective in preventing mortality and clinical disease, they are not able to block virus shedding following challenges with phylogenetically divergent field viral strains (Han *et al*. 2017; Miller *et al*. 2013). Consequently, in Asia for instance, where genotype VII NDV predominates, genotype-matched vaccines based on the currently prevailing NDV strains are developed to reduce the burden of the disease in the region (Aljumaili *et al*. 2020; Bello *et al*. 2020). However, in West Africa where several genetically distinct isolates exist (Bello, Yusoff, Ideris, Hair-bejo, *et al*. 2018; Snoeck *et al*. 2013), the optimal strategy for ND control is an adjuvanted multiepitope vaccine derived from conserved immunodominant epitopes of NDV strains in West Africa. This study utilized immunoinformatic tools to predict these epitopes from the fusion protein, aiming to design an effective region-specific vaccine.

The overall goal of NDV vaccination is to produce a sterilizing immunity or at least a strong immune response that substantially increases the amount of the virulent virus required to cause infection (Kapczynski, Afonso, and Miller 2013; Miller *et al*. 2013). A good ND vaccine should therefore, in addition to being effective in preventing disease, also be capable of blocking virulent virus challenges regardless of the infecting genotype. Although both F and HN surface glycoproteins of NDV are known to induce neutralizing antibodies in chickens (Ganar *et al*. 2014b), the contribution of F protein in protective immunity to NDV is by far greater (Hosseini *et al*. 2021; Rahmawati, Rantam, and Tyasningsih 2017). The F protein was chosen as the primary target for mapping conserved immunodominant epitopes in NDV isolates from West Africa, due to its abundance of neutralizing epitopes that can induce robust immune responses against the virus (Hosseini *et al*. 2021; Shahar *et al*. 2018). Based on in silico predictions, a total of 11 immuno-conserved epitopes (3 BCL, 2 CTL and 6 HTL) were obtained and used in the construction of the NDVaxWA vaccine.

The vaccine was constructed by sequentially linking CTL, HTL, and BCL epitopes using specific linkers. AAY linkers were used for CTL epitopes, GPGPG linkers for HTL epitopes, and bi-lysine (KK) for BCL epitopes. These flexible linkers enhance interaction between peptide domains while maintaining spatial separation (Chen, Zaro, and Shen 2013). The adjuvant: Avian Beta defensin-3 (80 aa) was fused with the N-terminal of the construct using the rigid linker EAAAK to improve immunogenicity. Based on our findings, the NDVaxWA vaccine, a 266-amino-acid construct, is highly antigenic, non-allergenic, thermally stable, and soluble. It effectively triggers both antibody-mediated and cell-mediated immune responses, making it a promising candidate against NDV strains in West Africa.

A critical component of the immunoinformatics design of vaccine candidates is the analysis of binding affinities of immunogenic molecules and selected vaccine peptides using molecular docking tools. Therefore, in the present study, we evaluate the interaction of TLR7 with the NDVaxWA vaccine candidate. TLR7 is an important receptor of innate immunity for its role in mediating the recognition of viral single-stranded RNA (ssRNA) including that of NDV (Diebold 2008; Mielcarska, Bossowska-Nowicka, and Toka 2021; Stevceva 2011) . Strong interaction was observed between the TLR7 and the vaccine as was indicated by the presence of hydrogen bonds.

An immune simulation was performed to observe the optimal behaviour and cell density parameters for successful target clearance and to find the best immunological response against the pathogen. The induction of memory B-cells populations and T-cells

populations were observed. Also, after the prime-boost antigen exposure, effective Ig induction was observed due to elevated helper T-cell populations, an indication of good humoral immune response. Satisfactory induction of the macrophage population was also observed, an indication of good antigen presentation to B-cells and T-Cells.

CONCLUSION

Taken together, an epitope-based vaccine was successfully designed using high-profile immunoinformatics pipelines to target the currently circulating NDV strains in Africa. Based on our robust in silico predictions, the vaccine candidate, NDVaxWA, is incredibly safe and capable of inducing strong antibody and cell-mediated immune responses in the vaccinated birds. NDVaxWA is therefore a potential countermeasure against the rising threat of NDV in West Africa. This study thus highlights the relevance of immunoinformatic approaches in the control of economically important transboundary animal diseases in West Africa.

Acknowledgement

This is to kindly appreciate the management and staff of CAMRET UDUS, Nigeria, for their support of this study.

REFERENCES

Absalón, A. E., V. Diana, Cortés-Espinosa, E. Lucio, P. J. Miller, and C. L. Afonso. (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. *Tropical Animal Health and Production* 51(5): 1033–48. doi:10.1007/s11250-019-01843-z.

Alexander, D J, E W Aldous, and C M Fuller. (2012)_. "The Long View: A Selective Review of 40 Years of Newcastle Disease Research." *Avian Pathol* 41(4): 329–35. doi:10.1080/03079457.2012.697991.

