Candidate name:

David Smith, PhD

Title:

Genetically engineering *Toxoplasma gondii* as a novel vaccine delivery system

Host Institution:

Universita degli studi di Perugia

Host collaborator:

Manlio Di Cristina, PhD

Dates of Fellowship:

05/07/2021 – 24/09/2021

I, David Smith, consent to this report being posted on the Co-operative Research Program website.

1. What were the objectives of the research project? Why is the research project important?

Toxoplasma gondii is the causal agent of toxoplasmosis, which is considered the third-most important foodborne disease by the World Health Organisation and European Food Standards Agency. The parasite infects more than one billion people worldwide and countless billions of livestock. Infection in humans is acquired through the ingestion of tissue cysts in undercooked meat or oocysts contaminating food or water. Livestock become infected following the ingestion of oocysts from the environment. This interlinked web of disease transmission makes Toxoplasmosis a classic "One Health" issue and methods to prevent infection in humans should consider cutting off disease transmission at sources. This includes the vaccination of meat animals to reduce the presence of tissue cysts in food.

Toxoplasmosis can be a devastating disease. In humans, infection in pregnant women can result in miscarriage or blindness, resulting from congenital toxoplasmosis. Infection in immunocompromised individuals can result in death. Furthermore, anti-*Toxoplasma* drugs are lacking. In small ruminants, toxoplasmosis is a major infectious cause of abortion in sheep and goats, making the disease a significant animal welfare issue, as well as a socio-economic one.

Disease prevention remains the most effective control measure and while there are currently no vaccines for use in humans, there is a live vaccine for use in sheep that is licensed in some countries (including UK, Ireland, France and New Zealand), which is based on an attenuated strain of the parasite that cannot persist within the host. This provides the theoretical basis for this project.

Toxoplasma gondii is very amenable to genetic modification and we know specific genes that are key to parasite persistence. This allows us to generate programmable parasites that can be engineered to no longer persist within the host. This would mimic the current *T. gondii* vaccine on the market, but will have been generated using more controlled and targeted approach. This modified, non-persistent strain would form the basis of the vaccine delivery system.

Toxoplasma sits alongside *Chlamydia abortus* as leading causes of abortion in sheep and there is a demand for a single vaccine that prevents against both pathogens. We reasoned that this could be achieved by modifying the *T. gondii* vaccine delivery strain to produce *Chlamydia* vaccine antigen. We are effectively looking to use *T. gondii* as the factory and the courier for our vaccine antigens. Targeting *C. abortus* antigens in this novel next generation vaccine delivery system would not only provide proof-of-concept for the system working to produce vaccine antigen from another pathogen, but would also lead to the development of a joint *Toxoplasma*-*Chlamydia* vaccine targeted at preventing abortion in sheep on a broader level.

The long-term aim is to use this system to incentivize animal producers to vaccinate their food animals against *T. gondii*, which is currently lacking. If the vaccine delivery system was programmed to protect against other pathogens that afflict food animals, we could simultaneously generate protection against *T. gondii* and thereby reduce tissue cyst burden in meat.

The main objectives of this project were to:

1) Engineer targeted gene knockout *T. gondii* strains that would be unable to persist within the host.
2) Develop the tools to express *Chlamydia abortus* vaccine antigen in the attenuated *T. gondii* background strain.

2. Were the objectives of the fellowship achieved?

The main objectives were achieved.

Single and double gene knockout *T. gondii* strains were successfully generated in a cystogenic parasite background. These will form the basis of the novel next generation vaccine delivery system.

Multiple *Chlamydia* antigen expression plasmids were successfully generated. It was decided that different antigen expression methods would be attempted in *T. gondii* and therefore different plasmids were generated that should result in antigen being trafficked in different ways (e.g. into the parasite cytoplasm, onto the parasite surface, into the parasitophorous vacuole and secretion into host cells). These plasmids were further modified to contain a drug-resistance cassette to facilitate the selection of modified parasites. The drug-resistance cassette was modified to contain flanking LoxP sites to allow for the removal of drug resistance at a later stage.

The next stage of the work is to confirm the modified *T. gondii* strain cannot persist *in vitro* and to then transfect this strain with the *Chlamydia* antigen plasmids, engineering the potential abortion vaccine strains.

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

A major achievement was the completion of key objectives. Specifically, generating the modified *T. gondii* strain that will be developed as a novel next generation vaccine delivery system and producing multiple plasmids for expression of a vaccine antigen, using different protein localization strategies. This latter point to test antigen delivery via different routes was an improvement on the original plan and arose from discussions between the leader of the host lab, Dr Manlio Di Cristina and myself.

