

The significance of recent highlights in heartwater research*

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Beaucoup des objectifs de la recherche sur la cowdriose, identifiés jusqu'à présent, ont été atteints pendant la décennie passée. Certains acquis, tels que la mise au point de sondes ADN spécifiques pour *Cowdria* et l'atténuation de l'organisme, sont encore au stade expérimental, mais d'autres, comme la culture *in vitro*, sont déjà des procédures bien établies dans nombre de laboratoires. Des techniques sérologiques sont plus généralement utilisées depuis que d'autres méthodes et d'autres sources d'antigènes sont disponibles. Néanmoins, des réactions croisées avec *Ehrlichia* continuent à compliquer l'interprétation des données épidémiologiques. Les études biochimiques pour identifier, isoler et caractériser les protéines antigéniques de *Cowdria* ont mis en évidence des protéines immunodominantes bien définies, qui pourraient convenir à des tests sérologiques. Malgré ces progrès importants de la recherche, les méthodes pratiques pour le diagnostic de la maladie et la lutte contre elle n'ont pratiquement pas changé. La lutte est toujours basée soit sur une lutte intensive contre les tiques, soit dans certains cas sur la création et le maintien de la stabilité endémique. La méthode d'infection et de traitement utilisant du sang infecté ou le traitement prophylactique par des tétracyclines continuent à constituer l'essentiel de la lutte contre la maladie en Afrique du Sud. La cowdriose reste une menace pour le continent américain tant que *Amblyomma variegatum* sera présent dans la région des Caraïbes, et l'éradication de la tique semble indiquée.

Mots clés : Cowdriose - *Cowdria* - Tique - *Amblyomma variegatum* - Sonde à ADN - Culture *in vitro* - Technique immunologique - Antigène - *Ehrlichia* - Protéine - Tétracycline - Afrique - Afrique du Sud - Caraïbes.

INTRODUCTION

I should like to start out by thanking the organizers of the conference for the invitation to speak at this symposium. I regard it as a great honour and appreciate the opportunity to participate.

The title of my talk namely *The significance of recent highlights in heartwater research*, is hopefully broad and vague enough to allow me to speak on what ever I think is recent or what I regard as highlights and to rely on my own interpretation of what the significance of these findings may be. I fully realise that it may differ from the interpretation of some of you present here today and I hope this will come out in the discussions to be held later during the week.

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* Texte introductif à la session Cowdriose.

During the 1986 workshop on heartwater, held in the Kruger National Park in South Africa a great deal of time was spent discussing the most urgent needs for future research (6). This was done mainly to structure future research on heartwater and to assist scientists in identifying gaps in our knowledge should they be interested in studying the disease. The majority of these goals were also recognized by CAMUS and BARRÉ in their very valuable review of 1982 (13).

This paper briefly looks at those goals again and judges the progress or lack thereof that took place since the proceedings of the previous workshop were published in 1987. It should therefore not be regarded as a complete review but merely a discussion on the progress made since the last workshop with regard to the goals set (table I). The most important findings will be highlighted and their significance briefly discussed.

DISCUSSION

The organism

Due to the fact that a great deal of studies on heartwater in the future will still depend on a constant supply of organisms from cell cultures, it is important that special effort should be made to address the remaining problems in this regard such as, quantification of infection, stabilizing and preservation of infection and even possible cloning of stocks. Information on the growth requirements of endothelial cells and *Cowdria* organisms in culture is also needed urgently. The establishment of a cell line that will support the growth of the Küm stock should also speed up comparative studies between stocks. It will also be interesting to know whether the *Ehrlichia*-like organism which has apparently transformed to a *Cowdria*, will be able to grow in culture (16, 17).

DNA studies on *Cowdria* are beginning to pay off as is evident from various reports on the subject. Genomic libraries were constructed (1) and DNA probes developed (36, 58, 62) and applied to demonstrate *Cowdria* antigen in various tick and animal tissues. The significance of this is that although a DNA recombinant vaccine for cowdriosis is not imminent, work toward this goal has begun. One should guard against unrealistic expectations regarding

TABLE 1 A summary of the progress made on heartwater research since the 1986 workshop held in South Africa.

