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Development of a Subunit Vaccine for Heartwater in Domestic Ruminants

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(3) Executive Summary:

The long-term research goal of this project was to develop a subunit vaccine for *Cowdria ruminantium*, which causes heartwater in cattle, sheep, and goats. Heartwater is endemic in most countries of Africa and occurs in the Caribbean Islands. The disease is a constraint to efficient production of meat and milk by domestic ruminants because it results in a very high morbidity and mortality in these animals. There is no effective vaccine that can be used in developing countries and, at the beginning of this PSTC project, the diagnostic tests for heartwater in animals and in the tick vector were very cumbersome and, therefore, not widely used.

Our project also had other goals, including providing PhD graduate training for an employee of the Kenya Agricultural Research Institute (KARI), our collaborating organization in the host country of Kenya; helping develop and support an animal disease biotechnology laboratory in Kenya; helping transfer recombinant DNA technology to Kenya; and developing long-term collaborations between host county and Washington State University researchers. These other goals were accomplished. Specifically, the PSTC project provided research support for Dr. Suryakant Waghela, a KARI employee, and he earned his PhD. The project supported his thesis research at the animal disease biotechnology laboratory in Kenya. Equipment was purchased for the laboratory, supplies were purchased and sent to the laboratory and Washington State University (WSU) faculty visited Kenya to provide technology transfer and research collaboration. The heartwater project continues in Kenya in collaboration with WSU faculty and includes a KARI employee working toward an MS degree.

The research accomplishments of the project include 1) the transfer of the *in vitro* cell cultures and technology for growth of *C. ruminantium* from Zimbabwe to Kenya, 2) construction of genomic DNA libraries from two *C. ruminantium* strains (Kiswani from Kenya and Crystal Springs from Zimbabwe), 3) identification of pCS20 and sequencing of the *C. ruminantium* DNA insert, 4) demonstration that the pCS20 DNA insert was specific to *C. ruminantium* and that it could be used as a DNA probe, 5) detection of *C. ruminantium* infected *Amblyomma variegatum* nymph and adult ticks with the DNA probe, 6) detection of *C. ruminantium* infected *Amblyomma hebraeum* ticks with the DNA probe, 7) detection of *C. ruminantium* infected sheep with the DNA probe, 8) identification of an immunodominant 21 kD *C. ruminantium* protein that is a candidate subunit diagnostic antigen and a subunit vaccine. Four research publications based on this work are published or are in press. This research was accomplished by collaboration among investigators from WSU, KARI, the University of Florida/USAID/SADCC Heartwater Research Project based in Zimbabwe, and the University of Utrecht, The Netherlands. These collaborations continue in an effort to accomplish all the long-term research goals defined in the PSTC project.

All the research techniques used during this PSTC project can be done by KARI personnel stationed at the KARI, Veterinary Research Laboratory, Kabete, Kenya. Also, the equipped animal disease biotechnology laboratory remains intact and serves as the nucleus for new projects including one on heartwater. WSU projects continue to do research and training in collaboration with KARI.

(4) Research Objectives:

Heartwater, caused by *C. ruminantium*, is an important rickettsial disease of cattle, sheep, goats and some wild ruminants (8). In sub-Saharan Africa, it is a very significant impediment to ruminant livestock production similar in importance to theileriosis, babesiosis, anaplasmosis and trypanosomiasis (25). Animals infected with highly virulent strains exhibit fever, diarrhea and nervous system involvement resulting in circling and disorientation (22). Mortality from heartwater can be high when susceptible ruminants are introduced into endemic areas (22,25).

C. ruminantium is transmitted biologically by ticks of the genus *Amblyomma* (9,10,18,23). *Amblyomma variegatum* is the most important of 12 *Amblyomma* species shown to transmit *C. ruminantium* because it is the most widely distributed species (35) and is an efficient heartwater vector. Even though ticks (*A. maculatum* and *A. cajennense*) capable of transmitting *C. ruminantium* are present in both North and South America (33,34) and heartwater occurs in the Caribbean (7,33), the disease has not been detected on the American continent.

Heartwater is a major constraint to cattle, sheep and goat production in large areas of Africa. An inexpensive, effective vaccine would reduce losses due to the disease, allow livestock movement into infected areas, lessen the need for tick control and make possible the introduction of exotic breeds to improve production in heartwater enzootic areas.

