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FUTURE PROSPECTS AND GOAL SETTING REGARDING RESEARCH ON HEARTWATER

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ABSTRACT

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This article highlights the most important research goals identified during the workshop on "Heartwater: Past, Present and Future," which was held from 8–11 September 1986 in the Republic of South Africa. An attempt has also been made to identify the most modern technology which is available for this purpose. All 60 papers presented at the workshop, together with other relevant information, are published in this number of the *Onderstepoort Journal of Veterinary Research*.

With regard to the causative organism it is crucial that research should be conducted on pure isolates. Moreover, existing culture methods should be improved in order to obtain better yields of organisms.

Research on the hosts and vectors of *Cowdria ruminantium* should aim to elucidate the ways in which vector ticks become infected in nature. For this purpose especially, it will be necessary to develop rapid tests (e.g. DNA probes) to detect the organism in living animals and ticks.

The nature of immunity and young animal resistance are still obscure and call for basic research. Mice and murino-tropic isolates of *C. ruminantium* should prove useful in this regard. Since cross-reactions with *Ehrlichia* occur, it is essential to give particular attention to the sero-epidemiology of *Ehrlichia* in conjunction with similar studies on *Cowdria*.

The development of a tissue culture vaccine offers the greatest chance of immediate success and should be actively pursued. Studies on a recombinant vaccine should, however, be initiated because of the potential long term advantages.

INTRODUCTION

One of the aims of this workshop has been to determine priorities and to set ourselves goals for future research on heartwater. This meeting has given me a unique opportunity to harness the wealth of expertise, knowledge and information available in the attempt to arrive at informed and wise decisions.

As a relatively large number of research goals have been identified it stands to reason that it will be impossible to give attention to all of them immediately, or even perhaps in the near future. It is perhaps best to regard this paper as a document to which reference can be made whenever information on research priorities on heartwater is required. It cannot, however, stand on its own and must be read in conjunction with the other papers printed in this number of the *Onderstepoort Journal of Veterinary Research*, which serves as the proceedings of the workshop.

The headings used in the scienific programme and in the index of the proceedings serve as a rough framework for this presentation. The goals identified under each heading are listed, as far as possible, in order of priority.

I. THE CAUSATIVE ORGANISM

1. We feel that it is necessary to compare the morphology of various isolates in culture. It is, for example, important to determine whether the empty forms and other morphological features, such as the slime layer observed in the Ball 3 isolate, also occur in other isolates.

Since antibiotics possibly have an influence on the morphological features, cultures with and without antibiotics should be compared.

2. As far as multiplication of the organisms is concerned, very few dividing forms have so far been seen and binary fission was regarded as the most important mechanism involved. For further clarification it is important that early cultures should be studied, or early infections in animals, and perhaps a time study should be done in order to find more dividing forms. Another question, for instance, is the significance of blebs. Is a bleb a form of budding or perhaps an antigenic mask? In morphological studies it may be necessary to concentrate on the dense forms, which are infectious in similar organisms and therefore probably most important.

3. It is imperative to determine whether there are soluble antigens in the culture media. They may be more immunogenic than the organisms themselves and therefore of potential practical significance. Rocket immunoelectrophoresis, other immunodiffusion tests or the ELISA should be used to demonstrate their presence. Purified isolates are absolutely essential for this work (see below).

Stability and preservation

1. Little attention has been given to studies on stabilizers. They are, however, extremely important since they affect almost every aspect of the studies on heartwater, such as purification of the organism, lyophilization and vaccine production. Substances such as gluamine, or ATP, may work wonders in this regard. It is felt that a breakthrough with stabilizers may well open a Pandora's box of exploitable research and development possibilities.

Cultivation

1. It is very important to standardize the cell culture methods used, for instance as far as pH, the effect of antibiotics and essential nutrients are concerned. Supplementation with a cocktail of aminoacids may be a useful boost to growth of the organism.