Goals set at workshop	Achievements	References
Re : THE ORGANISM Morphology of various stocks in culture	No comparative morphological studies were performed. However, no obvious differences were noticed.	(35)
Developmental cycle	<i>Cowdria</i> appears to have a <i>Chlamydia</i> -like developmental cycle.	(35)
Identification of antigenic proteins	Immunodominant antigenic proteins were identified. Western blot technique failed to demonstrate any soluble antigens in culture medium.	(30, 48) (56)
Improved methods to stabilize and preserve the organism	Successful lyophilization of infected : — mouse tissue (Kümm & Welgevonden) — sheep blood (Welgevonden & Ball 3) — cell cultures (Welgevonden)	(23) (23) (7)
Standardization of culture techniques	Much more standardized now but still more of an art than a science.	(5, 10, 11, 61)
Cloning and characterization of suitable cell lines	Nothing reported.	
Cloning and characterization of <i>Cowdria</i> isolates	Nothing reported. A difficult but great challenge.	
Attenuation of organisms in cell cultures	Spontaneous attenuation of the Senegal stock at Utrecht and confirmation at Guadeloupe. Other stocks did not attenuate.	(29, 38)
Establishment of more suitable cell lines, especially of ovine and caprine origin	A number of suitable endothelial cell lines of bovine and ovine origin have been established, and more than 15 different <i>Cowdria</i> stocks were cultivated in these cells.	(5, 8)
Purification of dense forms	Nothing specifically reported on the methods of purification. Infectivity of reticulate and elementary (electron dense) bodies of the Welgevonden stock were tested in mice.	(32)
Isolation of plasmids from dense forms	Nothing reported.	
Development of DNA probes	A number of <i>Cowdria</i> specific DNA probes were developed. Some were more sensitive than others. The one, pCS20, was used to demonstrate <i>Cowdria</i> antigen in ticks and animal tissues.	(36, 58, 62)
Establishment of a <i>Cowdria</i> metabolic unit	Nothing reported.	
THE HOST Reservoir status of wild and domestic animals	A real breakthrough by workers who demonstrated intermittent infectivity of blood for long periods after infection.	(2, 12)
Genetic resistance	More work was done on genetic resistance in Creole Guadeloupean goats. An attempt to establish an inbred line of mice resistant to <i>Cowdria</i> failed.	(40) (21)
Pathology : Pathogenesis Identification of antigen antibody complexes	Cr32 specific epitopes were demonstrated on the surface of elementary bodies by immuno-gold labelling with a specific monoclonal antibody. Immunohistochemical staining of <i>Cowdria</i> in tissue sections were achieved	(32) (9)

TABLE I A summary of the progress made on heartwater research since the 1986 workshop held in South Africa (continued).

