

## HEARTWATER SEROLOGY: SOME PROBLEMS WITH THE INTERPRETATION OF RESULTS

J. L. DU PLESSIS<sup>(1)</sup>, E. CAMUS<sup>(2)</sup>, P. T. OBEREM<sup>(1)</sup> and LETITIA MALAN<sup>(1)</sup>

### ABSTRACT

DU PLESSIS, J. L., CAMUS, E., OBEREM, P. T. & MALAN, LETITIA, 1987. Heartwater serology: some problems with the interpretation of results. *Onderstepoort Journal of Veterinary Research*, 54, 327-329 (1987).

Antibodies in the sera of domestic ruminants that have been infected with *Ehrlichia bovis* and other ehrlichial agents cross-react with the Kümm strain of *Cowdria ruminantium* used in the indirect fluorescent antibody test as antigen. These cross-reactions are also shown by the Elisa test in which the Ball 3 strain of the heartwater agent is used as antigen.

### INTRODUCTION

Until recently the indirect fluorescent antibody (IFA) test was considered to have a high degree of specificity (Du Plessis & Malan, 1987). This conclusion was based, first, on the negative reactions of some 100 sera collected from cattle in the Orange Free State where *Amblyomma hebraeum* does not occur. Second, no cross-reactions could be demonstrated between the mouse-derived peritoneal macrophage antigen and antibodies to several infectious agents that may be immunologically related: *Chlamydia psittaci*, *Rickettsia conorii*, *Rickettsia prowazeki*, *Rickettsia typhi* and *Coxiella burnetti* (Du Plessis, 1982).

The first indication that the sera of cattle infected with an agent related to *Cowdria ruminantium* might react positively in the IFA test, was the finding by one of us that the sera from cattle and small stock on some Caribbean islands where heartwater had never before been diagnosed, gave positive reactions. The specificity of the IFA test was further questioned when cattle sera from a region of Namibia where the tick vector of heartwater does not occur, also reacted positively in the test.

### MATERIALS AND METHODS

#### *Sera from Caribbean islands*

A joint United States-French-Dutch research project to study the prevalence of heartwater in the Caribbean has revealed the presence of the disease on the islands of Guadeloupe, Antigua and Marie-Galante (Burrige, Barré, Birnie, Camus & Uilenberg, 1984). On several other Caribbean islands (St. Lucia, Martinique, Dominique, St. Croix, Puerto Rico, Nevis, St. Kitts, St. Maarten/St. Martin, Vieques, Anguilla and La Desirade), where one of the vectors, *Amblyomma variegatum*, occurs, the disease has never been diagnosed (Uilenberg, Barré, Camus, Burrige & Garris, 1984). During the course of a serological survey to ascertain whether infection by *C. ruminantium* does occur on these islands, serum collected from cattle and small ruminants on several of these islands, was tested.

#### *Sera from Namibia*

In a preliminary trial to compare the specificity of the IFA test with that of the Elisa test (Neitz, Viljoen, Bezuidenhout, Oberem, Van Wyngaardt & Vermeulen, 1985), 2 bovine sera from Otjiwarongo, Namibia were included on the assumption that they could serve as negative controls since *Amblyomma* does not occur in that region of Namibia. When one of these sera gave a positive reaction with both tests, a further 43 sera from the same farm were subjected to both the IFA and Elisa tests.

#### *Sera to Ehrlichia spp.*

Sera against several species of *Ehrlichia* or suspected ehrlichial agents were submitted to the IFA test. Twelve sera positive to the agent of Jembrana disease, the causal agent of which is thought to be an *Ehrlichia* (Ressang, Budiarmo & Soeharsono, 1985); 9 caprine and 11 ovine sera to *Cytoecetes phagocytophila*; 12 bovine sera positive to an *Ehrlichia* sp. recently isolated in Kenya (Morzaria, Irvin, Kocan & Voigt, 1985); 2 ovine sera positive to *Ehrlichia ovina* were included.

#### *IFA test*

Commencing at 1:20, serial twofold dilutions of the sera were subjected to the IFA test as described elsewhere (Du Plessis & Malan, 1987).

### RESULTS

It can be seen from Table 1 that 3-8 % of sera from 5 of the Caribbean islands on which *A. variegatum* occurs in the absence of clinical heartwater gave positive reactions to the IFA test. On the other 2 islands (Martinique and St. Martin) considerably higher levels of sero-positivity were recorded. No clinical case of heartwater has ever been reported from any of these islands and heartwater-susceptible small ruminants inoculated intravenously with homogenates from ticks collected from cattle on these islands have consistently failed to develop clinical signs of the disease (Camus & Barré, 1986).

