OECD FELLOWSHIP SUMMARY REPORT

Dr Tim Bowden

CSIRO Australian Animal Health Laboratory

Subject Title

Development of a DIVA vaccine and companion ELISA for capripoxviruses

Theme 2: Managing Risks in a Connected World

Host Institution

National Centre for Foreign Animal Disease, Canadian Food Inspection Agency

Host Collaborator

Dr Shawn Babiuk

Dates of Fellowship

3 December 2017 – 15 January 2018

Consent

I agree to this report being posted on the Co-operative Research Programme's website.

1. What were the objectives of the research project?

The proposed project objectives were as follows:

1/ To establish and consolidate collaborative links with Dr Babiuk at the National Centre for Foreign Animal Disease in Winnipeg, Canada;

2/ To produce and characterise monoclonal antibodies against a recombinant ELISA antigen for the purposes of (i) developing a competition ELISA for capripoxviruses, and (ii) generating a new suite of high quality immunoreagents (for use in immunohistochemistry, immunostaining, immunofluorescence and Western blotting); and

3/ To generate proof of principle data, to assist in obtaining further funding to facilitate development and validation of a DIVA vaccine and companion ELISA, enabling us to pursue our long term research objectives of enhancing the capability to mitigate against, and control, the diseases caused by capripoxviruses (sheeppox, goatpox and lumpy skin disease).

Why is the research project important?

A significant deficiency in current disease mitigation and eradication response capabilities for sheeppox, goatpox, and lumpy skin disease of cattle is the lack of high-throughput and validated antibody detection tests to support post-outbreak surveillance and prove freedom from disease. We proposed to produce monoclonal antibodies to develop a competition ELISA built upon a recently developed indirect antibody detection ELISA. While indirect ELISAs often have high sensitivity (important for screening tests), competition ELISAs are usually more specific (better suited as confirmatory tests). Along with the development of a negative marker vaccine, these technologies are stepping stones towards the development of a comprehensive toolkit to enable differentiation of infected from vaccinated animals, so that vaccination could be used in control programmes without masking serological detection of infected animals, increasing the options available for disease mitigation and eradication.

2. Were the objectives of the fellowship achieved?

Or are they on the way to being achieved? If not, for what reasons?

Yes. Despite having to shorten the duration of the fellowship from 13 to 6 weeks due to changed work circumstances of the Fellow, the main (revised) project objectives were completed as planned. Almost 100 hybridoma supernatants were available for screening, 38 of which were shown to bind to the ELISA antigen in an indirect assay format. Subsequent evaluation of these hybridoma supernatants, using sera from sheep, goats and cattle, led to the identification of two that demonstrated utility for development of a competition ELISA that would be suitable for testing sera from all three host species (sheep, goats and cattle).

The analytical sensitivity of the ELISAs was assessed by determining the end point dilution of known positive sheep, goat and cattle sera, using a two-fold dilution series, and shown to be acceptable. The analytical specificity was also assessed using sera from sheep and goats that had been infected with orf virus, and no false positive reactors were detected.

Preliminary cut-off values were determined using naive sera from Canadian sheep, goats and cattle. Testing of sera from animals infected either naturally or experimentally, or vaccinated, was also undertaken, enabling preliminary estimates of diagnostic sensitivity and diagnostic specificity to be determined.

Evaluation of capripoxvirus infected and mock infected cell monolayers has also demonstrated the utility of one of the newly acquired monoclonal antibodies for immunostaining (detection of wild type virus) in cell culture by using a secondary anti-mouse antibody, conjugated to horseradish peroxidase, and 3-amino-9-ethylcarbazole (AEC) substrate to visualise bound antibodies by light microscopy.

3. What were the major achievements of the fellowship? (up to three)

The major achievements of the fellowship include the following:

1/ Generation of a new suite of high quality immunoreagents (monoclonal antibodies) for use in serological testing (by competition ELISA), immunostaining, immunohistochemistry, immunofluorescence and Western blotting;

2/ Development and preliminary validation of two competition ELISAs for detection of antibodies against capripoxviruses in sheep, goats and cattle; and

3/ Construction of a knock-out live-attenuated capripoxvirus vaccine isolate, based on the ELISA antigen, which is in the process of being purified to remove the wild type parental vaccine virus.

4. Will there be any follow-up work?

Is a publication envisaged? Will this be in a journal or a publication? When will it appear?

Not in the immediate future, as potential options for commercialisation are considered. In due course, in consultation with a commercial partner, publication of data in peer-reviewed journals would ensure that future policy decisions, related to achieving and maintaining food security in agricultural systems, are based on relevant scientific information.

Is your fellowship likely to be the start of collaboration between your home institution and your host?

Collaboration with Dr Babiuk and colleagues at NCFAD to mitigate against the biosecurity risks associated with the diseases of livestock caused by capripoxviruses (sheeppox, goatpox and lumpy skin disease) will continue. Ongoing evaluation and validation of the ELISAs at AAHL and NCFAD will be undertaken to confirm the preliminary estimates of diagnostic sensitivity and specificity using well-characterised sheep, goat and cattle sera. In addition we will use proof of concept data generated prior to, during and following the Fellowship to seek funding to support more comprehensive validation of the competition ELISAs and the DIVA vaccine. This will facilitate the production of new and improved vaccines for capripoxviruses, with DIVA capability, as well as high-throughput companion serological tests, that would more efficiently meet the requirements of national emergency disease control programmes. These tools would thus provide an enhanced level of preparedness for control of any disease outbreaks caused by capripoxviruses.