2. The unique E5 cell line should be characterized, but the cloning thereof is a prerequisite to meaningful characterization. Improved yields with the Ball 3 or other isolates, in the absence of prior irradiation of the cells, is the ultimate aim of this exercise.

3. Although no indication of attenuation in cell culture has been observed hitherto, this possibility should be investigated more actively using modern techniques such as the application of mutagens.

4. Since sheep and goats are more susceptible to heartwater than cattle, the development of an ovine or caprine endothelial cell line may be a profitable line of investigation.

Purification, genetics and metabolism

1. A method should be sought to purify the important dense forms on which metabolic and genetical studies could be done. This Workshop has shown that there is room for improvement of the methods of purification

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used. Filters with specific pore sizes might be useful in this regard.

2. The isolation of plasmids from the dense forms, using the studies done on *Coxiella* as a guide, could be very useful in the longer term. Plasmids are packages of DNA which would be invaluable for exploitation with recombinant technology.

3. Once purified dense forms can be regularly obtained, it should be feasible to start with genetic engineering studies such as the development of DNA probes for diagnostic and other purposes. Furthermore, the antigens of various isolates could be compared using immunoblot techniques.

4. An ideal development would be the establishment of a *Cowdria* metabolic unit somewhere.

II. The Hosts

Susceptibility and host range

1. We have already looked at the susceptibility of potential ruminant, bird, and rodent hosts to heartwater, but we still cannot answer satisfactorily the crucial question of how ticks get infected in nature, for instance in the Kruger National Park where the primeval epidemiological pattern of heartwater probably still exists. The same, of course, applies to stock on farms in various ecological regions in South Africa and elsewhere where the vector ticks occur. Nagging doubts about whether the individual wild animals which have been tested for their susceptibility to heartwater were in fact immune to start with make it necessary to repeat the studies with known susceptible animals. A DNA probe for heartwater could be particularly useful to identify susceptible animals.

2. The genetic resistance of domesticated ruminants to heartwater seems to be a promising field of investigation to pursue—I speak specifically about their resistance to heartwater rather than to ticks—particularly as far as managing the disease in Africa is concerned.

3. There was some discussion in the think-tanks about the terminology that should be used. Most participants thought that the term "isolate" rather than "strain" or "stock" should be used, the word "strain" perhaps being the most incorrect one of the three.

Pathology; pathogenesis

1. A specific search for antigen-antibody complexes using immuno-gold techniques in electron microscopic studies might be rewarding to explain some of the lesions such as vascular changes.

2. The possible role played by vaso-active substances in increased permeability of blood vessels should also be comprehensively studied. The main pathological features clearly point in that direction.

3. It is essential to develop a method to quantitate the infectivity of an inoculum. Only then will it be possible to determine if concepts such as ID50, sublethal dose and minimum infective dose apply.

Cell cultures or mice (only with murino-tropic strains) could possibly be used for this purpose.

Diagnostic methods

1. It would be useful if a rapid test could be developed to demonstrate the presence of the organism in the living animal. A DNA probe, which is rapid but not as sensitive as many people think, is a definite possibility. The ELISA test is already promising but could be developed further. It is just about as sensitive as a DNA probe but does not give the answer as quickly. We could also look for soluble antigens in sera, using a technique such as counter electrophoresis, for instance. The dot blot method, basically an ELISA test, which is being used successfully for the detection of African swine fever virus in the haemolymph of ticks, might also work in this case.

2. We also need an easier technique to obtain brain specimens from dead animals for diagnostic purposes without having to remove the brain first. The collection of brain material for diagnostic purposes through the foramen occipitale, as already published, or any other similar technique should be propagated amongst farmers and herdsmen. The aim would be to obtain large numbers of brain specimens for survey purposes. An accurate diagnosis is a prerequisite for any survey on the economic importance of the disease, which is long overdue.