A second major achievement was the advanced training component of the work, which enhanced my experience and capacity for genetically modifying *T. gondii*. In this regard, I received high-level training from Dr Manlio Di Cristina, as well as from experienced members of their team. This represents a significant skills development for me personally and a key advanced skills transfer to my lab and my host institute.

A third major achievement was cementing a collaboration between the host lab and myself. This will undoubtedly be a long-term cooperative arrangement and we are already discussing plans for regular PhD student and postdoctoral research exchanges between our two groups. We have also discussed plans for a number of new research projects to carry out between both our groups, which will manifest as a strong long-term collaboration. Finally, as part of this cooperative agreement, both Dr Di Cristina has invited me to contribute to a review article that is currently being written.

4. Will there be any follow-up work?

The work I performed in the host lab is part of an on-going project. The results gained from the OECD fellowship provides a springboard for an entire project on the development of a novel next generation vaccine delivery system. Work on this will be continued at The Moredun Research Institute where I am based. Other follow-up work includes plans for a number of projects to be carried out between both our research groups, a review paper worked on together and regular PhD and postdoctoral researcher exchanges between both groups.

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?

The project is focused on developing a new veterinary vaccine delivery system based on the development of an attenuated *T. gondii* strain that can be customized to express vaccine antigens of other pathogens. This has the potential to have multiple benefits, to both public health and livestock animal welfare. It would result in the protection of meat animals against *T. gondii* infection, thereby lowering tissue cyst burden in meat used for human consumption. This would lower parasite transmission to human populations. This is not achieved with the current *T. gondii* veterinary vaccine (based on a live-attenuated strain) as this is only used in reproductive animals (sheep) and there is no incentive for farmers to use this vaccine in animals used in meat production. I predict that the favorable immune response induced by an attenuated, non-persistent *T. gondii* strain would be beneficial for stimulating the immune response to antigens of other pathogens. The modified strain produced as part of this fellowship will be further customized to express and deliver antigens of other pathogens that are important for livestock health, with the aim of reducing disease and improving animal welfare.

6. How was this research relevant to:

Objectives of the CRP (Biological Resource Management for Sustainable Agricultural Systems):

A major objective of the CRP is stated to be influencing policy within agriculture and food production. The successful development of a novel and highly effective vaccine technology based on a genetically modified strain will strengthen the argument for future policy change surrounding the use of GM organisms in food production systems. The technology would be safer than the existing attenuated *T. gondii* vaccine strain, for which the basis of attenuation is unknown. The strain developed here has been engineered using precision-based genetic modification techniques, which offers greater control over the genetic changes and the outcomes of the modification.

Ultimately, the GM vaccine delivery system will increase food security and reduce carbon footprints by increasing animal protection against disease, which in turn reduces major losses that occur in livestock production that are attributed to infectious diseases. Prevention of disease in livestock also results in improved animal welfare. Taken together, this would represent a strong, positive case for the application of GM organisms in the food production system that are necessary for addressing major issues faced by society, namely food security and yield, climate change and greenhouse gas emissions and disease (including zoonoses).

The CRP research theme:

The research is strongly aligned to Research Theme 3: Transformational Technologies and Innovation, as it aims to result in the development of a novel and highly effective next generation vaccine delivery system with the potential for commercialization of a successful product that would arise from the research.

7. Satisfaction

- Did your fellowship conform to your expectations?

  Met expectations through facilitating a research visit to another lab to complete key research objectives. Exceeded expectations in terms of the extent of positive collaboration that came about and will continue in the long-tem.

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

  It is expected that this fellowship will directly improve my career opportunities as it has enabled the initiation of a project and the production of key results. This will be used as the basis of future large funding applications, which if successful would be transformational for my career. The cooperative arrangement between the host lab and myself will also boost our research outputs and improve our research portfolio.

- Did you encounter any practical problems?

  None of note. The host lab had all of the experience, tools and equipment necessary for carrying out the work and providing me with high quality training.

- Please suggest any improvements in the Fellowship Programme.

  None of note. Communication between the Fellowship Programme coordinators was very good throughout the process, from submission to approval of the proposal and throughout the duration of the fellowship. The coordinators were also very understanding of international travel delays due to Covid-19 and we were able to delay my visit by one month to fit in with an easing in travel restrictions, as well as factor in a 5-day isolation period upon arrival in Italy (as part of government instructions for international arrivals).

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?
  Email via ResearchConnect.