

**FINAL REPORT FOR USAID/RCSA COOPERATIVE AGREEMENT
no. 690-A-00-03-00038-00 RELATING TO THE HEARTWATER CONTROL GDA**

Background to the Heartwater Research Project

The UF USAID SADC Heartwater Research Project was initiated in 1985 with the aim of development of improved methods for the diagnosis and control of heartwater. The Project was funded by USAID through USAID Zimbabwe (1985-89), USAID Washington (1989-96) and USAID RCSA (1996-2002). The Project established a modern heartwater research laboratory in Zimbabwe which soon gained international stature, only to be closed in 2002 due to the political upheavals in Zimbabwe. However, prior to the laboratory's closure, the Project developed several technologies for control of heartwater including a diagnostic assay which was licensed to IDEXX of the United States, an inactivated vaccine, a recombinant vaccine and a tick decoy system for control of the tick vectors of heartwater.

Establishment of the GDA Project

Following the closure of the laboratory in Zimbabwe, it was decided to transfer the heartwater research laboratory and some of its personnel to South Africa so that two of the Project's technologies (the inactivated heartwater vaccine and the tick decoy) and a third technology developed at the University of Florida for tick control (the AppliGator[®] self-medicating applicator) could be commercialized for sale in southern Africa and beyond. Commercialization of these technologies formed the basis for the Heartwater Control GDA Project which began in October 2002 and ended in March 2005. This GDA Project had three partners: the University of Florida as the technical partner, Intervet International the private-sector partner and USAID the funding partner. The University of Florida held the US and foreign patents on the three technologies and, through several of its employees who invented the technologies, had the technical know-how. Intervet International was selected as the private-sector partner primarily for four reasons: (1) it is the largest manufacturer of large-animal vaccines in the world; (2) it has a very active subsidiary, Intervet South Africa, in South Africa; (3) it has excellent facilities for trials of vaccines and tick-control devices in South Africa; and (4) it is currently involved in heartwater control through sales of its acaricides deltamethrin and amitraz. Progress with commercialization of each of the three technologies will form the basis for this final report.

Commercialization of the Inactivated Heartwater Vaccine

Trial 1

The first vaccine trial was conducted at the Intervet Research Farm in Malelane from January to June 2003. The objective of the trial was to define an optimal vaccination regimen based on use of Intervet adjuvants: two Intervet adjuvants (saponin and GNE) were used, together with three antigen doses (10µg, 50µg and 150µg) and two dosing regimens (three vaccinations at four-weekly intervals or two vaccinations four weeks apart). The trial utilized 90 cattle divided randomly into six groups, with all animals receiving a lethal heartwater challenge following vaccination. While the survival rate in the control group was only 15%, it was 46-79% in the four treatment groups, with the best protection provided by the vaccine with a GNE adjuvant and 50µg of antigen given twice four weeks apart. It was concluded from this trial that GNE was a promising adjuvant for use in cattle.

Trial 2

The second vaccine trial was conducted at the Intervet Research Farm in Malelane in early 2004. The objective of the trial was to determine the efficacy of the GNE adjuvant in sheep. The trial utilized 80 sheep divided randomly into four groups; the three treatment groups received vaccine formulations containing different doses of antigen (25µg, 50µg and 100µg) in GNE adjuvant twice four weeks apart, whereas the negative control group received no vaccine. All 80 animals received a lethal heartwater challenge following vaccination. All vaccinated sheep, irrespective of antigen dose, and the control unvaccinated sheep responded similarly to tick challenge, sustaining similar high levels of morbidity and mortality. It was concluded that **the Intervet GNE adjuvant, while efficacious in cattle (see trial 1 above), did not work similarly in sheep in the inactivated heartwater vaccine.**

