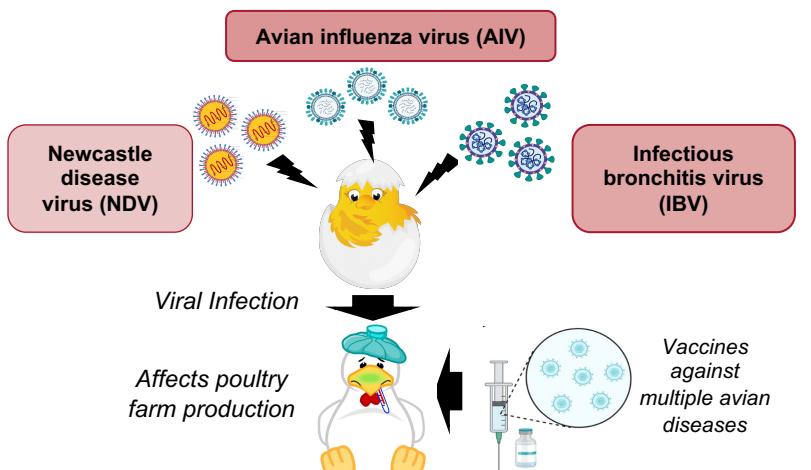


Introduction

Vaccines remains the primary means of disease prevention through immunisation schemes in the poultry sector. Novel approaches in vaccine development, such as reverse genetic systems and genome editing technologies (i.e. CRISPR/Cas9), are currently being utilized to overcome challenges in establishing an immunogenic platform that is safe and capable of inducing long-term immunity. The CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats associated protein nuclease 9) technology offers an effective, fast and simple novel approach to edit genomes for the development of viral vectors against poultry diseases such as Newcastle disease (ND), avian influenza (AI) and infectious bronchitis (IB).



Publications

- Rohaim, M. A., Clayton, E., Sahin, I., Vilela, J., Khalifa, M. E., Al-Natour, M. Q., Bayoumi, M., Poirier, A. C., Branavan, M., Tharmakulasingam, M., Chaudhry, N. S., Sodi, R., Brown, A., Burkhardt, P., Hacking, W., Botham, J., Boyce, J., Wilkinson, H., Williams, C., ... Munir, M. (2020). Artificial Intelligence-Assisted Loop Mediated Isothermal Amplification (AI-LAMP) for Rapid Detection of SARS-CoV-2. *In Vitro* (Vol. 12, Issue 9). <https://doi.org/10.3390/v12090972>
- Vilela, J., Rohaim, M. A., & Munir, M. (2020). Application of CRISPR/Cas9 in Understanding Avian Viruses and Developing Poultry Vaccines . In *Frontiers in Cellular and Infection Microbiology* (Vol. 10, p. 631). <https://www.frontiersin.org/article/10.3389/fcimb.2020.581504>

Methodology

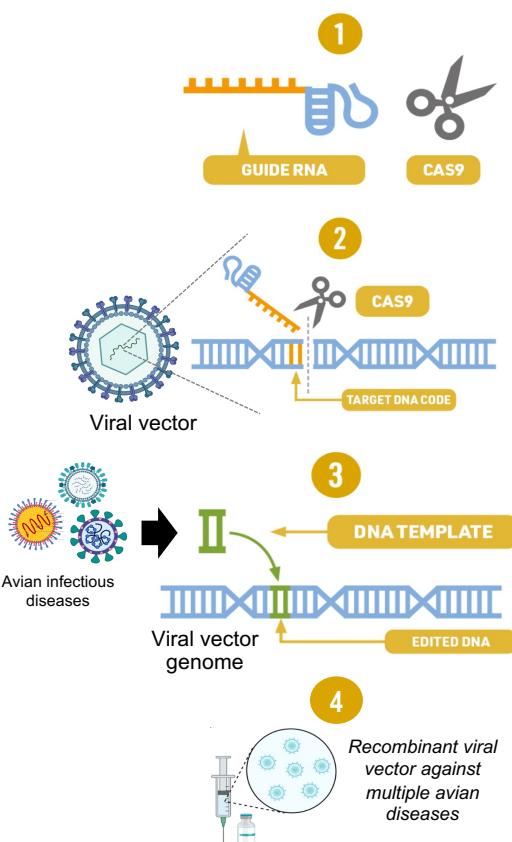


Figure 1. Schematic representation of the methodology used in this study. (1) We will be using two very important yet simple components- The guide RNA, which is a match of the DNA sequence we want to make a cut and a scissor protein, Cas9. (2) The guide RNA searches the viral whole genome for the target section of DNA and transports the scissor protein to it. The scissor protein cuts the DNA at this point. (3) Once the viral genome is cut, the template will be inserted in the cut site, this DNA template is a fragment of another virus, allowing us to create a viral vector that can carry other DNA fragments of other viruses such as AIV, NDV, IBV and develop a multivalent vaccines that will protect the chickens from becoming infected. (4) Using these technology viruses can be edited to carry antigens of other viruses to develop multivalent vaccines.