Aljumaili, O.A., M.B. Bello, S.K. Yeap, A.R. Omar, and A. Ideris. (2020). "Protective Efficacy of Inactivated Newcastle Disease Virus Vaccines Prepared in Two Different Oil-Based Adjuvants." *The Onderstepoort journal of veterinary research* 87(1). doi:10.4102/ojvr.v87i1.1865.

Awan, M. A., M. J. Otte, and A. D. James. (1994). "The Epidemiology of Newcastle Disease in Rural Poultry: A Review." *Avian Pathology* 23(3): 405–23. doi:10.1080/03079459408419012.

Bello, M. B, S.N.M Mahamud, K. Yusoff, A. Ideris, M. Hair-Bejo, B. P.H. Peeters, and A. R. Omar. (2020). "Development of an Effective and Stable Genotype-Matched Live Attenuated Newcastle Disease Virus Vaccine Based on a Novel Naturally Recombinant Malaysian Isolate Using Reverse Genetics." *Vaccines*. doi:10.3390/vaccines8020270.

Bello, M. B, K.Yusoff, A. Ideris, M. Hair-Bejo, B.P.H,, Peeters, and A. R. Omar. (2018a). "Diagnostic and Vaccination Approaches for Newcastle Disease Virus in Poultry: The

Current and Emerging Perspectives." *BioMed Research International* 2018: 7278459. doi:10.1155/2018/7278459.

Bello, M. B, K.Yusoff, A. Ideris, M. Hair-Bejo, B.P.H,, Peeters, A. H. Jibril, F.M. Tambuwal, and A. R. Omar (2018). "Genotype Diversity of Newcastle Disease Virus in Nigeria : Disease Control Challenges and Future Outlook." 2018.

Chen, X, J. Zaro, and W. Shen. (2013). "Fusion Protein Linkers: Property, Design and Functionality Xiaoying." *Adv Drug Deliv Rev*. 65(10): 1357–69. doi:10.1016/j.addr.2012.09.039.Fusion.

Desingu, P. A., S. Dayal Singh, K. Dhama, O. R. Vinodhkumar, K. Nagarajan, R. Singh, Y. S. Malik, and R. K Singh (2021). "Pathotyping of Newcastle Disease Virus: A Novel Single BsaHI Digestion Method of Detection and Differentiation of Avirulent Strains (Lentogenic and Mesogenic Vaccine Strains) from Virulent Virus." *Microbiology Spectrum* 9(3): e00989-21. doi:10.1128/spectrum.00989-21.

Dewidar, A. A., W. H. Kilany, A. A. El-Sawah, S. A. S. Shany, A. M. Dahshan, I. Hisham, M. F. Elkady, and A. Ali. (2022). "Genotype VII.1.1-Based Newcastle Disease Virus Vaccines Afford Better Protection against Field Isolates in Commercial Broiler Chickens." *Animals : an Open Access Journal from MDPI* 12(13): 1696. doi:10.3390/ani12131696.

Diebold, S.S. (2008). "Recognition of Viral Single-Stranded RNA by Toll-like Receptors." *Advanced drug delivery reviews* 60(7): 813–23. doi:10.1016/J.ADDR.2007.11.004.

Dimitrov K. M., Abolnik C., Afonso C. L., Albina E., Bahl J., Berg M., Briand F. X., Brown I. H., Choi K. S., Chvala I., Diel D. G., Durr P. A., Ferreira H. L., Fusaro A., Gil P., Goujgoulova G. V., Grund C., Hicks J. T., Joannis T. M., Torchetti M. K., Kolosov S., Lambrecht B., Lewis N. S., Liu H., Liu H., McCullough S., Miller P. J., Monne I., Muller C. P., Munir M., Reischak D., Sabra M., Samal S. K., Servan de Almeida R., Shittu I., Snoeck C. J., Suarez D. L, Van Borm S., Wang Z., Wong F. Y. K (2019). "Updated Unified Phylogenetic Classification System and Revised Nomenclature for Newcastle Disease Virus." *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 74: 103917. doi:10.1016/j.meegid.2019.103917.

Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007 Jan 5;8:4. doi: 10.1186/1471-2105-8-4

Ganar, K., M. Das, S. Sinha, and S. Kumar. (2014a). "Newcastle Disease Virus: Current Status and Our Understanding." *Virus Research* 184: 71–81. doi:10.1016/j.virusres.2014.02.016.

Han, Q., X. Gao, P. Wu, S. Xiao, X. Wang, P. Liu, L. Tong, *et al*. (2017). "Re-Evaluation the Immune Efficacy of Newcastle Disease Virus Vaccine in Commercial Laying Chickens." *Research in Veterinary Science* 111: 63–66. doi:10.1016/j.rvsc.2016.12.004.