Trial 3

The third vaccine trial was conducted at the Intervet Research Farm in Malelane, starting in July 2004. The objective of the trial was to test other Intervet adjuvants in the vaccine, namely a Quil A GNE combination and Quil A alone, with the Seppic adjuvant Montanide ISA50 as the positive control. Before initiation of the GDA Project, we had found Montanide ISA50 to be the best adjuvant available for the inactivated heartwater vaccine. The trial utilized 66 sheep which, following vaccination, were subjected to lethal heartwater challenge. The survival rate in the unvaccinated control sheep was 18%, in the Quil A adjuvant group 45%, in the Quil A GNE adjuvant group 70%, and in the comparable Montanide ISA50 adjuvant group 91%. It was concluded that the Montanide ISA50 adjuvant, which we had identified by the Heartwater Research Project in the late 1990s, was superior to any Intervet adjuvants for use in the inactivated heartwater vaccine, a conclusion with which Intervet concurred.

Trial 4

The fourth vaccine trial was conducted at the Intervet Research Farm in Malelane, starting in October 2004. The objective of the trial was to test the efficacy of the GNE-adjuvanted vaccine in cattle against field challenge at the specific request of Intervet. **Three groups of 15 cattle were used in the trial, one inoculated with GNE-adjuvanted vaccine, one inoculated with Montanide ISA50-adjuvanted vaccine, and one unvaccinated. The trial was conducted by Intervet over our objections since the challenge was by exposure to all ticks and thus all tickborne diseases, making interpretation of data difficult. In any event, the Montanide ISA50 vaccine protected 67% of the cattle while the GNE vaccine protected less than 50%, again demonstrating the superiority of the Seppic adjuvant over the Intervet adjuvant.**

Trial 5

The fifth vaccine trial was conducted in the Eastern Cape region of South Africa in early 2005. The objective of the trial was to test the efficacy of the Montanide ISA50-adjuvanted vaccine in Angora goats. Unfortunately, the heartwater challenge in the Eastern Cape, which had been so severe in 1999, was too poor to provide any meaningful data from the trial which has just been concluded.

Licensing of the Vaccine Technology

Intervet signed an option agreement for the inactivated heartwater vaccine in August 2001 and only last December renewed the option agreement to 1 September 2005. However, at a meeting in Johannesburg with the University of Florida and USAID RCSA on 9 March 2005, Intervet stunned us with the unexpected announcement that it was no longer interested in commercialization of the vaccine. With only 22 days left before the Project ended, we immediately looked for alternative vaccine manufacturers. Fortunately we have identified a South African company with great interest in the vaccine. That company is Onderstepoort Biological Products, the current manufacturer of the live heartwater vaccine. At the time of writing of this report, the University of Florida and Onderstepoort Biological Products were negotiating potential terms of a license agreement for commercialization of the inactivated heartwater vaccine.

Constraints Experienced with Intervet

It is worth noting some of the difficulties that the University of Florida experienced working with Intervet on commercial development of the inactivated heartwater vaccine since they might assist other projects in development of public private-sector collaboration. The primary problem related to management of the international company Intervet International. Even though the University of Florida was working with Intervet South Africa, all meaningful decisions had to be endorsed by Intervet International in The Netherlands which often took months to accomplish. Furthermore, Intervet International controlled access to the Intervet Research Farm in Malelane, and we could only conduct trials there when the facilities were not required for experiments by Intervet International. This meant that there were times when we had to wait many months before we could start a new trial. Finally, there was no way to determine the true interest of Intervet in the vaccine as can be seen by its renewal of an option agreement for the vaccine only a few months before it decided to terminate collaboration on commercialization of the vaccine.