The serum of 72 % of cattle from Namibia gave positive reactions to the IFA test (Table 2). The titres recorded varied between 1:20 and 1:320, the majority being in the lower range. Fifty-nine per cent of these animals were also positive when the Elisa test, in which the Ball 3 strain of *C. ruminantium* was used as antigen, was applied.

Only 3 out of 12 bovine sera positive to the agent of Jembrana disease were positive and only one out of 12 sera from cattle that had been infected with a Kenya strain of *Ehrlichia* gave a positive reaction. Only 2 out of 20 sera positive to *C. phagocytophila* gave a positive reaction to the IFA test (Table 2).

### DISCUSSION

There are two possible explanations for the cross-reactions between Kümm strain *C. ruminantium* IFA test antigen and the sera of large and small ruminants of the Caribbean islands on which *A. variegatum* ticks occur but which are free from clinical heartwater. Either the antigen cross-reacts with antibodies to an antigenically closely related infectious agent, or heartwater, possibly in a less pathogenic form of the disease without the characteristic clinical manifestations normally associated with the disease, does occur on these islands. Future research on these islands will be directed at isolating less pathogenic strains of the heartwater agent by inoculating susceptible small ruminants with further homogenates of

<sup>(1)</sup> Veterinary Research Institute, Onderstepoort, 0110

<sup>(2)</sup> Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, B.P. 1232, 97184 Point à Pitre, Guadeloupe.

Received 15 May 1987—Editor

## HEARTWATER SEROLOGY: SOME PROBLEMS WITH THE INTERPRETATION OF RESULTS

TABLE 1 IFA test results of cattle and small ruminants on Caribbean islands with *Amblyomma* but no clinical heartwater

| Caribbean Island | No. of +ive/No. tested |                 | Total  | % +ive |
|------------------|------------------------|-----------------|--------|--------|
|                  | Bovine                 | Small ruminants |        |        |
| St. Lucia        | 3/71                   | 2/36            | 5/107  | 5      |
| Martinique       | 23/226                 | 18/156          | 41/382 | 11     |
| Dominique        | 4/110                  | 0/13            | 4/123  | 3      |
| La Desirade      | 2/14                   | 0/39            | 2/53   | 4      |
| St. Kitts        | 5/60                   |                 | 5/60   | 8      |
| St. Martin       | 11/42                  |                 | 11/42  | 26     |
| St. Maarten      | 2/72                   | 2/32            | 4/104  | 4      |

TABLE 2 IFA test cross-reactions with Küm strain of *C. ruminantium*

| Ruminant species | No. of sera | Origin              | Antiserum to             | No positive     | % positive |
|------------------|-------------|---------------------|--------------------------|-----------------|------------|
| Bovine           | 43          | Namibia             | <i>Ehrlichia</i> sp.     | 31 (1:20-1:320) | 72         |
| Bovine           | 12          | Indonesia           | <i>Ehrlichia</i> sp.     | 3 (1:20-1:80)   | 25         |
| Caprine          | 9           | Scotland            | <i>C. phagocytophila</i> | 1 (1:80)        | 11         |
| Ovine            | 11          | Scotland            | <i>C. phagocytophila</i> | 1 (1:160)       | 9          |
| Bovine           | 12          | Kenya               | <i>Ehrlichia</i> sp.     | 1 (1:20)        | 8          |
| Ovine            | 2           | Via the Netherlands | <i>Ehrlichia ovina</i>   | 1 (1:80)        | 50         |

*A. variegatum* collected from cattle on Martinique and St. Martin where high percentages of seropositivity were recorded. It must be pointed out that on Guadeloupe heartwater was diagnosed with certainty (Perreau, Morel, Barré & Durand, 1980) only 150 years after the introduction of the tick vector from West Africa (Curasson, 1943).

Only limited cross reactions between *C. ruminantium* and several ehrlichial agents have been recorded in this study. The agent of Jembrana disease was one, but this disease has only been reported from Indonesia (Ressang *et al.*, 1985) and it is highly improbable that it occurs in the Caribbean. Only 2 out of 20 sera against *C. phagocytophila*, the causative agent of tick-borne fever, cross-reacted with *C. ruminantium*. The disease only occurs in Europe and its vector, *Ixodes ricinus* (Foggie, 1951) is not found in the Caribbean.

