R5623 – Heartwater: Developing diagnostic tests*

*Recombinant antigen production and sequence analysis of Cowdria ruminantium outer membrane protein antigens for diagnostic tests development

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Executive summary

• Heartwater, caused by Cowdria ruminantium, a tick-borne organism, is a serious disease in cattle, sheep, goats and wild ruminants.
• The disease has a major impact upon smallholder dairy schemes as higher performing exotic animals are highly susceptible.
• There is no effective test currently available to identify animals suffering from the disease and those at risk.
• This project developed sensitive and specific diagnostic tests for Cowdria ruminantium detection. These include:

  1. An indirect enzyme-linked immunosorbent assay (ELISA) that detects Cowdria ruminantium antibodies, the heartwater agent, thus enabling the identification of infected animals.
  2. The first ever rapid polymerase chain reaction (PCR) test for analysing and genotyping Cowdria from blood/culture samples.
  3. A rapid (pen-side) sero-diagnostic test, successfully tested in Swaziland, for monitoring field vaccinations.

• Genes identified and cloned during research may also be used to develop a heartwater vaccine.
• Project techniques and methods were transferred to relevant national institutions in Kenya, Gambia, South Africa, and Ghana and are now routinely used to diagnose and study heartwater epidemiology.

Project dates: April 1993 – March 1996

Background
Heartwater disease, caused by the tick-borne organism Cowdria ruminantium, affects cattle, sheep, goats and wild ruminants. In economic terms, it is the second most important tick-borne disease for livestock in
sub-Saharan Africa (theileriosis is first). The disease is a major development constraint to livestock owning communities as higher milk performing exotic animals are highly susceptible.

This problem is severe for small-holder cattle or goat dairy schemes where the vector tick is present. Exotic animal mortality can only be reduced with high levels of veterinary attention and treatment. Disease control is hampered by the absence of a specific, sensitive, diagnostic assay which can distinguish between animals recovering from *Cowdria* from those requiring protection.

**Objectives**

The project aimed to develop a specific, sensitive diagnostic test for *Cowdria ruminantium* detection through the isolation and expression of recombinant antigens. The novel genetic information obtained was also used to develop tests capable of differentiating *Cowdria* strains. This involved:

- Cloning genes encoding a *Cowdria* immunodominant antigen. This had a subsidiary benefit of making antigens available for use in identifying possible vaccine candidates.
- Expressing and purifying the antigen from recombinants.
- Identifying serological reactions between the recombinant antigen and sera from experimental and field cases of *Cowdriosis* and *Ehrlichiosis*.
- Comparing the sensitivity and specificity of enzyme-linked immunosorbent assays (ELISAs) and other immunoassay tests currently using this antigen.

Finally, the project also aimed to develop a rapid genetic method for differentiating and characterising *Cowdria* stocks from field outbreaks.

**Highlights**

Significant progress was made in the following areas:

Researchers identified and cloned an antigen of considerable importance in immune responses which may be the basis of a new vaccine – the 58 kDa heat shock protein of *C. ruminantium*.

An indirect ELISA was developed that uses the recombinant 58 kDa antigen to recognise the presence of antibodies to *Cowdria ruminantium*. This test was transferred to African laboratories in Kenya, Gambia, South Africa, and Ghana and is now routinely used in diagnostic and epidemiological studies on heartwater.

A rapid (pen-side) sero-diagnostic test was developed for the monitoring
of field vaccination. Successful trials were carried out in Swaziland (by FAO; 1995) and South Africa (1995).

The first ever rapid test for analysing and genotyping *Cowdria* was developed from blood/culture samples. This rapid polymerase chain reaction (PCR) based genetic differentiation test holds great epidemiological potential and was transferred to Ghana (1996) and Kenya (1996).

**Impact**

Project outputs included the transfer of techniques and methods to national agricultural research organisations (NAROs) and other regionally influential institutions in Africa (Muguga, Kenya; Onderstepoort, South Africa; and International Trypanotolerance Centre, The Gambia). These institutions are using the developed methods to undertake research activities which have national and international significance for the integrated control of heartwater with other tick-borne diseases, including the evaluation/monitoring of pilot immunisation programmes.

Animal health professionals will immediately benefit from the project as they will be able to identify animals suffering from or at risk from heartwater, which will lead to better animal health control. Ultimately, the work should lead to the development of a vaccine to protect cattle and goats against heartwater and allow the use of exotic breeds that can improve the milk productivity of small holder farms. This should dramatically affect their earnings and increase the amount of milk available to local communities.

From a capacity-building perspective, ELISA kits were sent to collaborating laboratories in Ethiopia, Ghana, and The Gambia. Sera or blood stabilates were received from institutes in Kenya, Ghana, South Africa, Guadeloupe, USA, Italy and The Gambia. Reagents and advice in establishing a serodiagnostic facility for heartwater was sent to Ghana (Accra), Kenya (Muguga), The Gambia (ITC, Banjul), Tanzania (Dar-es-Salaam), South Africa (Onderstepoort).

Personnel were trained in newly developed techniques in Malawi, Ghana, Mozambique, Nigeria, Trinidad, Brazil, Yemen, Zimbabwe, Botswana, Bolivia, Ethiopia, Panama, Kenya, Gambia, and Peru. Finally, technical advice about diagnosis and differentiation of *Cowdria* and *Ehrlichia* infections was provided to groups in Guadeloupe, Italy, Kenya, and the Gambia.

**Collaborators**

1. Kenya, National Veterinary Research Centre, Muguga  
2. Ghana, Accra Veterinary Laboratory, Department of Veterinary Services
Selected Publications

