Plan Overview

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Title: A non-replicative adenovirus vaccine platform for poultry diseases

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Template: IDRC - Open Research Data Initiative

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Project abstract:

Poultry are a vital village livestock asset and are particularly important for agro-economic independence of women in Sub-Saharan Africa (SSA). In times of crisis poultry become the most critical livestock asset due to their versatility and independence. Many diseases affect poultry however Newcastle disease (ND) is considered one of the most critical in SSA. Reporting is sporadic, however some countries in SSA have on average 10 ND outbreaks per year of this economically destructive disease. Commercial poultry worldwide is routinely vaccinated with inactivated or live vaccines which is protective, however the use of live vaccines leads to shedding of the virus which can lead to disease in non-vaccinated poultry. This is an issue given that current ND vaccine administration methods are not highly effective. Although cell culture production methods are being extensively used in vaccine manufacturing, current ND vaccines are produced in specific pathogen free (SPF) chicken embryonated eggs. Egg supply is expensive and in Africa limited by the need to import from Europe. In addition, egg production methods are not efficient and not adapted to rapid response in case of emerging threats. To alleviate the economic burden of ND and address the limitations of current ND vaccines production methods in SSA, a cost-effective more efficient ND vaccine is needed. We propose to use a non-replicative adenovirus-vectored vaccine which expresses the immunogenic glycoprotein HN and F of ND virus manufactured using HEK293 cell culture rather than eggs. Adenovirus (Ads) vectors are extensively used as recombinant vaccines with many advantages including cost-effective manufacturing, long-term stability, high efficacy, a well-established safety profile in humans and animals and demonstrated superiority over existing inactivated or attenuated egg produced. Importantly, we will contribute to build capacity at NVI, Ethiopia by transferring a robust and flexible technology platform for cell-culture manufacturing of veterinary vaccines

ID: 45836

Last modified: 20-02-2020

Grant number / URL: 108627

A non-replicative adenovirus vaccine platform for poultry diseases

Project and author details

Please enter your project title.

A non-replicative adenovirus vaccine platform for poultry diseases

Project description.

Newcastle Disease (ND) is considered one of the most critical diseases affecting poultry in Sub-Saharan Africa (SSA). Although reporting is sporadic, some countries have on average 10 outbreaks per year while immunizations with inactivated or live vaccines possess some well-known disadvantages such viral shedding that can lead to disease in non-vaccinated poultry. Current NDV vaccine administration methods are not highly effective. NDV vaccines currently available are produced in specific pathogen free chicken embryonated eggs. Egg supply is expensive and in Africa is limited by the need to import from Europe. In addition, egg production methods are not efficient compared to cell culture technologies and not adapted to rapid response in case of emerging threats.
The main goal of this project is to develop a vaccine manufacturing platform based on the use of a non-replicative human adenovirus vector, expressing the F and HN antigens from Newcastle Disease Virus (NDV) for production in HEK293 cells cultured in suspension, with a superior cost of goods and safer against ND compared to vaccines produced in eggs at the National Veterinary Institute (NVI) of Ethiopia.
Formulation options to evaluate for delivery include oral drench, eye drop, and ultimately in ovo administration. The protective capacity of rAd-NDV vaccine against ND is intended to be at least as robust as the existing vaccines produced by the NVI. The technology, developed equally by male and female scientists in Africa and Canada, provides a platform amenable to rapid adaptation to other avian viral threats in SSA (such as avian influenza) or diseases affecting small farm animals of economic importance.
The specific project objectives for the 30-month duration are:

Aim#1: to engineer recombinant replication incompetent adenovirus 5 vectors to express the F and HN antigens of NDV alone and in combination (rAd5-ND-F, rAd5-HN, and rAd5-ND-F-HN) and select the most effective antigens presentation.

Aim#2: to identify critical process parameters impacting the yield and quality of rAd5-ND vaccine using small-scale HEK293 culture systems.

Aim#3:to rationally define a process operating strategy allowing high-cell density productive infection in bioreactors.

Aim#4: to define downstream processing and final formulation steps for the oral drench, eye drop, or*in ovo*delivery.

Aim#5:to performanimal studies on humoral and cellular immune response to Ad-ND

Aim#6:to complete comparative animal studies on vaccine efficacy via the different delivery methods (oral, eye-drop,*in ovo*) relative to ND vaccines: live NDV vaccine Hichner B1/Lasota strain, NDV thermostable vaccine, and inactivated NDV LaSota strain vaccine currently produced by NVI.

