The immunogenic potential of heartwater (*Cowdria ruminantium*) grown in tick cell lines

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Project Dates: April 1999 – March 2002
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Executive Message

- Heartwater can cause high mortality amongst cattle, sheep and goats. It is caused by the tick-borne organism *Cowdria ruminantium*.
- The disease has a major impact upon the development of livestock owning communities especially those in small-holder dairy schemes.
- Existing vaccines against heartwater, a tick-borne disease which is a major constraint to small ruminant production in sub-Saharan Africa, are based on mammalian stages of the causative rickettsia *Cowdria ruminantium* and are expensive, potentially dangerous, and give poor protection.
- This project has shown that *Cowdria* grown in tick cell lines provides an alternative, possibly complementary source of antigenic material for immunisation and diagnostic tests. Under certain culture conditions, *Cowdria*-infected tick cells are highly immunogenic, protecting over 90% of sheep against experimental heartwater in the absence of clinical disease, thus proving the potential role of the cultures in improving current control measures.
- This development may eventually enable animal health workers to replace the current ineffective vaccines.
- Such new vaccines would greatly help many small-holders keeping goats or cows for milk in sub-Saharan Africa to improve their standard of living by allowing them to keep higher yielding animals, which are usually very susceptible to heartwater, in areas where the tick vector is present.

Background

The tick-borne rickettsial disease heartwater causes high mortality amongst susceptible domestic ruminants, including cattle, sheep and goats. Heartwater is considered the second most economically serious tick-borne disease of livestock in sub-Saharan Africa after theileriosis. It is caused by the tick-borne organism *Cowdria ruminantium*. The disease has a major impact upon the development of livestock owning communities because it restricts the introduction of higher performing exotic animals which are highly susceptible to Heartwater. This is a severe problem for small-holder dairy schemes involving cattle or goats where the vector tick is present.

Animals can be protected using vaccines based on the mammalian stages of *Cowdria* but are very costly, difficult to use and have problems of safety, efficacy, and cross protectivity. An alternative approach to vaccine production and the development of diagnostic tools is to use the tick stages of *Cowdria* grown in tick cell lines.

Objectives

This project was originally programmed for two year but was extended by 12 months. It’s aim was to contribute to safer, cheaper and more effective vaccines and diagnostic tools for heartwater by
developing and characterising *Cowdria/tick cell* culture systems which yield immunogenic material different from and complementary to that from mammalian culture systems.

The original outputs specified for the project were:

1. Developing a system for growing *Cowdria* in cell lines from one or more tick species
2. Increasing knowledge of *Cowdria* development in tick cells
3. Producing & testing useful quantities of immunogenic *Cowdria* material in tick cell cultures free from harmful contaminants

**Highlights**

All three outputs have been achieved:

1. Six different *Cowdria* isolates, have been successfully established in at least three each of ten tick cell lines. Two of the cell lines are derived from the vector tick *Amblyomma variegatum*, the remainder are from tick species not known to transmit *Cowdria* (*Ixodes scapularis, Ixricinus, Boophilus decoloratus, B.microplus* and *Rhipicephalus appendiculatus*). Initial infection of tick cells with mammalian stage *Cowdria* is achieved using cell lines from *Ixodes* or *Amblyomma, Boophilus* and *Rhipicephalus* lines have only been infected from other tick cell lines.

2. The morphology of *Cowdria* grown in tick cell lines was examined by light and electron microscopy, and found to resemble that previously found in intact ticks, while differing from that reported for mammalian stages. Western blotting of tick cell-derived *Cowdria* with polyclonal sera and monoclonal antibodies revealed that the immunodominant antigen in tick cell stages was not the 30-32 kD MAP1 protein found in mammalian stages, but a smaller 29kD protein. This antigen appeared to share some, but not all epitopes with MAP1. MAP1 is transcribed from one of a multigene family; analysis by RT-PCR (in collaboration with Utrecht University) revealed that, of those genes examined, only *map1* was transcribed in virulent mammalian stage cultures, whereas *map1-1*, and possibly *map1*, were transcribed in tick cell stages. The predicted size of the MAP1-1 protein is 29kD.

3. A range of tick cell line/Cowdria isolate combinations, grown under optimised conditions allowing production of up to 30 doses from two 75cm² culture flasks, were tested for infectivity/immunogenicity in sheep.