The research objectives as stated in the application for this project as submitted in August 1986 were:

1. Purify *C. ruminantium* from tick tissues
2. Construct a *C. ruminantium* genomic library
3. Select *E. coli* transformants expressing *C. ruminantium* surface proteins
4. Protect cattle by immunization with lysates of *E. coli* expressing *C. ruminantium* surface proteins

The innovative aspects of this proposal included transferring recombinant DNA technology to the Kenya Agricultural Research Institute's National Veterinary Laboratory, Kabete, Kenya and then the use of recombinant DNA technology to detect *C. ruminantium* organisms in infected ticks and animals. Finally, the technology was used to clone, express and sequence an immunodominant protein from *C. ruminantium* that is being tested for use as a subunit diagnostic antigen to detect antibody in animals with heartwater and for its potential to induce a protective immune response.

A significant part of this project was the training of Dr. Suryakant Waghela, a native of Kenya who completed his PhD with partial support from this project. Upon

completion of his PhD at Washington State University, Dr. Waghela resumed working for the Kenya Agricultural Research Institute at its laboratory at Kabete, Kenya. After one year in Kenya, Dr. Waghela continued his training by taking a post-doctoral position at Texas A&M University.

This PSTC-sponsored heartwater project has had significant help from two other USAID programs. The Small Ruminant Collaborative Research Support Program has a heartwater component as part of its animal health effort in Kenya with Travis McGuire as Principal Investigator. We have been in constant communication and collaboration with the University of Florida and the SADCC Heartwater Research Project. Personnel associated with this PSTC grant have published jointly with researchers working on the Heartwater Research Project.

In addition the DNA probe is being used by researchers in the Department of Parasitology and Tropical Veterinary Medicine, Institute of Infectious Diseases and Immunology, University of Utrecht, Utrecht, The Netherlands who work in West Africa and the Caribbean. The DNA probe also is being used by researchers at the Veterinary Research Institute, Onderstepoort, South Africa.

(5) Methods and Results:

Specific Aim 1: Purify C. ruminantium *from tick tissues*

A virulent Kenya isolate of *C. ruminantium*, designated Kiswani, was used to infect goats for a feeding of *A. variegatum* larvae. This isolate infects both calves and goats. Approximately 10,000 larvae that dropped off the infected goat during the height of the febrile period were collected. The larvae were allowed to molt into nymphs and batches of 2,000 were engorged on an uninfected goat.

For preliminary studies, midguts from 200 nymphs were dissected, pooled, minced and sonicated. The sonicated material was centrifuged at 200xg and the supernatant collected. The 200xg supernatant was centrifuged at 10,000xg and the supernatant and resuspended pellet were used to infect goats. Most of the infectivity was in the 10,000xg pellet. However, the *C. ruminantium* organisms were contaminated with large amount of tick material making the preparation unsuitable as a source of DNA cloning.

In collaboration with the University of Florida/USAID/SADCC Heartwater Research Project based in Zimbabwe, the technology for growing *C. ruminantium* in *in vitro* endothelial cell cultures was transferred to the National Veterinary Laboratory, Kabete, Kenya. *In vitro* cultures of irradiated bovine aortic endothelial cells (5,37) were inoculated with 2 ml of blood from goats acutely infected with Kiswani and Crystal Springs strains. Replication was monitored by the presence of cytoplasmic colonies with uninfected endothelial cell cultures as controls. To confirm the identity

of organisms in cell culture, infected cells were stained by IFA with sera from two goats infected with Kiswani and one with Crystal Springs strain. Homologous antibody titers ranged between 20,000 and 80,000 with the heterologous titers 2- to 4-fold lower. Preinfection sera titered less than 200 and infected goat sera did not react with uninfected endothelial cells.

Organisms released into 25 ml culture medium (26) were partially purified by differential centrifugation. Following centrifugation at 1,200 x g for 10 min at 4°C to remove cellular debris, organisms were pelleted at 10,000 x g for 15 minutes at 4°C and washed 3X with cold 0.01 M phosphate, 0.15 M sodium chloride, pH 7.4 (PBS). DNA was extracted and resuspended in 10 ml of 0.15 M sodium chloride, 0.1 M EDTA, pH 8.0 using described procedures (1,20). To determine if the partially purified organisms were infectious for goats, organisms from 25 ml of medium were resuspended in 1 ml of cold PBS, diluted 1:10, and 2 ml of each strain inoculated intravenously into each of two goats. All four of these goats died and all had *C. ruminantium* colonies in vascular endothelial cells of stained brain smears.

