PROCINORTE
(Canada, Mexico, United States)

Animal Health Task Force

Research Project
FINAL REPORT

Date: April 10, 2018

PROJECT TITLE	**Use of IFN-expressing vectors in control of Nipah and Ebola virus diseases in swine**
DURATION	2016- 2017
PROJECT LEADER and Professional Address	Hana Weingartl NCFAD, CFIA, 1015 Arlington St. ,Winnipeg, MB, R3E 3M4, Canada
COLLABORATORS and Professional Address	Teresa de los Santos PAIDC, ARS, USDA,

PROJECT SUMMARY (One Paragraph):

The aim of the proposed pilot studies is to provide proof of concept to:

A. Determine if pretreatment of swine with Ad5 vectored IFN protects swine against Nipah virus infection.

B. Determine if pretreatment of swine with Ad5 vectored IFN protects swine against Ebola-Zaire virus infection.

Milestones

April - June 2016

Selection and production (outsourced) of vectored IFN (PIADC consultation)

July – October 2016

EBOV pilot (animal experiment and analysis at NCFAD)

November 2016 – February 2017

NiV pilot (animal experiment and analysis at NCFAD)

March 2017

Report/(start of preparation of a publication, PIADC and NCFAD)

Due to administrative issues, the project was **funded in March 2017**.
The time shift generated conflict with other projects, and the Objective B. was started only in September 2017. The experimental component of the project was completed in January 2017, and one manuscript is in preparation (see below).

Experimental design

- Piglets will be inoculated with Ad5-poIFN-α or Ad5-poIFN-λ (10^{10} pfu). Serum will be collected 24h post Ad-IFN-α inoculation for cytokine and antiviral assay. At the same time all piglets will be oro-nasally challenged with 10^5 PFU of NiV (objective A) or 10^5 PFU of EBOV per animal (objective B).
- Swabs (oral and nasal) and blood (for PBMC and plasma harvest) will be collected at 1, 4 and 5 or 6 days post challenge (dpc) to assess virus shedding as correlates of protection.
  Selected cytokine levels will be determined in plasma, and aliquots of PBMCs will be subsequently analyzed for regulation of interferon stimulated genes (ISGs).
- Post mortem sample collection on day 5 or 6 dpc will include cerebrospinal fluid, olfactory bulb, brain, submandibular lymph nodes, bronchoalveolar lavage, lung, lung associated lymph nodes, turbinates, tonsil, and spleen for virus/viral RNA detection.

SHORT BACKGROUND INFORMATION (One Paragraph):

Background

Nipah virus and ebolavirus are zoonotic viruses infecting both humans and swine, often with fatal outcome in humans. Both viruses, if introduced into a swine population in North America would be difficult to detect immediately, as they do not cause severe and/or typical disease in swine. Thus, the likelihood of wide spread transmission from animals to humans prior to their detection is very high. The outbreak control by depopulation (assumed; note – this would be a CFIA or USDA program decision) would represent enormous logistical difficulties due to the nature of human infection.

Both viruses infect porcine immune cells, cause downregulation of IFN-alpha expression and consequent delay in the immune responses. The amount of the airborne virus shed by infected pigs is sufficient to infect other pigs and transmit to humans. Consequently, a swine vaccine able to abolish virus shedding is of veterinary and human health interest. ARS has developed a biotherapeutic strategy using adenovirus vectors expressing porcine type I or type III interferon (IFN) which could provide at least temporary protection against multiple swine diseases. If successful against EBOV and /or Nipah virus, the strategy could be employed as a mean how to slow down outbreaks and allow time for depopulation.

RESULTS:

Piglets inoculated with Ad5-poIFN-α were challenged 10^5 PFU **of EBOV** per animal 24 hrs post vector administration. Virus RNA shedding (oral swabs and nasal washes) was detected only in challenge control animal. RNA in tissues was detected only in challenge control animal, and to lower extent and in fewer organs from one IFN pretreated/challenged animal. Virus isolations are now

ongoing. Pathological changes indicative of EBOV infection on macroscopic and microscopic levels were not observed in IFN pretreated/challenged animals. Selected cytokine levels will be determined in plasma. RNA from peripheral blood mononuclear cells and cells pelleted from broncho-alveolar lavages were sent for microarray analysis, and the results will be analyzed for regulation of interferon stimulated genes (ISGs), and general cytokine/pathways expression changes. (Post mortem sample collection on day 5 or 6 dpc included submandibular lymph nodes, bronchoalveolar lavage, lung, lung associated lymph nodes, turbinates, tonsil, and spleen).
Preliminary data indicate that pre-treatment with IFN-alpha may offer early protection against EBOV.

Piglets inoculated with Ad5-poIFN-λ (10^{10} pfu) were challenged with 10^5 PFU **of EBOV** per animal 24 hrs post vector administration. Pathological changes possibly indicative of EBOV infection on macroscopic level were observed post mortem in challenge control animal as well as in three IFN pretreated/challenged animals out of four. Sample analysis indicated that the piglets were not protected against EBOV challenge (both real time RT-PCR and virus isolation were positive in collected samples).

The results from the EBOV pilot study are summarized in manuscript: "Potential use of IFN-expressing vectors in control of Ebola virus disease in swine: a pilot study" by Senthilkumaran C[1], Pickering B[1], Smith G[1], Carissa Embury-Hyatt[1], Collignon B[1], de los Santos T[3], Hana M Weingartl[1, 2,*].
The manuscript is currently being prepared for submission to Nature Scientific Reports.

Piglets inoculated with Ad5-poIFN-α were challenged 10^5 PFU of Nipah virus per animal 24 hrs post vector administration. Pathological changes possibly indicative of NiV infection on macroscopic level were observed post mortem in challenge control animal as well as in the IFN pretreated/challenged animals. NiV shedding was detected in all animals, and sample analysis indicated that the piglets were not protected against NiV challenge (both real time RT-PCR and virus isolation were positive in collected samples).

Brief communication will be prepared in future to report also these results.

BENEFITS:

There are no veterinary vaccines available for NiV or EBOV, due to reluctance to invest into their production and licensing. Use of adenovirus vectors expressing porcine IFN for control multiple swine diseases may serve as an incentive for production, licensing and stocking the product. Availability of this countermeasure tool would greatly enhance preparedness to control (or prevent large scale) outbreak control of NiV, should the virus be introduced to North America.

Dr. Teresa de los Santos visited NCFAD for the first animal experiment. This provided introduction to large animal work under BSL4 conditions (in preparation for the NBAF operation). NCFAD benefitted by introducing SC inoculation with adenovirus vectors into their repertoire of BSL4 animal work. The project will also provide further insight into pathogenesis and immune response to EBOV and NiV in pigs.

RECOMMENDATIONS:

The ***Ad5-poIFN-α*** gave very promising results as a possible countermeasure in case of EBOV outbreak in swine, and in combination with ***Ad5-poIFN-λ*** pre-treatment may be further explored to offer protection over a broader time-frame (effect of poIFN-λ is expected to have a later onset than poIFN-α).

Project Leader

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