The high percentage of cattle from Namibia that were serologically positive both with the IFA and the Elisa tests suggests that these animals had been infected with an infectious agent immunologically closely related to *C. ruminantium*. *E. bovis* may be responsible for these cross-reactions, since preliminary observations on an agent subsequently isolated from one out of 10 adult *Hyalomma truncatum* ticks collected from cattle on the same farm where these cross-reactions were recorded, have revealed features that are reminiscent of *E. bovis*. The agent was passaged in mice and calves and granular inclusions in Giemsa stained peritoneal macrophages of the mice and the monocytes of a calf were indistinguishable from those seen in *E. bovis* infection. A positive reaction to the IFA test on serum of the calf collected a month after being inoculated, was supportive evidence (J. L. du Plessis, unpublished data, 1986).

The finding that only one out of 12 sera from Kenyan cattle infected with an agent suspected of being *E. bovis* was positive in the IFA test, however, questions *Ehrlichia* as the cause of the cross-reactions. The large percentage of serologically positive Namibian cattle may suggest that some strains of *E. bovis* have more antigens in common with *C. ruminantium* than others.

There is further evidence that *Ehrlichia* is immunologically closely related to the heartwater agent. Using goat neutrophils infected with 4 strains of *C. ruminantium*, Holland, Logan, Mebus & Ristic (1987) detected antibodies to *Ehrlichia canis* and *Ehrlichia equi* in an IFA test. The fact that identical results were obtained

with neutrophils infected with the Küm (Du Plessis, 1982), Gardel (Uilenberg, Camus & Barré, 1985), Kwanyanga (MacKenzie & Van Rooyen, 1981) and Mali (Linda Logan, unpublished data, 1986) strains, suggests that different species of *Ehrlichia* share common antigens with several strains of *C. ruminantium*.

The cross-reactions between antibodies to ehrlichial agents and *C. ruminantium* questions the value of the IFA and the Elisa tests in epidemiological studies on heartwater in those areas where both *Amblyomma* and *Hyalomma* occur. Limited data would indicate that *E. bovis* is transmitted by *Hyalomma* (Donatien & Lestoquard, 1936; Rousselot, 1953) and possibly also by *A. variegatum* (Rioche, 1966). Since *E. bovis* is implicated as the cause of these cross-reactions, studies to characterize different strains of this agent are needed. The prevalence and distribution of this agent and the ticks responsible for its transmission should be studied. It may infect large proportions of herds as suggested by the sera from the Namibian cattle. There is, however, indirect evidence that it does not occur in all heartwater endemic areas. At the Mara Research Station, for example, where experiments on heartwater have been conducted (Du Plessis, Bezuidenhout & Lüdemann, 1984) and are still in progress, 26 serologically positive 9-month-old Hereford calves that had been exposed to tick infection, have been found to be solidly immune to challenge with *C. ruminantium* infected sheep blood (J. L. du Plessis, unpublished data, 1986). Had the positive serological reaction of any of these animals been due to infection by *E. bovis*, they would have reacted to the challenge, because cattle that have recovered from infection with *E. bovis* are fully susceptible to heartwater (Donatien & Lestoquard, 1936; Girard & Rousselot, 1945; Morzaria *et al.*, 1985). It must be borne in mind though, that the high *C. ruminantium* infection rate of the cattle may have masked the presence of antibodies to *Ehrlichia*.

While the application of the IFA test to the epidemiology of heartwater is complicated by the cross-reactions with *Ehrlichia*, the test remains a valuable tool to monitor the artificial infection of experimental animals. It shows a high degree of sensitivity in experimentally-infected cattle and small ruminants (Camus & Barré, 1987; Du Plessis & Malan, 1987) and there is a good correlation between resistance to challenge and the seropositivity of cattle after having been infected artificially or naturally through the tick, in that serologically positive cattle

have consistently been found to be immune (Du Plessis & Malan, 1987). Should it be proved that *E. bovis* is not transmitted by *Amblyomma*, the inoculation of individual *A. hebraeum* ticks into mice and the subsequent detection of antibodies to *C. ruminantium* in the serum of the mice (Du Plessis, 1985) will continue to be the only method of determining the infection rate of ticks.

ACKNOWLEDGEMENTS

We wish to thank Profs A. A. Ressang and G. Uilenberg and Drs S. P. Morzaria and G. R. Scott for kindly supplying us with *Ehrlichia* positive sera.