Goals set at workshop	Achievements	References
Role of vaso-active substances in increased permeability	Nothing reported.	
Quantification of infectivity	A mouse lethal dose assay was developed to test the infectivity of the Kwanyanga stocks of <i>C. ruminantium</i> in goats and ticks.	(25)
Diagnostic methods Rapid test for diagnosis of heartwater in live animals	DNA probe detected <i>Cowdria</i> in infected sheep blood.	(36)
Easier technique to obtain brain tissue from dead animals	Collection of brain material from dead animals using a hammer, 15 cm nail and a syringe with needle was described.	(37)
Serological techniques Differentiation between <i>Cowdria</i> and <i>Ehrlichia</i>	Cross reactions between <i>Cowdria</i> and most <i>Ehrlichia</i> spp. are present no matter what source of antigen is used, even in the competitive ELISA with a monoclonal antibody against the Cr32 protein. Complete absence of cross immunity between <i>E. phagocytophila</i> and <i>Cowdria</i> .	(19) (34)
Comparison of serological tests	Tests were compared and good correlations found in sensitivity and specificity, however there are : — differences in the ease of antigen production and use, — cross reactions with something other than <i>Cowdria</i> .	(39) (19)
Isolation of monoclonal antibodies	A few monoclonal antibodies were produced.	(32)
Immunity Elucidation of non specific immunity in young animals	Confirmation that there is no difference in the resistance of calves born to cows fully susceptible to heartwater from that of calves bred in a heartwater endemic area.	(20)
Intra uterine infection	1 suspected case reported.	(55)
Passive immunity	All effects to neutralize infectivity of <i>Cowdria</i> by exposure to immune sera failed.	(59)
Nature of immunity	Conclusive evidence that immunity to heartwater in mice is largely cell-mediated.	(18)
Antigenic diversity and cross immunity between stocks	Marked antigenic diversity exists between stocks. Antigenicity of stock sometimes varied in different host species. No single stock has yet been identified that will protect against all other stocks. Not even a combination of stocks seems to enhance immunity.	(22, 24, 31, 33, 50)
THE VECTORS Distribution, biology and ecology Training of tick taxonomists	Not aware of any definite commitments made in this regard.	
Ecological studies on vectors of cowdriosis	In Zimbabwe the results of studies on the role of CO ₂ and attraction/aggregation/attachment pheromone in host finding were utilized to improve sampling techniques and to develop a novel method for tick control. The ecology and biology of <i>A. variegatum</i> in the Caribbean especially with regards to the factors responsible for the spread of the tick were studied in depth. Wild hosts of <i>A. hebraeum</i> and <i>A. marmoratum</i> were identified in South Africa.	(41, 43, 44, 63) (3) (47)
Development of a tick vaccine against <i>Amblyomma</i>	Limited success with midgut and nymphal tissues.	(15, 27, 52)

TABLE 1 A summary of the progress made on heartwater research since the 1986 workshop held in South Africa (continued).

Goals set at workshop	Achievements	References
Development of methods to determine the infection rate in ticks	Three additional methods for determining the infection rate have been published.	(25, 42, 62)
Sources of infection for ticks/reservoir status of hosts/transovarial transmission	See also above. Additional findings, demonstrating the constant absence of organisms in ovarian tissues, indicates that transovarial transmission is highly unlikely to occur or play a role in the epidemiology of the disease.	(4, 42, 57, 62)
Characterization of <i>Cowdria</i> stages in the salivary gland	<i>Cowdria</i> is seldom seen in the salivary glands of infected ticks and therefore very difficult to obtain for further studies.	(26)
CONTROL Treatment Wonder drug for treatment	Rifampicin is effective against <i>Cowdria</i> infection but has not been registered for treatment.	(45)
Screening of compounds on infected cell cultures	Nothing reported.	
Improved supportive treatment	Nothing reported.	
Vaccination Improved live vaccine	Subcutaneous implant of doxycycline to control vaccination reactions (Doximplant*).	(46)
Easier route of administration	Nothing reported.	
Development of a DNA recombinant vaccine	Nothing reported.	
Development of a method to increase natural immunization through increasing the infection rate of ticks	Nothing reported. However, use of the aggregation/attachment pheromone to increase transmission rates to livestock proposed.	(42)

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the utilization of these probes as diagnostic tools, especially in certain countries in Africa. Under these conditions, farmers and stockmen get to know the disease so well that they seldom bother to have the diagnosis confirmed. The chances that they will pay for such a diagnosis is remote. Also the absence of a proper infrastructure makes the collection and testing of material, no matter what the origin may be, very difficult.

On the other hand, it is realised that DNA probes will prove invaluable in elucidating heretofore unaccessible epidemiological aspects of the disease. Such information especially the exact infection rate in ticks and the factors that influence it is important for the construction of epidemiological models which will ensure effective control strategies. The application of biotechnology for the diagnosis and control of ticks and tick-borne diseases was recently reviewed (51).