Aim#7: Evaluate rAd-ND vaccine stability relative to live NDV vaccine Hichner B1/Lasota strain, NDV thermostable vaccine, and inactivated NDV LaSota strain vaccine. To perform toxicology studies in non-target organisms.

Aim#8: to conduct sustainable technology transfer which builds the capacity of the NVI.

Presently, different variants of recombinant adenoviral vectors expressing the F and HN antigens of NDV (encoding sequences from recent Ethiopian field isolates) were constructed under different regulatory elements and rescued following a novel and straightforward approach. The expression of the encoded antigens was demonstrated *in vitro* and their characterization was completed. The identification of critical parameters for optimal rAds production at high cell densities in HEK293 serum-free cells has been conducted, followed by process intensification for production at cell densities over 4-6M/mL and by process development in bioreactors, assessments of purification and formulation. The viruses have been prepared at high titers (over 1 x 1011 infective particles per mL), stocked, and used in animal immunization experiments for the identification of the most immunogenic candidate and evaluation of functionality *in vivo*. The evaluation by ELISA and hemagglutination inhibition assays (HIA) of the humoral immune response elicited in mice and chicken by the different adenoviral vectors demonstrated the induction of specific antibodies against the F- and HN- antigens alone or in combination, with detection of neutralizing antibodies against NDV confirmed by an *in vitro* seroneutralization assay. The development of a final process for the rAd-NDV vaccine production at larger scale (3L controlled bioreactors) is also currently in progress. Overall,to support the technology transfer and deployment of the vaccine production process at NVI and IPT, a set of reliable and efficient bioreactor monitoring tools and control strategies has been developed. Some innovations of the project in course are: a new process development for Ads production at high cell densities in HEK293 serum-free cells including an improved protocol for viral rescue and amplification, a new viral vector-based vaccine against NDV based on the sequence of local Ethiopian isolates, and a new technological platform for the rapid development of novel anti-viral vaccines for poultry.

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Data description

Describe the datasets you will submit.

Description	Format and justification	Volume
First data to be submitted is related to the generation of various adenoviral vectors and their production as vaccine candidates in cells cultured in suspension. Data will include characterization of such vectors.	In terms of vectors sequence, the data will be submited as Fasta format. This includes the foreign sequences inserted and additional sequences for identfication of each vector and the expression cassette. The functionality of the vectors was also assessed by the biochemical characterization of the recombinant products expressed in different cell lines. Data to be submitted will specify product size, antibodies used, and expression levels. This information will be submitted as TIFF files and microsoft word documents.	Up to 1Gb
New viral rescue method proposed, which resulted in a considerable improvement in the adenovirus production process.	Cell density values expressed as total cells per ml and virus titers showing the kinetics of amplification and production will be the data submitted in its original form as Excel sheets. Data on the characterization of these primary adenovirus stocks includes analysis of integrity and purity (by HPLC for determination of viral particles [VP] per ml) and measurement of infectious viral particles [IVP] per ml using a cell-based ELISA for the detection of the hexon protein. These data will be provided as as TIFF files and as Excel sheets. Datasets with TCID50 (median tissue culture infectious dose) values performed in adherent HEK 293A cells for virus titration will be also provided in the form of Excel sheets.	Up to 5Gb
Data from the different batch and fed-batch cultivation experiments conducted will be submitted. It consist in a range of operating culture conditions for rAd production in suspension, to achieve viral production at cell densities over 4-6M cells per ml in shake culture flasks. The experimental conditions mimicked in a first approach the perfusion-type of culture, with infection at different cell densities following centrifugation and exchange for fresh culture medium. In subsequent approaches, cells in suspension were cultured in batch until reaching certain cell densities and Ad infection was initiated at each of these cell densities. Different fed-batch strategies were conducted in order to investigate whether the presence of additives, starting at the time of infection or before the time of infection, at different regimens, could increase the viral production capacity. Data sets will be submitted for all the culture conditions assayed.	The parameters submitted as datasets for the analysis of each experimental condition are: cell density expressed as total cells per ml, total viable cells per ml, viability (expressed in percent), cell diameter and daily time points comprising the whole duration of every experiment. This will be submitted in the form of Excel data sheets. A detailed description of the cultivation conditions will be submitted in form of word document. The experimental conditions were tested by infection with one recombinant Ad5 vector expressing GFP, intended for the final calculation of both volumetric virus productivity and cell specific viral yield. The assessment of GFP expression was conducted by flow cytometry, using the disruption and culture supernatants from the original flasks for further infection of adherent HEK293 in 96-well plates. Datasets from flow cytometry measurements will be submitted for each condition. They are expressed as total number of cells according to the gating strategy employed, number of positive cells expressing GFP, number of infectious viral particles per ml and number of infectious viral particles per cell. These datasets may be submitted in their original format (FCS files, which require an specialized software) or in the form of Excel data sheets.	Up to 5Gb