REFERENCES

BURRIDGE, M. J., BARRÉ, N., BIRNIE, E. F., CAMUS, E. & UILENBERG, G., 1984. Epidemiological studies on heartwater in the Caribbean, with observations on tick-associated bovine dermatophilosis. *Proceedings of XIIIth World Congress on Diseases of Cattle*, 1, 542-546.

CAMUS, E. & BARRÉ, N., 1986. Epidemiology of heartwater in Guadeloupe and in the Caribbean. *Onderstepoort Journal of Veterinary Research*, 54, 453-460.

CURASSON, G., 1943. *Traité de Protozoologie vétérinaire et comparée*. Tome I. Trypanosomes. Vigot Frères, Paris, p. 272.

DONATIEN, A. & LESTOQUARD, F., 1936. *Rickettsia bovis*, nouvelle espèce pathogène pour le boeuf. *Bulletin de la Société de Pathologie Exotique*, 39, 1 057-1 061.

DU PLESSIS, J. L., 1982. Mice infected with a *Cowdria ruminantium*-like agent as a model in the study of heartwater. D.V.Sc. Thesis, University of Pretoria.

DU PLESSIS, J. L., 1985. A method for determining the *Cowdria ruminantium* infection rate of *Amblyomma hebraeum*: Effects in mice injected with tick homogenates. *Onderstepoort Journal of Veterinary Research*, 52, 55-61.

DU PLESSIS, J. L. & MALAN, LETITIA, 1987. The application of the indirect fluorescent antibody test in research on heartwater. *Onderstepoort Journal of Veterinary Research*, 54, 319-325.

FOGGIE, A., 1951. Studies on the infectious agent of tick-borne fever in sheep. *Journal of Pathology and Bacteriology*, 63, 1-15.

GIRARD, H. & ROUSSELOT, R., 1945. La rickettsiose bovine à *Rickettsia bovis* au Soudan français. *Bulletin de la Société de Pathologie Exotique*, 38, 64-77.

HOLLAND, C. J., LOGAN, L. L., MEBUS, C. A. & RISTIC, M., 1987. The serological relationship between *Cowdria ruminantium* and certain members of the genus *Ehrlichia*. *Onderstepoort Journal of Veterinary Research*, 54, 331.

MACKENZIE, P. K. I. & VAN ROOYEN, R. E., 1981. The isolation and culture of *Cowdria ruminantium* in albino mice. *Proceedings of International Congress on Tick Biology and Control, Rhodes University, Grahamstown, 1981*, 33-39.

MORZARIA, S. P., IRVIN, A. D., KOCAN, K. M. & VOIGT, W. P., 1985. Isolation and characterization of an *Ehrlichia* sp. from cattle in Kenya. *Workshop on diseases caused by leukocytic rickettsiae of man and animals*. Illinois, U.S.A., p. 030.

NEITZ, A. W. H., VILJOEN, G. J., BEZUIDENHOUT, J. D., OBEREM, P. T., VAN WYNGAARDT, W. & VERMEULEN, N. M. J., 1985. Detection of antibodies to *Cowdria ruminantium* in serum and *C. ruminantium* antigen in *Amblyomma hebraeum* by an enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, 52, 39-41.

PERREAU, P., MOREL, P. C., BARRÉ, N. & DURAND, P., 1980. Existence de la cowdrose (heartwater) à *Cowdria ruminantium* chez les ruminants des Antilles françaises (La Guadeloupe) et les Mascareignes (La Réunion et Ile Maurice). *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 33, 21-22.

RESSANG, A. A., BUDIARSO, I. T. & SOEHARSONO, S., 1985. Jembrana disease. Its similarity to bovine ehrlichiosis. *Workshop on diseases caused by leukocyte rickettsiae of man and animals*. Illinois, U.S.A., p. 039.

RIOCHE, M., 1966. La rickettsiose générale bovine au Sénégal. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 19, 485-494.

ROUSSELOT, R., 1953. Notes de parasitologie tropicale. Parasites du sang des animaux. Tome I. Vigot Frères, Paris, p. 87-108.

UILENBERG, G., BARRÉ, N., CAMUS, E., BURRIDGE, M. J. & GARRIS, G. I., 1984. Heartwater in the Caribbean. *Preventive Veterinary Medicine*, 2, 255-267.

UILENBERG, G., CAMUS, E. & BARRÉ, N., 1985. Quelques observations sur une souche de *Cowdria ruminantium* isolée en Guadeloupe (Antilles françaises). *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 38, 34-42.