Commercialization of the Tick Decoy Technology

Trial 6

The first trial was conducted at the Intervet Research Farm in Malelane from March to June 2003 using tick decoys fabricated at the University of Florida. The objective of the trial was to identify the best Intervet acaricide for use in the decoys. The trial included four groups of five cattle with one a negative control group without any decoys, one a treatment group with decoys containing the acaricide amitraz, one a treatment group with decoys containing the acaricide deltamethrin and one a treatment group with decoys containing both acaricides. All 15 animals in the three treatment groups received one ear decoy and one tail decoy. After decoy attachment, all 20 cattle were released into tick-infested pastures. Tick counts were made on each animal before decoy attachment and thereafter at weekly intervals for 14 weeks. All groups had high mean counts ranging from 552-626 adult ticks at the start of the trial. The ticks were primarily the brown ear tick *Rhipicephalus appendiculatus* (a primary vector of East Coast fever) and the bont tick *Amblyomma hebraeum* (the vector of heartwater). The tick counts on the cattle in the

negative control group continue to be so high that these animals had to be treated with acaricide on days 0, 14, 36 and 84 of the trial to prevent permanent tick damage. In contrast, the mean tick counts on the animals with decoys declined to reach their lowest values by day 77 when there was an average of 1 tick per animal on those with deltamethrin decoys, 20 ticks per animal on those with amitraz decoys and 21 ticks per animal on those with amitraz deltamethrin decoys. It was concluded from this trial that deltamethrin was the acaricide of choice for use in the tick decoys.

Trial 7

The second trial was conducted in Malelane from January to March 2004 to determine, at Intervet's request, the importance of the *Amblyomma* tick pheromones and the necessity of the tail decoy for successful tick control using the decoy technology. Decoys were fabricated at the University of Florida for the trial which included four groups of five cattle: a group with both ear and tail decoys containing both pheromone and deltamethrin acaricide, a group with both ear and tail decoys containing only deltamethrin acaricide, a group with only ear tags containing both pheromone and deltamethrin acaricide, and a negative control group without any decoys. The trial showed conclusively that both pheromone and tail decoys were necessary for effective tick control.

Licensing of the Tick Decoy Technology

In light of the two positive trials, the University of Florida Office of Technology Licensing initiated discussions with Intervet regarding a license agreement for the tick decoy technology. Surprisingly, Intervet decline to proceed with commercialization of the technology. Consequently, the University of Florida searched for an alternative licensee and found one in Insect Science, a company based in Tzaneen, South Africa, which specializes in marketing environmentally sound and effective means of insect control including use of pheromones. At the time of writing of this report, the University of Florida and Insect Science were negotiating terms of a license agreement for commercialization of the tick decoy technology.

Commercialization of the AppliGator[®] Technology

Trial 8

Prototype AppliGators[®], self-medicating applicators designed at the University of Florida for tick control on wild animals, were manufactured in the United States for the University of Florida by Acme Plastics of New Jersey and shipped to South Africa. The trial was conducted at Mauricedale Game Ranch in Malelane from June to October 2003 with the objective of evaluation of the durability of AppliGator[®] under extreme conditions. The AppliGators[®] were attached to the lips of the troughs from which African buffaloes (*Syncerus caffer*) were feeding, allowing the AppliGators[®] to be exposed multiple times daily to the pressures of feeding buffaloes weighing up to 2,000 lbs each. After 17 weeks, the AppliGators[®] which had been primed with amitraz for mite control were still functioning well, demonstrating an outstanding durability for the device.

Licensing of the AppliGator[®] Technology

Intervet agreed that the trial demonstrated that the AppliGators[®] was ready for commercial production. After lengthy negotiations, Intervet International signed a license agreement on 14 February 2005 to market the AppliGator[®] technology for control of ticks in Africa. At the time of writing of this report, Intervet indicated that it hoped to launch the AppliGator[®] technology in South Africa in late 2005.

Summary of Achievements of the GDA Project

1. Inactivated Heartwater Vaccine: Negotiations underway with Onderstepoort Biological Products of South Africa for marketing of this vaccine in Africa.
2. Tick Decoy Technology: Negotiations underway with Insect Science of South Africa for marketing of this tick control technology in Africa.
3. AppliGator[®] Technology: Technology licensed to Intervet International in February 2005 for control of ticks in Africa.

Michael J. Burridge
Suman M. Mahan
University of Florida
30 June 2005