The analyses with the recombinant adenoviruses encoding the NDV antigens have also involved differential nutrient composition, and differential multiplicity and time of infection. In conjunction with the experiments described previously, the initiation of the scale-up and validation in 1-3L bioreactors is presently in progress.	Datasets to be submitted will comprise batch and fed-batch bioreactor process parameters from bioreactor runs already finished, such as speed of agitation for cells growth, pH, dissolved oxygen concentration, temperature, capacitance values (pF/cm) along the process (to monitor changes in cells undergoing the productive infection phase), as well as additional operational parameters. They will be submitted in the form of Excel data sheets supported by TIFF additional files and word documents with detailed explanations. The kinetics of growth, nutrient consumption, product synthesis values, viral particles per ml of culture, infectious viral particles per ml of culture and specific productivity will be also provided as datasets in the form of Excel data sheets supported by TIFF additional files. Finally, data on the biochemical, structural and functional characterization of the adenoviruses produced and their encoded antigens will be also provided by means of TIFF and word files. In addition, datasets on the VP/ml and IVP/ml will be provided for the simplified streamlined downstream processing being developed for the Ad5 vectors in the form of Excel datasheets.	Up to 5Gb
Data generated from the evaluation of the immunogenicity and protective efficacy of the vectors produced includes assessments of immunogenicity in the target species, with measurements of specific humoral and cell-mediated immune responses specific to NDV, comparative studies on vaccine protective efficacy, immunization regimens and routes of delivery and safety in the target animal species. Data from the functionality of the adenoviruses in vivo suche as their immunogenicity and protective efficacy in animals is presently being generated.	The datasets will consist of titers values derived from analysis by ELISA of specific anti-NDV responses, virus hemagglutination inhibition titers and data derived from the intracellular staining of cytokines aimed at the characterization of the Th type of cell-mediated responses. These data will be submitted in the form of Excel datasheets supported by the use of TIFF files. Data on clinical parameters measured in vaccinated and challenged chicken will be also provided summarized in the form of Excel datasheets.	Up to 1Gb

Describe how the data was collected.

A detailed description of the methods and technical analyses will be submitted together with each dataset, to support the quality of the experiments and measurements conducted. The data were collected as follows:

The kinetics of cell growth and virus production runs briefly described previously, evaluated in shake flask experiments and 1-3L bioreactors were analyzed by the Vi-Cell XR cell counter (Beckman Coulter, USA). This instrument works on the principle of Trypan blue dye exclusion, captures 50 images and provides an average value for viable cell density, percentage of viable cells, and cell diameter. The accuracy of the instrument is ±10% in the range of 0.2–10 106 cells/mL.

Flow cytometry acquisition was performed on a three-laser BD Fortessa-X20 cytometer, and results were analyzed by using the software FlowJo v10.2. Culture supernatant and cell disruption supernatants were collected, mixed and used for infection and quantification of GFP expression in adherent AD293 cells (with infection at different virus dilutions). They were used for infection of HEK293 adherent cells at different dilutions, aiming for quantification of GFP expression (by flow cytometry) and determination of IVPs/ml and specific IVPs/cell.

The method for the TCID50 performed on HEK293 (in assays in which no GFP is present) is described elsewhere. To prevent the effect of interday variability, all titres determined for each one of the experimental condition assayed were processed and titrated on the same assay. Also, the TCID50 value shown for the titres at the time of infection was calculated from the titre of the vial used to infect the culture. When evaluating different culture conditions, TCID50 was calculated from the mixture of culture and disruption supernatants or from the disruption supernatant, depending on the type of experiment.