Based on these results, the DNA for *C. ruminantium* infected cultures was used in Specific Aim 2 to construct genomic libraries.

Specific Aim 2: Construct a C. ruminantium *genomic library*

For cloning, DNA from each strain was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and partially digested with Sau3A to a median size of 2 kilobase pairs. Then 300 ng of digested DNA was ligated, using T4 DNA ligase, to 100 ng of BamHI cleaved and dephosphorylated pUC19 (19). *Escherichia coli* JM 109 cells were transformed with ligated plasmids using a calcium chloride method (19) and transformants selected on medium (0.8% tryptone, 0.8% yeast extract, 0.5% sodium chloride, 1.5% agar) containing 100 μg/ml ampicillin, 0.004% 5-bromophenol-4-chloro-3-indol ß-D-galactosidase(X-gal) and 0.002% isopropyl-ß-D-thiogalactopyranoside (IPTG). Plasmid DNA from 74 transformants (34 from Kiswani and 40 from Crystal Springs DNA) was prepared by alkaline lysis (6), digested with EcoRI and electrophoresed on 0.6% agarose gels. Those plasmids with inserts were digested with KpnI and XbaI, Southern blotted (31) and probed with infected and uninfected endothelial cell DNA labeled with biotin-7-dATP (16). Probe binding was detected with a streptavidin-alkaline phosphatase conjugate and a color substrate (BlueGene, GIBCO BRL, Gaithersburg, MD). Two recombinant plasmids, pCS20 from Crystal Springs DNA and pCR9 from Kiswani DNA, hybridized only to *C. ruminantium* infected cell DNA.

Southern blots of the plasmid inserts were probed with biotin-7-dATP-labeled DNA isolated from Kiswani strain-infected and uninfected endothelial cells. Inserts in two recombinant plasmids, pCS20 (Crystal Springs) and pCR9 (Kiswani), hybridized to Kiswani strain-infected cell DNA, but not to uninfected cell DNA. Gel

electrophoresis of restriction endonuclease digested DNA fragments demonstrated that pCS20 and pCR9 contained inserts of approximately 1,500 and 750 base pairs, respectively.

Specific Aim 3: Select E. coli *transformants expressing* C. ruminantium *surface proteins*

Before identifying DNA clones expressing *C. ruminantium* surface proteins, the inserts in pCS20 and pCR9 were investigated as DNA probes. This was done because *C. ruminantium* could spread from endemically infected areas of sub-Saharan Africa and certain Caribbean islands to other countries, including the United States, in which *Amblyomma* ticks exist. Also, demonstrating *C. ruminantium* in ticks and in live animals is difficult and often requires subinoculation into susceptible animals. *C. ruminantium* differs from several other rickettsia and protozoa because it is not detectable by light microscopic examination of stained blood smears even during times when the blood is infectious for other ruminants (8,14). After infection of ruminants, organisms multiply in the cytoplasm of endothelial cells (8,11,14,24) and possibly leukocytes (17,28). Definitive diagnosis of heartwater in animals was made by finding colonies of *C. ruminantium* in endothelial cells of stained brain smears (27) obtained by biopsy or at post mortem. In infected ticks, colonies of the organism were found in thick or thin sections of midgut by light and electron microscopy (4,15,36). Since current procedures were unsuitable for rapid detection of *C. ruminantium* in either live animals or ticks, a method to screen large numbers of samples for *C. ruminantium* was needed. One method for detecting rickettsia and other infectious organisms in host samples, including arthropod vectors, is nucleic acid hybridization. Usually a RNA or DNA probe derived from cloned DNA fragments of an organism is used in laboratory and field studies (2,12,21,30,32).