Hosseini, S. S., Aghaiypour Kolyani, K., Rafiei Tabatabaei, R., Goudarzi, H., Akhavan Sepahi, A., & Salemi, M.. (2021). "In Silico Prediction of B and T Cell Epitopes Based on NDV Fusion Protein for Vaccine Development against Newcastle Disease Virus." *Veterinary Research Forum* 12(2): 157–65. doi:10.30466/vrf.2019.98625.2351.

Izquierdo-Lara, R., Chumbe, A., Calderón, K., Fernández-Díaz, M., & Vakharia, V. N. (2019). "Genotype-Matched Newcastle Disease Virus Vaccine Confers Improved Protection against Genotype XII Challenge: The Importance of Cytoplasmic Tails in Viral Replication and Vaccine Design." *PLoS ONE* 14(11): e0209539. doi:10.1371/journal.pone.0209539.

Kalita, J. K., K. Chandrashekar, R. Hans, P. Selvam, and M.K. Newell. (2006). "Computational Modelling and Simulation of the Immune System." *International Journal of Bioinformatics Research and Applications* 2(1): 63–88. doi:10.1504/IJBRA.2006.009194.

Kapczynski, D. R., C. L. Afonso, and P. J. Miller. (2013). "Immune Responses of Poultry to Newcastle Disease Virus." *Developmental and Comparative Immunology* 41(3): 447–53. doi:10.1016/j.dci.2013.04.012.

Mielcarska, M. B., M. Bossowska-Nowicka, and F. N. Toka. (2021). "Cell Surface Expression of Endosomal Toll-Like Receptors—A Necessity or a Superfluous Duplication?" *Frontiers in Immunology* 11(February): 1–13. doi:10.3389/fimmu.2020.620972.

Miller, Patti J., Claudio L. Afonso, John El Attrache, Kristi M. Dorsey, Sean C. Courtney, Zijing Guo, and Darrell R. Kapczynski. (2013). "Effects of Newcastle Disease Virus Vaccine Antibodies on the Shedding and Transmission of Challenge Viruses." *Developmental and Comparative Immunology* 41(4): 505–13. doi:10.1016/j.dci.2013.06.007.

Ong, E., X. Huang, R. Pearce, Y. Zhang, and Y. He. (2021). "Computational Design of SARS-CoV-2 Spike Glycoproteins to Increase Immunogenicity by T Cell Epitope Engineering." *Computational and Structural Biotechnology Journal* 19: 518–29. doi:10.1016/j.csbj.2020.12.039.

Rahmawati, I. L., F. Abdul Rantam, and W. Tyasningsih. (2017). "Amino Acid Analysis of Fusion (F) Gene and Prediction of Epitope B-Cell Newcastle Disease Surabaya Isolate As Vaccine Candidate." *KnE Life Sciences*. doi:10.18502/kls.v3i6.1140.

Rapin, N., O. Lund, M. Bernaschi, and F. Castiglione. (2010). "Computational Immunology Meets Bioinformatics: The Use of Prediction Tools for Molecular Binding in the Simulation of the Immune System." *PLOS ONE* 5(4): e9862. doi:10.1371/JOURNAL.PONE.0009862.

Roohani, K., S. W. Tan, S. K. Yeap, A. Ideris, M. Hair Bejo, and A. R. Omar. (2015). "Characterisation of Genotype VII Newcastle Disease Virus (NDV) Isolated from NDV Vaccinated Chickens, and the Efficacy of LaSota and Recombinant Genotype VII Vaccines against Challenge with Velogenic NDV." *Journal of Veterinary Science* 16(4): 447–57. doi:10.4142/jvs.2015.16.4.447.

Shahar, E., R. Haddas, D. Goldenberg, A. Lublin, I. Bloch, N. B. Hinenzon, and J. Pitcovski. (2018). "Newcastle Disease Virus: Is an Updated Attenuated Vaccine Needed?" *Avian pathology : journal of the W.V.P.A* 47(5): 467–78. doi:10.1080/03079457.2018.1488240.

Snoeck, C. J., A. A. Owoade, E. Couacy-Hymann, B. R. Alkali, M. P. Okwen, A. T. Adeyanju, G. F. Komoyo, *et al*. (2013). "High Genetic Diversity of Newcastle Disease Virus in Poultry in West and Central Africa: Cocirculation of Genotype XIV and Newly Defined Genotypes XVII and XVIII." *Journal of Clinical Microbiology* 51(7): 2250–60. doi:10.1128/JCM.00684-13.

Stevceva, L. (2011). "Toll-Like Receptor Agonists as Adjuvants for HIV Vaccines." *Current Medicinal Chemistry* 18(33): 5079–82. doi:10.2174/092986711797636027.