Of 99 sheep inoculated with live *Cowdria*-infected tick cells only one developed clinical heartwater, and none showed any other adverse effects beyond mild transient fever. The tick cell lines were all shown to be mycoplasma-free; foetal calf serum was the only mammalian-derived component. Certain combinations of cell line and isolate were found to be highly immunogenic; for example, 25/27 sheep inoculated with freshly harvested Gardel isolate grown in the *A.variegatum* cell line AVL/CTVM13 were fully protected against subsequent virulent homologous or antigenically-related (Ball 3 isolate) challenge.

Under specific conditions, the *Cowdria/tick cell* culture system developed by the project produces highly immunogenic material and thus protects sheep against lethal experimental challenge. The project achieved protection with four *Cowdria* isolates using a single i/v dose of live culture which means it compares favourably with other live vaccines. Incorporating tick cell-derived *Cowdria* antigens into inactivated vaccines may actually improve their current poor performance.

Initial work by the scientists indicate that antigen giving protection is the highly conserved MAP1-1 gene is transcribed in tick cells in vivo and in vitro, and in attenuated, but not virulent, mammalian stage cultures. Because of its conserved nature this gene, and its associated protein, could help in improved diagnostic tests.

In addition to the *Cowdria* studies, the project produced the first all-British tick cell line, established from embryonic *Ixodes ricinus* provided by CEH Oxford.

**Impact**

Existing vaccines against heartwater, a tick-borne disease which is a major constraint to small ruminant production in sub-Saharan Africa, are based on mammalian stages of the causative rickettsia *Cowdria ruminantium* and are expensive, potentially dangerous, and/or inadequately protective. The results of this project means that the *Cowdria/tick cell* culture system developed will be useful in the development of any improved heartwater vaccines and diagnostic tools. This project has shown that *Cowdria* grown in tick cell lines provides an alternative, possibly complementary source of antigenic material for immunisation and diagnostic tests. Under certain culture conditions, *Cowdria*-infected tick cells make a highly effective ‘vaccine’, protecting over
90% of sheep against experimental heartwater. This means the cultures produced do have a potential role in improving current disease control measures.

As detailed below there is still some way to go to produce the improved heartwater vaccines needed by animal health professionals and farmers. This project has however made much progress towards a new, simpler, cheaper, safer and more effective vaccine that will eventually improve the performance of livestock belonging to poor people by controlling diseases such as heartwater and improving its detection.

Next Steps

This scientific research has thrown up several issues requiring more work including:
- Identifying which fractions of the crude, live antigen are immunogenic
- What role the tick cell material plays
- Whether the immunogenic fraction will be useful in an inactivated form
- Determining the nature and role of the immunodominant 29kD protein. If this protein is MAP1-1, it may be useful for diagnosis as well as immunisation.
- Further characterisation of the tick cell/Cowdria systems to fully investigate the behaviour of Cowdria in tick cells in vitro. Comparison with development of Cowdria in ticks would indicate how far the in vitro systems were representative of the in vivo situation.

Some of the above issues will be addressed under an EU-funded project "Integrated diagnostic and recombinant vaccine development for cowdriosis and anaplasmosis" . This involves a consortium of 11 laboratories in Africa, The Caribbean, Latin America and Europe and includes CTVM as a partner.

Capacity building

One aspect of a DFID sponsored project like this is to enhance the capacity of scientists in developing countries to pursue future research in the same area. Specific training has already been provided by the project to build such expertise and capability up at the ITC, The Gambia for collaborative and in-country heartwater research. The project also provided tick cell lines and training in their maintenance to the following: Glasgow University; IAH, Pirbright; NERC-CEH, Oxford; Liverpool University; Institute of Aquaculture, Stirling University; Marseille University; Free University of Brussels; Freiburg University; ILRI, Kenya; Veterinary Services Department, Ghana; CIRAD-EMVT, Guadeloupe.

Dissemination

Selected Publications


Presentations

Kenya: CTVM/KARI/DFID workshop on heartwater, Egerton University, May 1999 Papers on in vitro cultivation of Cowdria and isotype-specific ELISAs.

Holland: Seminar at Utrecht University Vet Faculty Nov 1999.