The host

In the case of host related studies there are three aspects of which recent findings have great significance. Firstly, the demonstration of a prolonged intermittent carrier state in domestic and some wild animals provides the evidence that was needed to explain how enzootic stability is created and maintained in heartwater endemic areas. Secondly, the persistent cross reactions with *Ehrlichia* in all serological tests, even in the case of the competitive ELISA which makes use of a monoclonal antibody directed specifically against the Cr32 protein (19). These findings, unfortunately, cast a shadow over all serological results of tests or epidemiological studies performed in areas where the presence of *Ehrlichia* can not be excluded. Serological surveys will therefore remain of little value until such time that the two organisms can be diffe-

rentiated accurately. Thirdly, but perhaps most importantly, are the findings that there exist marked antigenic differences between the different stocks of *Cowdria*. This stands in sharp contrast with earlier findings where no such differences, with the exception of the murinotropic stocks, could be demonstrated. There is presently not a single stock available that will give full protection against all other stocks. It appears that not even a combination of stocks will enhance the immunity (22). A further important finding is that there now exists conclusive evidence that in mice at least, immunity to heartwater is largely cell-mediated (18). This is probably also true for other animal species and will have a profound effect on the type of molecular engineered vaccine that should be developed for effective control of the disease.

The vector

Since the last workshop, vector related aspects of the disease received a surprising amount of attention and which has really produced a remarkable amount of new information. An additional experimental vector of heartwater, the American reptile tick *A. dissimile*, was identified (28). Demonstration of the infection in ticks was done using a variety of methods (25, 36, 42, 63). This no doubt is the beginning of accurate methods to determine the infection rate in ticks and which is so badly needed for the development of epidemiological models who would form the basis of more effective control strategies. A great deal of research was conducted on the role of CO₂ and attraction/aggregation/attachment pheromones in host finding. The results of these studies were utilized to improve sampling techniques for nymphs and adults of *A. hebraeum* and *A. variegatum* and for the development of a novel method for tick control (41, 43, 49, 60). The biology and ecology of *A. variegatum* in the Caribbean were studied in great detail (3). These results, to a large extent, make it possible to explain and predict the way and rate in which the vector tick and the disease have spread in that region.

Control

Despite the significant progress on almost all aspects of the disease, the treatment and control of the disease remain virtually unchanged. Apart from a real lack of information on the pathogenesis which hampers the development of improved methods for supportive treatment the lack of progress on control is ascribed to a concentration of research on new potential areas of diagnostics probes and biochemical aspects of the organism while improvement of control measures currently in use were left aside in the meantime. However the recent findings regarding attenuation of at least one stock of *Cowdria*, successful freeze-drying and improved culture techniques could, within a relatively short period of time, be combined to replaced the present blood vaccine.

CONCLUSION

Many of the goals set at the 1986 workshop were addressed during recent years and a great deal of additional information became available, some of it of real significance. The overall significance of these findings is that a strong scientific basis has been laid on which sound strategies for future control measures can be built (53, 54).

This explosion of knowledge is timely because of the ever increasing threat that the disease may spread from the Caribbean to the American mainland. Apart from a solid understanding of the epidemiological factors that are involved in this unfortunate situation, an efficient dead vaccine is what is urgently needed to protect the susceptible animal population in those threatened areas or countries.

It is becoming more and more clear that the two parts in the world where heartwater poses a problem or a threat, namely Africa and the Caribbean, need different control strategies. In the case of the Caribbean heartwater will remain a real threat to the American mainland for as long as the disease is present on any island in the region. Moreover the tick on its own or together with its capability to transmit dermatophilosis is already a major threat to the cattle population in Latin America and certain parts of the USA. Eradication of the tick rather than control of the disease appears to be the option of choice. Conclusions from research on Guadeloupe on *A. variegatum* are that eradication of the tick appears technically difficult but possible, economically profitable but socially completely utopian (14).

In Africa the eradication of the disease is not possible and therefore the strategy to limit the impact of the disease should rather focus on the creation of enzootic stability. In this instance a living vaccine would perhaps be as good or even better than a non-living vaccine because of its potential to multiply in the host and to increase the infection rates in ticks. High infection rates in ticks are one of the cornerstones of the concept of enzootic stability.

