Research Stimulus Fund

Final Report

Bovine neosporosis – Epidemiological and proteomic steps to improved control

DAFF Project Ref No: RSF 06-366
Start date: 03/11/2006
End date: 31/04/2010 (with extension).

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Other Principal Collaborating Researchers: Insert names and institutions

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Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDAMENTAL  APPLIED/PRE COMMERCIAL

×

Key words: (max 4) Neospora, cattle, abortion, bovine
1. Rationale for Undertaking the Research

*Neospora caninum* is a protozoan parasite that causes a significant proportion of bovine abortions in Ireland and worldwide. Even though there has been much new knowledge developed over the past decade in relation to the epidemiology of bovine neosporosis, there are still significant gaps around the advice on control that can be given to farmers (Williams et al., 2009).

We know that whereas vertical transmission within herds in important, and gives rise to problems in successive years, a *Neospora* problem in a herd often first manifests as an “abortion storm”, indicating an external point-source transmission to an immunologically-naïve population. Domestic dogs are definitive hosts of the parasite, and are implicated in some, but not all such outbreaks. We set out, therefore, to assess whether wild carnivores could be an additional source of infection.

Another knowledge gap exists in relation to determinants governing parasite virulence and host susceptibility. Through a proteomics approach, we wished to investigate whether we could identify parasite and/or host determinants of susceptibility/resistance.

The rationale for carrying out this research was to improve understanding of *Neospora caninum* infection as a cause of abortion in cattle in Ireland. The approaches taken included an appraisal of the potential role of wildlife in the transmission cycle of the parasite, a study of virulence/attenuation following *in vitro* culture of the parasite, an assessment of individual animal factors, related to the immune response, affecting the susceptibility and occurrence of abortion, and dissemination of relevant findings to stakeholder groups.

Specifically, the project addressed the following questions:

1. Are there wild carnivores in Ireland which are definitive hosts of *N. caninum*?
2. Are there proteomic markers of changes following long-term cultivation which can be potentially associated with changes in parasite virulence?
3. Are there proteomic biomarkers of individual response variation that can be linked to variation in susceptibility of individual animals?
4. How can these findings best be communicated to relevant stakeholders.
2. Research Approach

Specify the research methodologies employed, emphasising novel techniques and also outline any modifications from the original approved project proposal.

A bank of serum, faecal and brain samples from foxes, minks and badgers was collected and examined to establish whether there are wild carnivores in Ireland which are definitive hosts of *N. caninum*.

In order to assess the potential for evolutionary change in virulence isolates, placental samples from confirmed cases of *Neospora*-associated bovine abortion were cultured with VERO cells in efforts to obtain fresh isolates. Cells were observed daily for 60 days in order to monitor appearance of tachyzoites. In addition, a low-passage number of a dog-isolated, virulent strain, WA-K9 was obtained from Western Australia.

For proteomic analysis, four different methods for the extraction and solubilisation of *Neospora caninum* proteins were evaluated by comparing protein yield, specific antigenicity and relative protein abundance in the electrophoresis profile. These included extraction with SDS and sulphobetaines together with two sonication methods.

Antigen preparations from WA-K9 were characterized using both ID-gels and immunoblots. Large-scale 2D gel mapping was optimised for comparison of field and attenuated laboratory isolates.

A serum bank from a large dairy herd from Co. Wicklow with a recent history of *N. caninum* abortion was obtained. Sera from this herd were evaluated by ELISA and by 1D and 2D immunoblotting over a 12-month period in order to map and evaluate the fine-specificity of immune responses in this herd.

Bioinformatic analysis of antigens identified as potentially of interest as determinants of qualitative markers of individual animal susceptibility/resistance were sequenced and subjected to bioinformatics analysis using the *N. caninum* genome database NeoDB, maintained by the Wellcome Trust’s Sanger Centre. Where proteins could not be matched to an annotated protein in NeoDB they were characterized, where possible, as homologues of proteins in the Sanger Centre’s ToxoDB, the *Toxoplasma gondii* genome database.
3. Research Achievements

Our results indicate that 1% of mink and 1% of fox samples were positive for *N. caninum* by IFAT. According to PCR analysis of DNA extracted from brain tissue, 3% of the mink, 4% of the otters and 6% of the foxes examined were infected with *N. caninum*. All fecal samples tested negative for *N. caninum* DNA. The implications of these findings are that *N. caninum* is capable of infecting a range of wild carnivores common in Ireland, specifically foxes, mink and otters. The fact that no oocysts or *N. caninum* DNA was detected in faecal samples from these species does not rule out their role in either direct or indirect transmission to cattle, since oocyst shedding even in domestic dogs is comparatively low, intermittent, and difficult to detect.

