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Development of a vaccine production platform for poultry diseases in Africa: Newcastle Disease Virus non-replicative adenovirus-vectored vaccine

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A vaccine production platform for poultry diseases in Africa: Newcastle Disease Virus adenovirus-vectored vaccine

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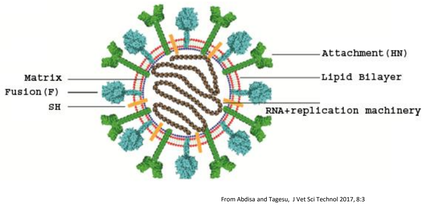
Background

✓ Poultry are a vital village livestock with important economic impact in sub-Saharan Africa.
✓ Newcastle Disease (ND) is a highly contagious and endemic poultry infectious disease.
✓Recurrent outbreaks provoke heavy losses every year.
✓ND virus (NDV) is a negative-sense single-stranded RNA virus from the genus Avulavirus, family *Paramyxoviridae*.
✓Current NDV vaccines are only partially protective and are produced in specific pathogen-free chicken embryonated eggs, whose supply is expensive and problematic.
✓ A recombinant adenovirus-based Foot and Mouth Disease vaccine has been licensed for production and use in US in emergency situations.

Schematic of a paramyxovirus



From Abdisa and Tagesu, J Vet Sci Technol 2017, 8:3

Objective

Implement at the National Veterinary Institute (NVI), Ethiopia, a technological platform for the production of veterinary vaccines, by developing a recombinant non-replicating adenoviral vector vaccine against NDV using the human adenovirus serotype 5 (Ad5) and serum-free suspension HEK293 adapted cells.

Specific aims and methodologies

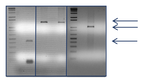
Molecular Phylogenetic analysis and characterization of currently circulating strains in the region

Maximum Likelihood method
MEGA v7.0

Representative strains isolated in recent years in Ethiopia belong to genotypes VI, and VII of class II.

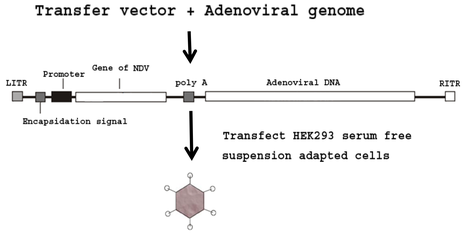
LaSota and B1 strains (included in current classic vaccines) were isolated many years ago and belong to genotype II, class II.

Amplification of F gene fragments from Ethiopian NDV field isolates



Strains in current inactivated or attenuated vaccines

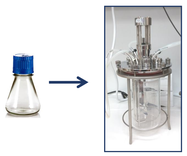
1- Generating recombinant adenoviruses type 5 vectors expressing the F and HN antigens under different regulatory sequences.



2- Establishing critical process parameters impacting the yield and quality of rAd5-ND vaccines at cells densities >6x10^{6}cells/mL.

- Basal serum-free media, additives.
- Kinetics of growth, nutrient consumption, product synthesis.
- Vaccine product: infectivity, antigen activity.
- Cell mass, cell productivity.

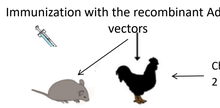
3- Defining a process operating strategy allowing high-cell density productive infection in bioreactors as well as a downstream processing and formulation.



- Scale-up in bioreactors: → 1-3L → 20L.
- Physiological changes, nutritional requirements, feeding rate.
- Downstream processing.
- Thermal stabilization → additives.
- Formulations for: oral drench /eye drop.
- Lyophilization defined as primary choice for eye-drop and oral presentations.

Maximized rAd5 product yields in HEK293 serum free cells

4- Performing animal studies on the immunogenicity (humoral, cell-mediated responses to the rAd-ND) and comparative studies on vaccine efficacy.



- Mucosal administration (compared with inactivated vaccines).
- Two different rAd doses.

- Neutralizing antibodies, HIA
- Lymphocyte proliferation assay, CTL, intracellular staining of cytokines.
- Vaccination and challenge experiments (chicken) with NDV.
- Cell mass, cell productivity.
- Assays: HIA, neutralization, TCID$_{50}$ and qPCR for virus quantification after challenge.
- I ntracerebral pathogenicity index (ICPI) for randomly selected animals.

5- Evaluate vaccine stability relative to live NDV vaccines and perform toxicology studies in non-target animals.

6- To conduct sustainable technology transfer for building capacity at the NVI, Ethiopia.

- Toxicology studies and biodistribution (GFP-expressing rAds) in non-target animals.
- TCID$_{50}$ and qPCR for virus quantification.

- Develop SOPs required, repository and common access.
- Scale-up of the process validation and document Process Analytical Technologies.
- Technology transfer to NVI, Ethiopia.