Data generated during the bioreactors runs were extracted from the corresponding interface softwares from the companies Applikon Biotechnology for the 1L and 3L bioreactors and Aber Instruments in the case of the capacitance datasets.

ELISA measurements for the detection of Ad5 viral particles was conducted following the manufacturer’s directions (QuickTiter™ Adenovirus Titer Immunoassay Kit, Cell BioLabs). The detection of specific antibodies to NDV was conducted by using the NDV IDvet Reference kit for F protein detection (NDVS-10P) while hemagglutination inhibition assays were performed following standard procedures described elsewhere.

What is the value of your data to the research community?

The value for the research community comes from the fact that vaccine production based on viral vectors or virus production for gene therapy often requires of advanced process strategies in order to overcome limitations of conventional cultures. It is also a frequent fact that laboratory research is hardly able to reach the industry because of limitations in the establishment or scale-up of a production process. Overcoming such limitations and arriving to a scalable process with a specific fed-batch strategy instead of using perfusion bioreactors constitutes a significant technological achievement that provides new opportunities for adenovirus vaccine

production. Fed-batch cultivation, as encountered in the experiments performed, using the media and conditions identified, alleviates in part the difference in metabolic requirements of HEK293 during the phases of cell growth and virus production. It is operationally simpler than perfusion and is reliable and flexible for implementation in different types of facilities. It is a means for manufacturing costs reduction compared to processes that require medium exchange. Data compiled also accounts for a new streamlined downstream processing, monitoring methods, control strategies for quantification of the viral products and advanced process characterization. Thus, the strategies leading here to a production process of a new NDV adenovirus vaccine are part of a new technological platform that can be replicated (for the expression of antigens from phylogenetically distant isolates, for instance) or rapidly adapted to other viral diseases affecting poultry (i.e. avian influenza) or small farm animals of economic importance in African or developing countries. The process scale-up is being completed and is generating a considerable and complete data set that will serve as reference for the development of similar processes in the area of viral vectors production or can be certainly valid for the re-usage of the information for a variety of purposes (*i.e.* study of HEK293 cells monitored by capacitance values before and after viral infection, and during the virus production phase). Further process intensification is possible and can be also explored by potential data users. The scientific/industrial community interested in process development and vaccine production are potential users of these data as well as academic and industry Institutions related to the recipients of this technology.

What documentation and metadata will accompany the data? Please consider the use of metadata standards when explaining your data.

Data will be clearly described, with supporting information accompanying the datasets to be submitted. It will include complete documentation such as standard operating procedures supporting how the data was generated, processed and preserved. Appropriate references of technical documentation such as operator or instrument manuals will be also provided. Megadata documentation will deepen the details on how data was obtained, how it was created and organized (for each experiment described), why and when, and how to access it. It will provide stable identifiers for submitted datasets and will allow public access to data without unnecessary restrictions. Any additional information providing clarity on the meaning of the datasets, which make them understandable, interpretable and re-usable by the research community, will be joined to the submission.

Ethical and Legal issues

Are there any ethical issues that will complicate the publication of the data? If so, what provisions have been made regarding the sharing of research results?

There are no ethical issues related to the publication of the data. The animal experiment protocols were signed and approved by the corresponding Institutional Committees of Ethics

Is there an obligation to re-submit your project to an ethical review to assess whether the data from the project can be shared?

There is no motive that justifies a re-submission.

Are there any issues regarding Intellectual Property Rights (e.g., copyright, database rights etc.) associated with your data? How will the data be licenced?

There are no issues of intellectual properties related with the publication of the data.

Data sharing/openness

How will you provide access to the data? We strongly recommend depositing it in a public repository. If this is your plan, plese name the intented repository. Please suggest several repositories you feel are relevant, and explain your logic behind the choice.

Data will be accessible by depositing it in a public repository. We have identified as potential repositories the use of generalist repositories, which can handle a wide variety of data, since a data-type specific repository for the type of data generated in the project does not seem to be available. A generalist repository may also be appropriate for archiving associated information or analyses that will help to interpret and re-use the datasets. The repositories proposed are, by order of interest: *i)* Figshare, which provides 100 GB free per scientific data manuscript and goes up to 1 TB of maximum size per dataset, *ii)* the Harvard Dataverse,

which provides 2.5 GB per file, 10 GB per dataset as maximum size without fees, and ***///)*** the Open Science Framework, which allows 5 GB per file, and multiple files can be uploaded.