Attempts to prepare a fresh isolate from placenta and brain samples from aborted calves from a confirmed outbreak of neosporosis, using a protocol that had been previously been used successfully to prepare a *Toxoplasma gondii* isolate, were ultimately unsuccessful. Because of these difficulties, an alternate strategy of using a recent canine isolate, WA-K9, as a proxy for a virulent, non-lab adopted strain, was adopted (McInnes et al, 2006). Following some initial difficulties this strain was established and successfully grown in our laboratories.

A series of 2D-gels from each *N. caninum* isolate were used to identify a number of candidate antigens that were downregulated in the attenuated isolate. These spots were subjected to mass-spec analysis and compared by Blast-P with proteins in NeoDB, and where no match was evident, in ToxoDB. Bioinformatics analysis of these proteins indicated them to be mostly associated with essential parasite metabolism and/or motility. Unlike the proteins identified as immunodominant antigens they were not either surface or secreted antigens, or those identified as putative candidate vaccine antigens.

2D gel electrophoresis followed by immunoblotting allowed the identification of 37 highly immunodominant antigens recognised by cows in the immediate aftermath of an initial exposure to *N. caninum*, followed by abortion. Evidence from our work shows that all of these are immunodominant antigens recognized by cows shortly after abortion where a point source of infection has been introduced to a herd – i.e. a previously naïve population. These correspond in large part to immunodominant antigens identified by other authors (Zhang et al., 2011), and, as expected, they are all, unlike the majority of antigens in Table 1, surface-expressed and/or secreted molecules. Our work did not identify an absolute difference between either susceptible and resistant individuals in terms of the immunodominant antigens identified. However, based on bioinformatics analysis of
the immunodominant antigens identified, we did propose a number of mechanisms whereby genetic predisposition to *Neospora* abortion in cattle could be predicted.

4. Impact of the Research

*Provide a summary of outcomes of research and outline the benefits of the research to end users, e.g. industry, consumers, regulatory authorities, and scientific community etc*

Evidence of *N. caninum* infection in wild carnivores.

Our work has shown for the first time evidence of *N. caninum* infection in carnivorous wildlife in Ireland. Based on the known life-cycle of *N. caninum*, and the evidence for frequent point-source introduction of infection into previously-naïve cattle herds, we postulate a potential role of wild carnivores, and particular foxes and mink, in the origin of some *Neospora*-related abortion storms in cattle herds in Ireland. This is supported by the failure to demonstrate involvement of domestic dogs in some such cases (personal communications). The fact that we did not demonstrate oocyst shedding in faecal samples from such species does not invalidate this reasoning, as oocyst shedding in domestic dogs and in coyotes, the only other carnivores where it has been demonstrated, is notably intermittent, involves relatively small numbers of oocysts, and is difficult to demonstrate.

The impact of these findings is to alter the advice to herd owners to include preventing access of these wildlife species to cattle housing and feed stores, in addition to preventing such access to domestic dogs.

Evidence for antigenic variation between laboratory-adapted and wild-type *N. caninum* isolates.

Demonstration of immunodominant antigens in cows that recently suffered acute abortion in comparison with those experimentally-infected and resistant to abortion: Our work has provided evidence of and putative reasons why immune responses to a set of immunodominant antigens in cows who aborted following infection with *N. caninum* are not in themselves protective, but may yield clues to host genetic variants that may demonstrate enhanced resistance, based on reduced affinity for parasite lectin-binding proteins. This work may be exploited in future work.
The project’s scientific impact is encompassed in three scientific publications, with two more in preparation.