To detect *C. ruminantium* in tick vectors and animals, DNA probes were made from *C. ruminantium* DNA isolated from endothelial cell culture. Two clones were evaluated; pCS20 from Crystal Springs (Zimbabwe) strain DNA had a 1306 base pair insert and pCR9 from Kiswani (Kenya) strain DNA had a 754 base pair insert. Probe specificity was evaluated by hybridization with DNA isolated from *C. ruminantium*-endothelial cells, uninfected endothelial cells, goat leucocytes, calf thymus, *Anaplasma marginale*, *Trypanosoma brucei*, *Babesia bigemina*, *B. bovis*, *Ehrlichia risticii*, a new bovine *Chlamydia*-like organism, *E. coli*, *C. pneumoniae*, *Campylobacter jejuni*, *C. fetus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Clostridium perfringens*, *Actinobaccilus pyogenes*, and *Listeria monocytogenes*. Both DNA probes detected 1 ng of Crystal Springs DNA, but the pCS20 probe had a 10 fold greater ability to discriminate between *C. ruminantium* DNA and DNA from other organisms. Also, the pCS20 probe did not hybridize to 400 ng (highest amount tested) of the DNA from bovine cells, three protozoa, three rickettsia and 12 bacteria listed above.

To obtain ticks for DNA probe studies, a tick colony was derived from an engorged adult female *A. variegatum* tick collected from a cow in the Narok district of Kenya. To obtain infected nymphs, larvae were applied to skin patches five, six and seven days after inoculating the goat with the Kiswani strain (3,13). Control nymphs were prepared by feeding larvae on uninfected goats. Engorged larvae dropping off goats 7-11 days after attachment were collected and allowed to moult into nymphs for 16-21 days at 28°C and 80% relative humidity. Nymphs were hardened for seven days under the same conditions, engorged on an uninfected goat for 7-12 days, and hardened again for 13 days. Nymphs were then washed with distilled water and 70% ethanol, the midguts removed and stored individually at -70°C. Infected adult ticks were obtained by feeding uninfected nymphs on infected goats. Controls were fed on uninfected goats. After moulting from nymphs, adult ticks were hardened for 20 days, dried for one day at 37°C, washed with water and ethanol, the midguts removed and stored individually at -70°C.

Of 25 goats inoculated with Kiswani stabilate for tick feeding, all became ill and one recovered. Confirmation of the infected status of tick nymphs fed as larvae on infected goats was obtained by transmission of heartwater to 10 goats during nymphal engorgement. Five goats used to engorge nymphs previously fed as larvae on uninfected goats remained asymptomatic. Analogously, the presence of *C. ruminantium* in midguts of adult ticks previously fed as nymphs on infected goats was confirmed by parenteral inoculation of a midgut homogenate into eight goats. All three goats inoculated with midgut homogenate from adults fed as nymphs on uninfected goats remained asymptomatic.

For hybridization, individual tick midguts were solubilized (12) for 18 hours at 37°C in 200 μl of PK buffer (0.1 M Tris-HCl at pH 7.5, containing 0.15 M sodium chloride, 0.012 M EDTA, 1% sodium dodecyl sulfate, and 100 μg/ml proteinase K) and centrifuged for 2 min in a microcentrifuge. DNA was extracted from the supernate (19), precipitated with ethanol, resuspended in 200 μl of TE buffer, denatured with 0.3 M NaOH, and neutralized with 1 M ammonium acetate. Fifty microliters representing 25% of the midgut DNA was applied to nitrocellulose membranes with a vacuum manifold, baked for 2 hours at 80°C, hybridized with pCR9 probe (16) and processed for autoradiography (12). In one experiment, the midgut DNA from adult ticks was resuspended in 100 μl of TE buffer, 10 μl applied to nylon membranes, and hybridized with pSC20 probe. In results from all experiments, *Cowdria ruminantium* DNA was detected in midguts from 99 of 160 *A. variegatum* nymphs infected as larvae and in midguts from 38 of 80 adult ticks infected as nymphs, but not in midguts from control nymphs and adults. The presence of *C. ruminantium* in nymphs and adults was confirmed by transmission of heartwater to goats.

The DNA probe also hybridized with *C. ruminantium* DNA in organs of laboratory-infected *Amblyomma hebraeum* ticks (the major southern African vector

of heartwater). The probe hybridized with *C. ruminantium* DNA in 46 of 49 midguts from male ticks and 26 of 29 from females. Corresponding salivary glands were less heavily infected; infections were more numerous in glands from males. Infection in ticks was confirmed by transmission of the disease to susceptible goats. The probe did not hybridize with DNA from uninfected ticks or with DNA from a spotted fever group rickettsia commonly associated with *A. hebraeum* in Zimbabwe. The *C. ruminantium* specific pCS20 DNA probe can be applied to determine accurately the infection rates in the two major vectors of heartwater and the risk of exposure of ruminants in endemic areas.

