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Heartwater, one of the most important livestock diseases of Africa, is currently being studied in Zimbabwe, both in the laboratory and field, with a view toward control. The current status of this research project, sponsored by USAID and administered by the University of Florida and the Government of Zimbabwe, is reviewed 2½ years after its inception. Significant progress has been made in the cultivation of the organism *in vitro* (*Cowdria ruminantium*) and isolation of its DNA from cell cultures. Proteins possibly relevant to immunity in heartwater have been identified. Field studies (epidemiology and vector ecology) have resulted in the production of 9 research manuscripts describing off-host behaviour of vector ticks, means for stimulating and attracting them to traps, methods for identifying and quantifying infection in ticks naturally infected with heartwater, the role of intermittently feeding male ticks in disseminating the disease among multiple hosts, and a prolonged carrier status for domestic and wild ruminants.

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WORK SHEET

CURRENT RESEARCH ON HEARTWATER IN ZIMBABWE

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Heartwater (*Cowdria ruminantium* infection), an acute, non-contagious, tickborne disease of ruminants, was first recognised 150 years ago in South Africa (Provost and Bezuidenhout, 1987) and for most of this century has been considered one of the most important livestock diseases in Africa (Mare, 1984). In Zimbabwe it causes significant losses in commercial cattle herds of the lowveld where the main vector, *Amblyomma hebraeum*, is not rigidly controlled by intensive dipping. Further north, in the Zambezi drainages, the principal vector is *Amblyomma variegatum*. The great majority of experimental work on the aetiology, natural history, transmission, pathology, pathogenesis and control of the disease has been done in South Africa. A recent volume resulting from an international workshop held in Kruger Park on all aspects of heartwater research documents historical and recent findings (Bigalke, 1987).

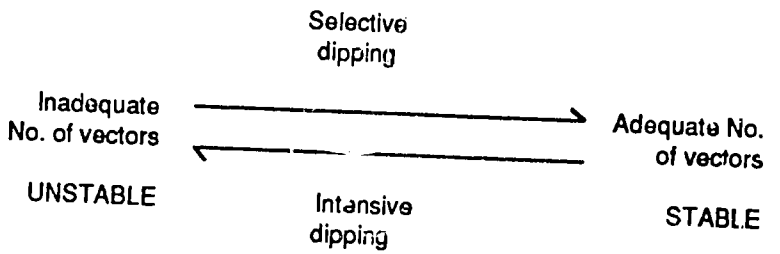
The University of Florida/USAID/Zimbabwe Heartwater Research Project

In 1985 the Government of Zimbabwe requested and received research and training assistance in the area of heartwater, through the United States Agency for International Development (USAID). Motivating this request was a realization of the need to reduce reliance on expensive chemical acaricides and, at the same time, to exploit naturally acquired immunity to tickborne diseases through integrated and selective control of ticks and pathogens. The contractor selected by the Government of Zimbabwe and USAID, the University of Florida's Tropical Animal Health Center, provided three long-term experts in immunology, ticks and tickborne diseases and epidemiology. In addition, it provided specialised laboratory equipment and vehicles as well as short-term training for Zimbabwean technical personnel, in areas relevant to Zimbabwe, at the International Laboratory for Research on Animal Diseases, Nairobi, Kenya. The host organisation, the Veterinary Research Laboratory of the Department of Veterinary Services, Harare, provided physical facilities and furnishings, additional equipment and field and laboratory assistants.

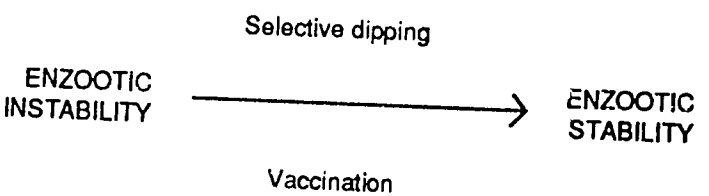
The project commenced in December of 1985, local staff were hired, a cell culture laboratory was set up and field work began shortly thereafter at the Government Quarantine Station at Mbizi.

Enzootic Stability and Research Goals of the Project

Control of ticks and tickborne diseases in Zimbabwe has in the past been based on intensive dipping programmes, which, while highly successful, strain foreign currency reserves, stress livestock and create herd susceptibility to tick-borne diseases (enzootic instability). When intensive dipping is interrupted for any reason, resurgence of ticks and tickborne diseases can be expected (Norval, 1978). Enzootic stability (Callow, 1977) can be achieved by allowing tick populations to increase to a predetermined level and controlling them at that level with acaricides (selective dipping).