Is your research likely to result in protected intellectual property, novel products or processes?

Since DIVA vaccines and companion serological tests for capripoxviruses are not currently available anywhere in the world, we would seek to have commercial uptake of both so that they would be available internationally to support disease control and eradication programmes.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

The DIVA vaccine and companion ELISA(s) are about enhancing disease preparedness, supporting control and eradication programmes, and underpinning any claims regarding disease-free status that may arise within the international animal health community, thereby enabling countries to return to trade in the shortest time frames possible. As the suite of tools we ultimately aim to produce will allow the differentiation of infected animals from those which have been vaccinated, the competition ELISA(s) and DIVA vaccine will provide policy makers with an alternative to widespread culling to control the spread of sheeppox, goatpox and lumpy skin disease.

In order to make the competition ELISA and DIVA vaccine available internationally, to support disease control and eradication programmes, we intend to seek commercial partners to ensure their large scale production and widespread availability. In addition, publication of data in peer-reviewed journals, in due course, would ensure that

the relevant scientific information generated through this project could be utilised to inform future policy decisions relating to their acceptable use for achieving and maintaining food security in agricultural systems.

6. How was this research relevant to:

The objectives of the CRP?

The proposed project aligns well with the Co-operative Research Programme objective of strengthening scientific knowledge, as the tools we aim to produce will increase our understanding of basic capripoxvirus biology and the immunological response of livestock to these viruses. By developing new and innovative tools to support control and eradication programmes for sheeppox, goatpox, and lumpy skin disease, which are the most serious poxvirus diseases of production animals, the project outcomes will provide pertinent scientific information, advice and tools that will help to inform future policy decisions and disease incursion response strategies related to the sustainable use of livestock for food.

The CRP research theme?

The proposed project, considering its overall context, delivers directly into the sustainability, food security and nutrition space, which are key outcomes of the Co-operative Research Programme. Sheeppox, goatpox, and lumpy skin disease viruses are exotic, and therefore pose a biosecurity risk, to many of the developed countries around the world, including Australia and Canada. By seeking to reduce, through improved surveillance and more effective disease mitigation and eradication response capabilities, the social, economic and environmental impacts posed by these viruses, this project aligns particularly well with the sub-programme "Invasive Species and Biosecurity" in Theme II (Managing Risks in a Connected World).

The innovative DIVA vaccine and companion assays will enhance the capability of all countries to prepare for, respond to and manage outbreaks of sheeppox, goatpox and lumpy skin disease. These new tools will assist countries to maintain healthy livestock populations, which is essential to securing the social, environmental and economic wellbeing of farmers, and the communities that rely on them for sustainable production of food, as well as enhancing the opportunities for free trade and market access. In developing countries, these tools would also contribute to increasing food availability, raising economic returns, and making production outputs more reliable. Ultimately, the proposed project will facilitate greater food and economic security through providing specific tools to improve animal health and biosecurity.

7. Satisfaction

Did your fellowship conform to your expectations?

Yes, very much so. The fellowship enabled me to spend a very productive 6 weeks working, collaboratively, in the laboratory of an internationally recognised capripoxvirus researcher, with minimal interruption from my routine day-to-day work commitments. A tremendous amount of preliminary work was undertaken by colleagues at my host institute (particularly in relation to production of the monoclonal antibodies) prior to my arrival, without which the project could not have progressed as rapidly or successfully during my visit. I am particularly grateful to my host (and his wife), who were extremely generous in ensuring my stay was as comfortable and productive as possible, especially considering the timing (and duration) of my visit, which coincided with extreme winter weather conditions. I am also very grateful to the OECD for willingly accepting and accommodating the change in my work circumstances, which necessitated a variation to both the duration and scheduling of my fellowship. In this regard the administrative support was excellent.

Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.

Collaborating with NCFAD has, and will continue to enable, CSIRO's knowledge and expertise to be utilised in a way that would simply not be possible in Australia due to the regulatory restrictions that prohibit work with live capripoxviruses in the country, and in a manner that will ultimately benefit Australia, Canada and the international

animal health community. The proof of principle data generated will be invaluable in seeking additional funding to support more comprehensive validation of the competition ELISAs and the DIVA vaccine. Obtaining additional funding would enable us to pursue our longer term research objectives of enhancing the capability to mitigate against, and control, the diseases caused by capripoxviruses (sheeppox, goatpox and lumpy skin disease), and thereby increase my career opportunities indirectly.

Did you encounter any practical problems?

Not with the fellowship itself - only the change in my work circumstances, which necessitated a variation to both the duration and scheduling of my visit. In this regard both my host and the OECD were very accommodating, for which I am particularly grateful.

Please suggest any improvements in the Fellowship Programme.

I, and my host, found the fellowship to be a very positive, productive and worthwhile experience, and would encourage any eligible applicant to consider this Fellowship Programme as part of their professional career development. Perhaps some additional consideration needs to be given to the relative costs of living (and exchange rates) in the host country compared to the country of origin, when calculating the fellowship stipend, especially when accommodation in major cities is required.

8. Advertising the Co-operative Research Programme

How did you learn about the Co-operative Research Programme?

I was notified of the CRP Call for Fellowship and Conference Applications, via an all staff email, by a senior manager within CSIRO.

What would you suggest to make it more "visible"?

Advertising at scientific meetings, conferences and workshops, mass emailing to eligible universities and research organisations, and perhaps by having a presence on Facebook as well.

Are there any issues you would like to record?

No.