Double-strand DNA of plasmid inserts was sequenced by dideoxy nucleotide chain termination (29) using Sequenase (United States Biochemical). Oligonucleotide primers specific to pUC19 were used in the first sequencing reactions. Additional primers were then synthesized based on the sequence obtained. The GenBank accession number for the pCS20 nucleotide sequence derived from *C. ruminatium* (Crystal Springs strain) DNA is X58242 while the GenBank accession number for the pCR9 nucleotide sequence derived from the Kiswani isolate is X58243. The 1306 base pair insert in pCS20 contained 2 long open reading frames of 513 (No. 205-717) and 459 (No. 717-1175) nucleotides. There were no obvious ribosomal binding sites associated with the open reading frames and it is not known whether they are transcribed. The 737 base pair insert in pCR9 (not shown) had one long open reading frame of 243 nucleotides. Even though the open reading frame started with an ATG codon, there was no obvious ribosomal binding site upstream of the ATG. Both insert sequences contained approximately 70% A+T which caused numerous potential prokaryotic promoter consensus sequences to be found.

After isolating *C. ruminantium* organisms and making genomic DNA libraries, efforts turned to identifying surface proteins. In collaboration with Dr. Tony Barbet at the University of Florida and Dr. Frans Jongejan at the Utrecht Faculty of Veterinary Medicine, immunoscreens of genomic DNA libraries were done with dilutions of a serum from a heartwater infected goat that survived infection and was rechallenged.

A recombinant *E. coli* colony, F5.2, was identified from a pUC13 library of *C. ruminantium* genomic DNA by immunoscreening with hyperimmune sheep serum. The F5.2 plasmid insert DNA programmed *in vitro* synthesis of a protein of approximately 21 kD that was immunoprecipitated by immune sheep serum. Sera from rabbits immunized with recombinant F5.2 *E. coli* recognized a 21kD protein in all seven strains of *C. ruminantium* tested. By immunoblotting the 21kD protein was immunodominant and consistently recognized by sera from infected sheep, goats and cattle.

The 2773 bp cloned DNA insert contained two long open reading frames (ORF's) of 627 bp and 831 bp, both potentially encoding proteins containing an N-terminal signal peptide. The 627 bp ORF encoded a 23.6kD polypeptide or a mature

protein of 21.4kD. The 831 bp ORF was incomplete, being interrupted at the 3' end by plasmid vector sequences. Deletion experiments suggested that the 627 bp ORF encoded the immunodominant 21kD *C. ruminantium* protein. A 2125 bp EcoRI fragment of the cloned DNA hybridized to genomic DNA prepared from *C. ruminantium* strains from Zimbabwe, South Africa, Nigeria and the Caribbean island of Guadeloupe and did not hybridize to *Anaplasma marginale, Babesia bigemina, Babesia bovis, Trypanosoma brucei,* bovine, ovine or caprine DNA.

The immunodominant nature of the 21kD protein and its consistent recognition by sera from heartwater infected animals are reasons for further testing of this recombinant protein in subunit vaccines and diagnostic tests.

Specific Aim 4: Protect cattle by immunization with lysates of E. coli *expressing* C. ruminantium *surface proteins*

Current efforts are to test the recombinant 21kD protein in goats. Efforts are also continuing to identify additional surface proteins using both sera from infected goats and new monoclonal antibodies reactive with other surface proteins.

(6) Impact, Relevance and Technology Transfer:

We have solidified the transfer of this technology to Kenya through the provision of equipment and the training of Kenya citizens. The work is still proceeding in the same laboratory, based largely on the efforts of a graduate student funded through the Small Ruminant Collaborative Research Support Program. This project was an important step in the continuing development of veterinary science research in Kenya.

The DNA probe derived from pCS20 is being used in the developing countries (particularly Kenya and Zimbabwe) for epidemiological studies of *C. ruminantium*. In addition, the 21 kD recombinant protein is being evaluated as the basis for a subunit diagnostic test for detecting serum antibody in ruminants with heartwater. If any of the surface proteins identified from the genomic libraries induces a protective immune responses, a subunit vaccine could be developed using the identified recombinant protein.