Results

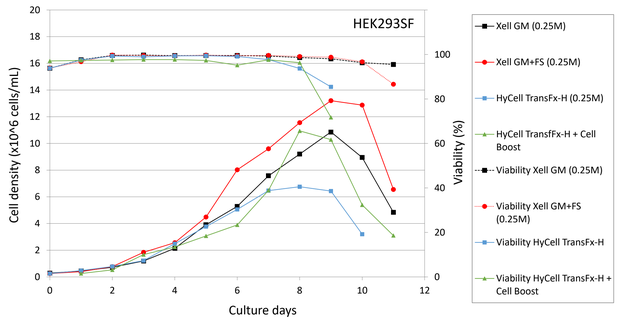
Transfer / expression vectors constructed:

pAdCMV-F, pAdCMV-HN, pAdCMV-F-HN: express the F and HN from NDV under the human cytomegalovirus immediate early enhancer.
pAdCMV-GFP-F, pAdCMV-GFP-HN: express additionally the GFP under the human cytomegalovirus immediate early enhancer / promoter.
pUC-βactin-F, pUC-βactin-HN, pUC-βactin-F-HN: express the F and HN from NDV under the human CMV enhancer + chicken β-actin promoter.

Table 1. Adenoviral vectors generated / in construction.

rAd5 generated	Foreign coding gene	Regulatory elements	Status
AdCMV-F	NDV F protein	Human cytomegalovirus immediate early enhancer / promoter (hCMV)	Primary Ad stock generation in HEK293 cells established / rAds in amplification steps
AdCMV-HN	NDV HN protein		
AdCMV-F-HN	NDV F and HN protein		
AdCMV-GFP-F	NDV F protein + GFP		
AdCMV-GFP-HN	NDV HN protein + GFP		
Adβact-F	NDV F protein	• Human CMV enhancer • Chicken β-actin promoter • Chimeric intron from chicken β-actin and rabbit β-globin	Primary Ad stock generation in HEK293 cells to be established
Adβact-HN	NDV HN protein		
Adβact-F-HN	NDV F and HN protein		
AdGFP	GFP protein	hCMV	Primary stock established

Figure 1. Effect of different culture media, feeding supplements and feeding strategies on the cell density and viability of HEK293 cells in suspension.



• HyCell™ TransFx™-H Media (Hyclone Laboratories Inc) was supplemented with GlutaMAX™ at 4-6 mM and Kholliphor P188 at 0.1% .
• Xell GM (Xell AG, Germany) was supplemented with GlutaMAX™ at 4-6 mM.

Figure 2. Analysis by flow cytometry of GFP expression under different transfection conditions in HEK293 serum-free cells (for adenovirus stock generation).

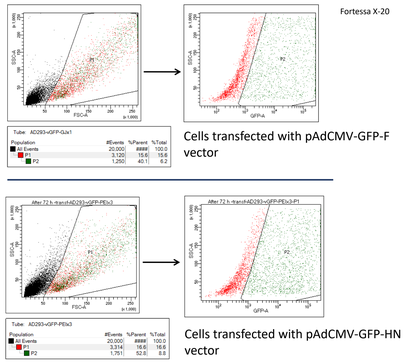
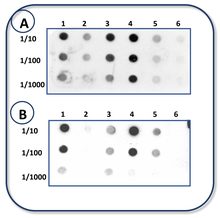


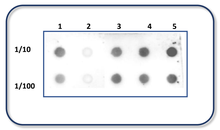
Figure 3. Expression analysis in HEK293 and HEK293 serum-free cultured cells of the recombinant F and HN antigens using polyclonal and monoclonal antibodies against NDV.



Cells lysates from adherent HEK293 transfected cells:

1- HEK293 cells transfected with pAdCMV-GFP-HN.
2- HEK293 cells transfected with pAdCMV-GFP-HN (cells in PFA 2%).
3- HEK293 cells transfected with pAdCMV-F.
4- HEK293 cells transfected with pAdCMV-HN.
5- HEK293 cells transfected with pAdCMV-GFP-F (cells in PFA 2%).
6- HEK293 cells transfected with pAdT-GFP (GFP verified by flow cytometry).

Panel A) Polyclonal serum anti-NDV
Panel B) Monoclonal anti-HN antibody
(GFP expression verified by flow cytometry).



Cells lysates from serum free HEK293 transfected cells in suspension:
Dot blot using polyclonal serum anti-NDV

1- HEK293 cells transfected with pAdCMV-GFP-F.
2- HEK293 cells transfected with pAdCMV (GFP).
3- HEK293SF cells transfected in suspension with pAdCMV-F.
4- HEK293SF cells transfected in suspension with pAdCMV-HN.
5- HEK293 cells transfected with pUC-βactin-F.

Concluding remarks

- The ND adenovirus vaccine constructed, to be produced at elevated cell densities in HEK293SF (expressing protective antigens from NDV), will provide an efficient and cost-effective platform able to address the limitations in efficacy and manufacturing associated with current conventional vaccines.
- This promising platform technology for vaccine production and delivery will benefit the NVI of Ethiopia and will enhance its central role as the Pan African Veterinary Vaccine Control Center, producing and supplying vaccines to the Preferential Trade Area countries of Eastern, Western and Southern Africa.

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