However, during the transition, as tick levels increase, losses are bound to occur and herds must be protected by vaccination. This combination of selective dipping and vaccination (integrated control) permits repeated exposure to naturally infected ticks with maintenance of immunity in the herd (enzootic stability). Enzootic stability of heartwater has already been observed in southern Zimbabwe (Norval, 1981).



Therefore, with a view toward improved control of heartwater through regulated exposure to ticks, two major research goals of the project, which can be categorised as immunological and epidemiological, were formulated:

Immunology: To develop a safe, efficacious and easily administered vaccine against heartwater. Immunization at present is achieved only by the cumbersome and risky technique of infection and treatment.

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Epidemiology: To gain an understanding of the natural history of the disease, including the ecology of the vector. This is an essential objective for two reasons: when a vaccine is developed it must be used in a rational manner and effective enzootic stability cannot readily be achieved without this knowledge.

Progress to Date

Immunology: When the project was conceived, the causal agent of heartwater had not been grown successfully in the laboratory and large amounts of antigen were unavailable. However, by late 1985, workers at the Veterinary Research Institute, Onderstepoort, South Africa, had devised a means of growing the organism in cultured cells (Bezuidenhout *et al.*, 1985). We have duplicated their results using a South African strain of heartwater organism and are now attempting to repeat this with a Zimbabwean strain, because the former strain may not be completely cross-immunogenic with other strains of heartwater. To facilitate this we have established serially propagating lines of bovine and ovine endothelial cells derived from vascular endothelium from aorta and pulmonary artery. These lines were tested and some were found to support excellent growth of the organism.

Figure 1: A technologist (Ms Farirayi Chikomo) of the University of Florida/ USAID/Zimbabwe Heartwater Research Project, prepares growth media for cell cultures infected with *Cowdria ruminantium*, the causal agent of heartwater. Ms Chikomo and co-workers have developed cell lines from ruminant blood vessels which are susceptible *in vitro* to infection with the organism.

Another method we are pursuing to obtain large quantities of *Cowdria* antigen is to harvest the organism from infected ticks. Ticks are fed as nymphs on heartwater-infected sheep and, after the ticks moult to the adult stage, large numbers of *Cowdria* colonies can be seen in their gut cells. We are attempting to harvest these colonies free from gut constituents.

Meanwhile, we have isolated several proteins from two different heartwater-infected source materials, cell cultures and infected sheep blood, which proteins do not appear in uninfected control materials. One protein in particular was identified in both sources and could be a key to the induction of immunity to heartwater. Once a protein has been isolated, and is proved to induce immunity, it will be developed as a vaccine by means of recombinant DNA technology to be carried out at the University of Florida (UF). Consultants visiting the Project from UF have isolated *Cowdria* DNA from our cell culture fluids, quantified it and shown that it is relatively pure, i.e., free from excessive contamination with host-cell DNA. We now know how