Serological techniques; serology

1. The discovery of serological cross-reactions between *Cowdria* and *Ehrlichia* is one of the most important issues to emerge at this conference. This casts considerable doubt on the purity of the *Cowdria* isolates used as antigens in serological tests. Although in my opinion there is a definite possibility that many of our isolates are in fact not contaminated with other rickettsial organisms, they will have to undergo a purification process in order to do credible serology. The limited dilution or plaqueing technique could be used for this purpose. Both have the production of clones as objectives. Comparative serological tests with known *Ehrlichia* and available *Cowdria* isolates might provide useful information on the nature, extent and cause of the cross-reactions.

Only when known pure isolates are available can genetic engineering work such as the development of genomic libraries, DNA probes and sub-unit vaccines receive the necessary attention, not to mention other studies in which the organism will be used. Several isolates, such as Welgevonden and Ball 3, should be purified, not only one.

2. There are indications from studies on other organisms that high antibody titres could be associated with specificity and that a cut-off point could be determined for non-specific reactions using existing non-purified antigens. Comparative studies with known *Ehrlichia* antisera and/or antigen seem, however, essential for determining such cut-off points.

The ELISA test may be a better proposition than the IFAT. The 2 tests should nevertheless be compared on a set of sera. The antigens currently used for the ELISA could also be improved.

3. Monoclonal antibody studies can be initiated once known pure strains are available. In the meantime a start could be made by looking for common protective antigens in purified material using polyclonal sera. The demonstration of common protective antigens would hopefully lead to the eventual development of a sub-unit vaccine protecting against all possible antigenically different isolates.

Immunity

1. The occurrence and nature of so-called non-specific resistance in young animals needs further elucidation in cattle, sheep and goats. If this phenomenon is also present in mice it could speed up research in this regard considerably. Fundamental studies on the nature of this resistance are required. There may for instance be a relationship between the pathogenesis of heartwater and calf resistance. One could start with more fundamental studies on the pathogenesis of heartwater in cattle and work backwards towards young calf resistance. Acute phase reactions, of which conglutinin is one, may be involved in this process. They should be looked at as a group though. One might even manipulate them and thus find a practical application for these studies.

It is also important to determine to what extent non-specific resistance occurs in the different species and how long it lasts.

In respect of young animal resistance it is felt that we should have another good hard look at passive immunity. It may play a role after all. The same applies to intra-uterine infection, particularly if one considers the fact that non-specific resistance apparently exists for an appreciable period.

2. It is very important that fundamental studies on the nature of immunity be vigorously pursued. The mouse model should be useful but a more typical isolate such as the Welgevonden isolate should be employed. Cross-immunity studies may cast light on some of the epidemiological features such as deaths despite vaccination with, for example, the Ball 3 strain. They will also help with studies on the nature of the immunity. Modern techniques for the detection of cellular immunity, such as lymphocyte transformation, should also be brought into these studies.

III. The Vectors

Distribution, biology and ecology

1. There is a dearth of tick taxonomists and it is therefore imperative that more should be trained.

2. We know that animals whose resistance is down because of stress harbour more ticks than non-stressed ones. It is therefore important that ecological studies on ticks should include counts of free-living stages. Ecological studies on the bont ticks should continue despite the fact that manipulation of the ecology to control ticks is not a very practical proposition.

3. Another point made is that the African buffalo may well be the original host of *Amblyomma*. Is it not perhaps also the original reservoir of *Cowdria*?

Biochemical studies

1. It is felt that if a vaccine against the *Amblyomma* tick is a high priority, biochemical studies should be aimed in that direction. If it is not such a high priority, and there is reason to believe that this is the case, then it might well be more profitable to concentrate the biochemical studies on *Cowdria*, as indicated above.

Natural transmission

1. One of the highest research priorities is to find a method to determine the infection-rate in ticks. If a DNA probe could be developed for this purpose it would herald considerable progress in our understanding of the epidemiology, and hence management, of the disease. The molecular biological techniques developed for *Anaplasma* by Barbet & McGuire could prove very useful as a guide in this respect.