It is therefore clear that, although there are definite areas of research that overlap between these two control strategies such as the development of methods to determine the infection rate in ticks accurately and to identify carrier and immune animals, the emphasis on other aspects may differ sharply. For enzootic stability it is very important not to have a highly susceptible animal population and the identification of breeds or populations of animals with a high resistance to the disease is thus an important aspect for future research.

When it comes to the eradication of the disease, it would have been better to work with highly susceptible animals in order to monitor the presence of the disease more easily. Methods to ensure effective tick control are of course much more critical in an eradication program than in the case of enzootic stability where tick control is aimed only at controlling excessive tick numbers in order to prevent tick worry.

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J.D. Bezuidenhout

BEZUIDENHOUT (J.D.). The significance of recent highlights in heartwater research. *Revue Elev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 101-108

Many of the objectives identified earlier, with regard to research on cowdriosis, have been achieved during the past decade. Some contributions such as the development of *Cowdria* specific DNA probes and the attenuation of the organisms are still in the experimental stage but others, such as *in vitro* cultivation, are already well established practices in many laboratories. Serological techniques are now more widely used since other methods and other sources of antigen became available. However, persistent cross reactions with *Ehrlichia* still complicate the interpretation of epidemiological data. Biochemical studies to identify, isolate and characterize antigenic proteins present in *Cowdria* organisms revealed definite immunodominant proteins that could prove to be suitable antigens for serological tests. Despite these significant research advances, practical methods to diagnose and control the disease have remained virtually unchanged. Control is still based on either intensive tick control or in some cases on the establishment and maintenance of endemic stability. The infection and treatment method using infected blood or prophylactic treatment with tetracyclines, remains the backbone of disease control in South Africa. Heartwater will remain a threat to the American mainland for as long as *Amblyomma variegatum* is present in the Caribbean and eradication of the tick from that region seems indicated.

Key words : Heartwater - *Cowdria* - Tick - *Amblyomma variegatum* - DNA probe - *In vitro* culture - Immunological technique - Antigen - *Ehrlichia* - Protein - Tetracycline - Africa - South Africa - Caribbean.

BEZUIDENHOUT (J.D.). Importancia de descubrimientos recientes en la investigación de la cowdriosis. *Revue Elev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 101-108

La mayor parte de los descubrimientos concernientes a la cowdriosis se llevaron a cabo durante el siglo pasado. Algunos aspectos, como el desarrollo del ADN específico para *Cowdria* o la atenuación de los organismos, se encuentran aún en un estadio experimental. Otros, como el cultivo *in vitro*, son prácticas ya establecidas en muchos laboratorios. El uso de las técnicas serológicas ha aumentado con el acceso a otros métodos y otras fuentes de antígenos. Sin embargo, las reacciones cruzadas persistentes con *Ehrlichia*, dificultan la interpretación de los datos epidemiológicos. Mediante estudios bioquímicos de identificación, aislamiento y caracterización de las proteínas antigénicas presentes en los organismos del género *Cowdria*, se han encontrado proteínas inmunodominantes, que podrían actuar como antígenos adecuados para los tests serológicos. A pesar de estos avances, los métodos prácticos de diagnóstico y control de la enfermedad no han cambiado en forma importante. El control se basa principalmente en el control intensivo de la garrapata y, en algunos casos, en el establecimiento y mantenimiento de una estabilidad endémica. El método de infección y tratamiento, mediante el uso de sangre infectada, o el tratamiento profiláctico con tetraciclinas, son los principales métodos de control de la enfermedad en Sudáfrica. Mientras *Amblyomma variegatum* se mantenga presente en el Caribe, la cowdriosis representará una amenaza para el Continente Americano, por lo que se recomienda la erradicación de la garrapata de esta región.

Palabras claves : Cowdriosis - *Cowdria* - Garrapata - *Amblyomma variegatum* - Sonda de ADN - Cultivo *in vitro* - Técnica inmunológica - Antígeno - *Ehrlichia* - Proteína - Tetracilina - Africa - Sudáfrica - Caribe.