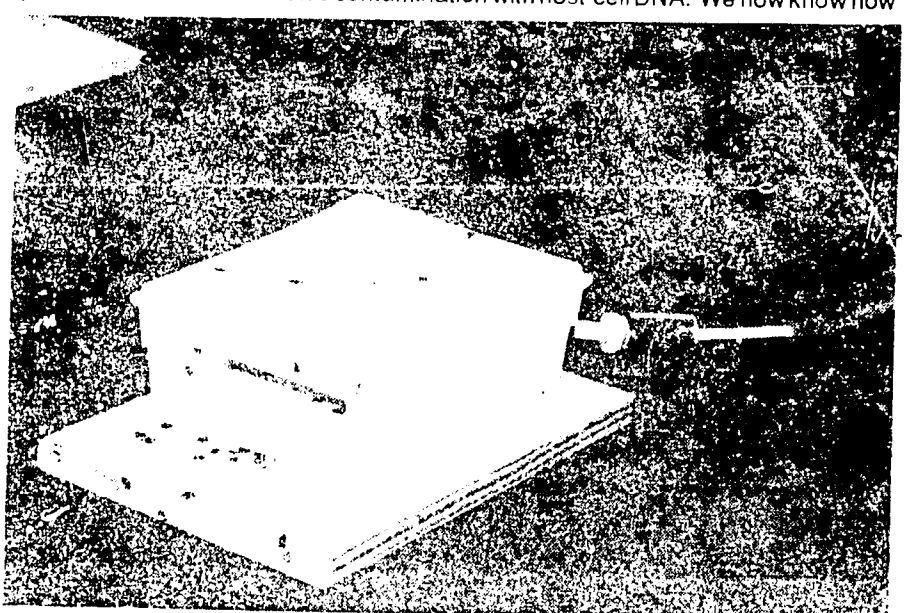

Figure 2: The bont tick, *Amblyomma hebraeum*, main vector of heartwater in southern Africa, is attracted to a trap devised by consultants and staff of the University of Florida/USAID/Zimbabwe Heartwater Research Project. Unlike many related species of ticks, which passively rest on vegetation while awaiting contact with a suitable host, the bont tick actively seeks out its host when one is sensed nearby. The trap emits carbon dioxide gas which has passed over a tube containing fed male bont ticks. The latter secrete volatile pheromones which attract wild ticks to the trap.

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much infected culture material will be needed to prepare a DNA library and are stockpiling toward this end. The production of this library is anticipated before the end of this year and will provide the genomic sequences from which the immunogenic protein or proteins can be selected. In addition, this library will provide material for the development of a sensitive diagnostic probe for heartwater. At present the only means of diagnosing the disease is by examination of brain material, usually after death.

The immune response of the ruminant host to heartwater is also under study. All efforts to neutralize infectivity of infectious *Cowdria* stocks by exposure to immune serum have failed (de Gee, Andrew and Semu, pers. comm.), indicating that other mechanisms, such as cell-mediated immunity or interferon, may be more important than the humoral response. These mechanisms are currently under study.

Epidemiology: A number of factors have enabled us to make substantial progress in this area:

a) the existence at the Government of Zimbabwe's Veterinary Research Laboratory of expertise and facilities for the production of large numbers of *Amblyomma* ticks
b) the ability to commence field activities, including the isolation of Zimbabwean strains of heartwater and the study of seasonal population dynamics of vector ticks, without waiting for equipment and supplies to arrive
c) the use of a 3000 hectare Government of Zimbabwe veterinary field station at Mbizi, in the lowveld, where we may perform experiments and collect data in a natural focus of infection
d) the avid interest and help of ranchers whose herds are affected by heartwater, who have provided us with much information and even the use of their animals.

Early in 1986, using susceptible Merino sheep transported from the highveld to Mbizi as sentinel animals, we obtained our first isolate of heartwater from ticks which they attracted. Frozen stocks were prepared from this isolate and additional isolates were subsequently recovered. We now have 10 stocks from various Zimbabwean localities where heartwater is enzootic.

Surprisingly, two isolations were made from *A. hebraeum* ticks collected in the highveld of northern Zimbabwe, in Zwimba and Mhondoro communal lands. *A. hebraeum* is characteristically a southern lowveld species and is usually supplanted by *A. variegatum* in more northerly areas. However, heartwater-bearing *A. hebraeum* now appears to be well established in the northern areas. The search for geographically different isolates of the heartwater organism was in part prompted by our need to determine if antigenically different strains occur in the field. If strains were found that failed to confer immunity against others, it would affect our

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choice of candidate vaccine strain. Preliminary tests carried out to date indicate that most Zimbabwean strains are cross-protective.

Prior to the commencement of this project little was known about the behaviour of the vector tick in nature when not attached to a host. Nor were any figures available, or practical means to obtain these figures, of the degree to which the ticks are naturally infected with heartwater. We have produced nine scientific research manuscripts describing off-host behaviour of the ticks, means for stimulating and attracting them to traps, methods for identifying infection in and quantifying infection rates of ticks with heartwater, the role of intermittently feeding male ticks in disseminating the disease among a number of hosts and the ability of domestic and wild ruminants to transmit heartwater to uninfected ticks for long periods after recovery. By Project's end (May 1989) we will have documented the seasonal dynamics of natural populations of the tick and gained a firm idea of the economic impact of heartwater and of the relative costs to control it through dipping, vaccination or natural exposure and treatment. We also expect to be able to provide the farmer with a simple method of maintaining the immune status of the herd to heartwater by natural means in enzootic areas, as distinct from marginal areas.

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