Several individuals, laboratories, departments, and institutions have participated in the project and benefitted from it. Dr. Suryakant Waghela received part of his PhD training under the auspices of this project. Research in Africa is continuing at KARI, in South Africa, and in Zimbabwe. We have joint publications with the University of Florida's SADCC Heartwater Research Project based in Zimbabwe and with the Department of Parasitology and Tropical Veterinary Medicine, University of Utrecht, The Netherlands. In particular, we have shared technology and *C. ruminantium* isolates that resulted directly in the development of the DNA probe.

The project has contributed equipment to a biotechnology laboratory at the KARI, National Veterinary Laboratory, Kabete, Kenya. This laboratory continues to function under the direction of Dr. F.R. Rurangirwa and continues to do heartwater research. The current research involves 1) an M.S. research project by Leah Ndungu, a KARI employee with a B.V.Sc., on neutralizing antibody to heartwater surface proteins, 2) a project to make additional monoclonal antibodies to heartwater surface proteins to screen existing genomic DNA libraries, and 3) collaboration with S.M. Mahan and L.A. Wassink (University of Florida/USAID/SADCC Heartwater Research Project based in Zimbabwe), and A.F. Barbet (University of Florida), to evaluate the 21 kD protein as a subunit diagnostic antigen and for its ability to induce a protective immune response.

There are several KARI employees who are graduate students on other projects whose research has been greatly enhanced by the equipment, reagents and scientific exchanges provided by the PSTC heartwater project. The effect on their training and on the quality of the research by the biotechnology laboratory on several projects is a longlasting and documentable effect of the project.

(7) Project Activities:

This project paid for regular communication between Washington State University and the research site in Nairobi. Communication included travel by the Principal Investigator (Dr. Travis McGuire) to Kenya and the site coordinator in Nairobi (Dr. Fred Rurangirwa) to the United States.

Part of the training of Dr. Suryakant Waghela, who earned his PhD at Washington State University in 1989 while working on this research, was included in this project.

This project provided the basis for the research currently being conducted by Leah Ndungu, who is earning a master's degree at Washington State University. She is continuing the effort to develop a subunit vaccine and diagnostic test for heartwater.

Project Publications:

Waghela, S.D. Demonstration of a protective immune response against heartwater by immunization with surface antigens of *Cowdria ruminantium*. PhD thesis, Washington State University. Pullman, Washington, 1989.

Waghela, S.D., Rurangirwa, F.R., Mahan, S.M., Yunker, C.E., Crawford, T.B., Barbet, A.F., Burridge, M.J. and McGuire, T.C. A cloned DNA probe identifies *Cowdria ruminantium* in *Amblyomma variegatum* ticks. Journal of Clinical Microbiology. 29:2571-2577, 1991.

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Yunker, C.E., Mahan, S.M., Waghela, S.D., McGuire, T.C., Rurangirwa, F.R., Barbet, A.F. and Wassink, L.A. Detection of *Cowdria ruminantium* in infected bont ticks, *Amblyomma hebraeum*, by means of a DNA probe, pSC20. Epidemiology and Infection. Accepted for publication.

Mahan, S.M., McGuire, T.C., Jongejan, F., Rurangirwa, F.R. and Barbet, A.F. Molecular cloning of a *Cowdria ruminantium* gene expressing an immunodominant 21kD protein. Submitted for review.

(8) Project Productivity:

The project did not accomplish one of its long-term goals--testing of a subunit vaccine. The research did, however, provide a DNA probe to detect *C. ruminantium*in ticks and in sheep, goats and cattle. The project also identified a 21 kD protein that is now being tested as a subunit diagnostic test and as a subunit vaccine. Genomic libraries and monoclonal antibodies were made and are being used to identify other candidate proteins.

(9) Future Work:

As indicated in (6) above, the work is continuing toward development of subunit vaccines and diagnostic tests for heartwater. Our research site in Nairobi is still the scene of research on this topic, based mainly on the work of a graduate student from Kenya earning a master's degree at Washington State University. We are also collaborating with scientists in Zimbabwe, at the University of Florida, and in South Africa on heartwater research.

Dr. Guy Palmer, an Associate Professor in this department, has a PSTC project on heartwater pending with USAID. This project would be based in Kenya and provide substantial support for Leah Ndungu's master's degree research as well as continuing work on subunit vaccines and diagnostic tests after she completes her degree.

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