5. **Exploitation of the Research**

Outline the outcomes of the research that have commercial or economic importance and provide details of Intellectual Property / licences / patents generated. Details of outputs adopted by industry should also be provided.

The immediate exploitation of this research is provided by the more accurate and complete advice that can be provided to farmers in relation to potential sources of transmission to their herds. An invention disclosure has been made to NOVA UCD in respect of one antigen where host genetic variation would be expected to affect susceptibility.

6. **Summary of Research Outputs**

(a) Intellectual Property applications/licences/patents
   1. Invention disclosure to NOVA UCD in relation to glycoprotein-associated variations in host susceptibility.

(b) Innovations adopted by industry
   1. Standard advice to vets and farmers in herd health planning in relation to neosporosis now includes advice re preventing access of foxes and mink to feed stores and cattle sheds.

(c) Number of companies in receipt of information

   We have had informal discussions with Pfizer Animal Health (now Zoetis) on our findings re immunodominant antigens potential of host genetic variants to influence susceptibility to abortion. This has not progressed to the stage where confidentiality agreements would be warranted.

(d) Outcomes with economic potential
   1. Outcomes under 6(a) and 6(b) have economic potential.

(e) Outcomes with national/ policy/social/environmental potential
1. Outcome 6(a) has immediate impact in terms of advisory/social/animal health potential.

2. 

(f) Peer-reviewed publications, International Journal/Book chapters.

   *Parasitology* **140**:296-302.
3. (Submitted for publication). Glycoprotein variation may mediate susceptibility to *Neospora caninum* infection in cattle. 
   Read, C., Zintl, A. and Mulcahy, G.
4. (In Preparation) 
   Characterisation of a *Neospora* abortion outbreak in a previously uninfected dairy herd in Ireland.  
   O’Grady, L.E., Read, C., Sekiya, M., More, S. and Mulcahy, G.

5. (in preparation) Proteomics of *N. caninum* with a focus on changes associated with attenuation.

(g) Scientific abstracts or articles including those presented at conferences


(h) National Report
1. Nil.
2. 

(i) Popular non-scientific publications
1. Nil
2.
(j) Workshops/seminars/ open days at which results were presented (excluding those in (g))
1. UCD Herd Health Discussion Group, UCD
2. Research Open Day, School of Agriculture, Food Science and Veterinary Medicine, 2010
3.

7. Permanent Researchers

<table>
<thead>
<tr>
<th>Institution Name</th>
<th>Number of Permanent staff contributing to project</th>
<th>Total Time contribution (months)</th>
<th>Average time contribution per permanent staff member (%)</th>
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<tr>
<td>NUIG</td>
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<td>15.9</td>
<td>10</td>
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<td>UCD</td>
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Total 5 23.1 7.5

8. Researchers Funded by RSF

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<td>Contract Researchers</td>
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<td>PhD postgraduates</td>
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<td>Masters postgraduates</td>
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<td>Temporary researcher</td>
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<td>Other</td>
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9. Postgraduate Research

Total Number of PhD theses: 1
Stuart, P. October 2010, NUIG
Investigating the role of Wild Carnivores in the Epidemiology of Parasitic Disease
Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Total Number of Masters theses: __Nil__

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

10. Project Expenditure

Total expenditure of the project: € 457,010

Total Award by RSF € 450,591.00

Other sources of funding (specify) €

1. Nil

2.

Breakdown of Total Expenditure

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<th>Name Institution 3</th>
<th>Name Institution 4</th>
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11. **Future Strategies**  
*Outline development plans for the results of the research.*  
Dr. Annetta Zintl, has recently joined the School has lecturer in Veterinary Parasitology. She is a protozoologist of some repute, having worked on related apicomplexans such as Cryptosporidium and Toxoplasma. In collaboration with the UCD Herd Health Group, she will have designated responsibility for continuing on with the Neospora research and further developing the intellectual property and scientific findings obtaine in this project.

12. **Industry Collaboration**  
*Summarise details of industry collaboration in the research project.*  
Preliminary discussions with Pfizer Animal Health (now Zoetis) have been held on the prospects for improved neospora diagnostics and vaccines.