2. It is also very necessary to determine how ticks become infected in nature because of the implications for the epidemiology of the disease. All indications are that reacting cattle, goats and sheep cannot be the only source of infection for ticks. The information on possible wild animal reservoirs is so scanty that this pivotal issue deserves considerable further research inputs.

The organisms in neutrophils appear to be the source of infection for ticks and this information, as well as the culture techniques described for neutrophils, should be kept in mind when the infectivity of the blood of animals is studied. Xenodiagnostic studies with larval and nymphal ticks should always be used in combination with subinoculation of blood to determine if reacting animals, recovered animals, re-infected immune animals and wild animals could serve as a source of infection for ticks.

It is felt that transovarian transmission of *C. ruminantium* in *Amblyomma* spp. should be re-investigated. If it occurs more commonly than we believe, it opens up a new dimension in the epidemiology of the disease.

3. It might be well worth while to characterize the stages in the salivary glands of ticks in terms of their antigenic structure. It might eventually be possible to produce a genetically engineered vaccine which blocks the infection, if the immunogens can be identified. Investigations could be conducted from both the molecular biology and immunology angles, which will hopefully eventually meet.

IV. Epidemiology

Several research priorities which refer to the epidemiology of heartwater have already been mentioned. There is, however, one additional very important point:

In view of the fact that some isolates of *C. ruminantium* are probably not pure and that there are cross-reactions with *Ehrlichia*, it is felt that more attention should be directed towards *Ehrlichia* sero-epidemiology. As many *Ehrlichia* antigens as possible should be obtained from overseas and local sources. These should be tested for cross-reactions with sera which are already available in serum banks and the results compared to those already obtained with *Cowdria* antigens. Such well-controlled studies should enable us to determine to what extent the serological work already done is valid.

V. Control

Treatment

1. The pharmaceutical and chemical industries should continue their good efforts to look for a wonder drug. Apart from mice, cell cultures can now be used as a screening system.

2. It is also important to continue with studies on supportive treatment. It is clear that these should be based on studies on the pathogenesis of the disease.

Vaccination

1. There is much room for improvement of the available live vaccines. One of the biggest problems encountered when using the tick vaccine is shock in young animals, especially goats and lambs. This vaccine is much cheaper to manufacture than the blood vaccine and has considerable potential for use in Africa if the shock problem could be eliminated. For the moment there is a great potential for the development of a cell culture vaccine, which seems to be a higher priority. But we may well have to come back to the tick vaccine.

2. The vexed question of the route of administration of the live vaccine should also be addressed. The subcutaneous route, for example, might work better with a cell culture vaccine, which should have a higher infectivity. It is also necessary to investigate the histological changes at the inoculation site of a vaccine containing brain-tissue, which seems to be highly infective when injected subcutaneously. Other commercially available substances such as hyaluronidase, dimethylsulphoxide and bradykinin should be investigated for the same reason.

3. It has already been mentioned that studies on the possible development of a recombinant vaccine are necessary. The fact that dead antigens do not induce immunity might make the development of an effective recombinant vaccine a hard nut to crack. Perhaps it will work if the combination of the right antigen or antigens, i.e. immunogens, can be identified.

Alternative methods of control

If a method could be found to increase the percentage of infected ticks it may not be necessary to vaccinate calves, which possess young animal resistance. Perhaps the infection rate in a tick population can be increased by intensively vaccinating calves and not dipping them for 6

months. In this way highly infected paddocks could be created through which the herd could be circulated at the right time. A DNA-probe to detect infected ticks would probably be a pre-requisite for such studies.

ACKNOWLEDGEMENTS

In conclusion I wish to express my sincere appreciation to everybody who attended this Workshop. It was quite clear that the speakers had come well-prepared for their tasks. Particularly gratifying, however, were the lively and in-depth discussions in which everybody participated.

Questions and comments were clearly aimed at the clarification of uncertainties and the identification of unanswered problems and priority research fields. The spirit of the entire meeting was indeed constructive. I am particularly indebted to those people who worked many hours at night and between sessions to assist me to identify the most important research goals outlined above.

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