Results

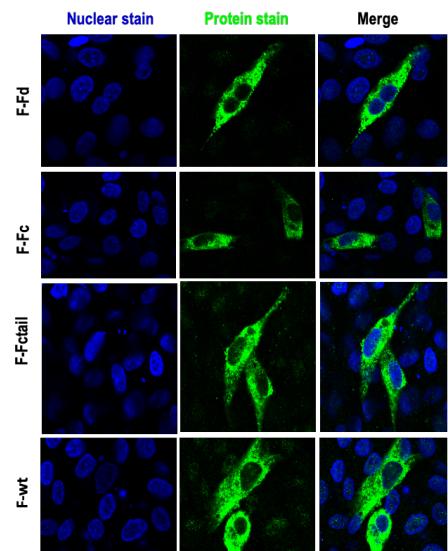


Figure 3. Development of recombinant viral vector expressing Fusion (F) protein of NDV. LMH cells were transfected with the following plasmids: donor with DNA template, CRISPR protein, guide RNAs. Under the fluorescent microscope, donor plasmid will fluoresce red and viral vector will fluoresce green. Red fluorescent protein (RFP) as control (Panel 1). Panel 2 shows red fluorescence of the donor plasmid (red). Panel 3,6,9 shows the bright field. After 36hrs LMH cells were infected with the viral vector. Panel 4 shows the GFP expression (viral vector) and Panel 5 shows DsRed expression (donor plasmid). Plaques (recombinant viral vector) co-expressing GFP and DsRed were selected for further passaging. Panels 7 and 8, shows passage number 3 of the putative recombinant viral vector carrying NDV F gene.

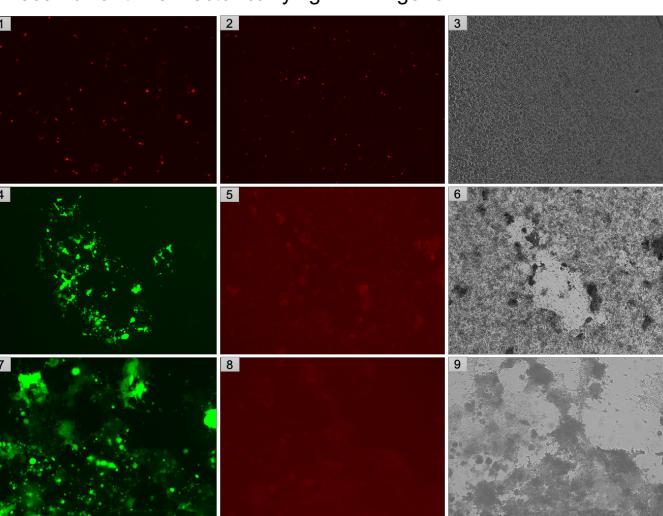


Figure 2. Confocal microscopy analysis for the NDV F gene donor plasmids. The DNA template will be introduced into the viral vector genome using donor plasmids. Detection of NDV F expression in CEF using Immunofluorescence assay (IFA). Expression was detected using ANTI-FLAG followed by Goat anti-Rabbit IgG labelled with Alexa fluor 488 (bright green). All four (4) F gene donor plasmids were expressed in CEF cells as visualized in the protein stain (middle panel). These donor plasmids will be used to generate recombinant viral vector carrying NDV F gene.

Summary

- Evaluated four (4) putative candidate plasmids carrying the NDV F gene.
- Developed recombinant viral viruses putatively carrying NDV Fusion proteins for the development of vaccine against Newcastle disease.

References

- Atasoy, M. O., Rohaim, M. A., and Munir, M. (2019). Simultaneous deletion of virulence factors and insertion of antigens into the infectious laryngotracheitis virus using NHE-CRISPR/Cas9 and cre-lox system for construction of a stable vaccine vector. *Vaccines* 7:207.
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- Vilela, Julianne, Mohammed A. Rohaim, and Muhammad Munir. 2020. "Application of CRISPR/Cas9 in Understanding Avian Viruses and Developing Poultry Vaccines ." *Frontiers in Cellular and Infection Microbiology* 10: 631.