

# The serological response to heartwater immunization in cattle is an indicator of protective immunity

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LAWRENCE (J.A.), TJORNEHOJ (K.), WHITELAND (A.P.), KAFUWA (P.T.). La réponse sérologique à l'immunisation contre la cowdriose constitue un indicateur effectif du degré de protection immunitaire. *Revue Elev. Méd. vét. Pays trop.*, 1995, 48 (1) : 63-65

Les auteurs ont mis en évidence une corrélation significative chez des bouvillons croisés Frison entre la réponse sérologique à des vaccinations antérieures avec la souche Ball 3 de *Cowdria ruminantium*, et le développement de la protection immunitaire contre un isolat de la souche Kalota du Malawi. Des 10 animaux dont la séroconversion a été constatée après la vaccination, tous étaient complètement ou partiellement immunisés contre la souche d'épreuve. Dix des 14 animaux qui n'ont pas effectué de séroconversion étaient immunisés mais cette proportion n'était pas significativement différente de celle des témoins non vaccinés (4/10). Enfin, sur 29 animaux vaccinés et traités simultanément avec un implant de doxycycline, 13 n'ont effectué aucune séroconversion et, parmi eux, 4 étaient totalement sensibles à l'isolat.

Mots clés : Bovin - Cowdriose - *Cowdria ruminantium* - Immunologie - Vaccin - Antibiotique.

## INTRODUCTION

Heartwater (*Cowdria ruminantium* infection) was first recognized as an important disease of domestic ruminants in South Africa during the 19th Century (8). Its prevention depended entirely on control of the vector ticks, *Amblyomma* spp., until the 1940's when Neitz and Alexander (6, 7) developed a method of immunization involving the inoculation of infected sheep blood and treatment of the ensuing disease process, where necessary, with sulphonamides or, more recently, tetracyclines (1).

Lawrence, Whiteland, Malika, Kafuwa and Jongejan (5) reported the use of indirect immunofluorescence, with infected endothelial cells grown in culture as antigen, for the evaluation of the immune response in cattle to such a vaccine. They demonstrated differences between various batches of vaccine and between various regimens of administration of the same batch of vaccine and postulated that these reflected differences in immunogenicity. However, as it has been suggested that protective immunity to heartwater is cell mediated rather than antibody dependent (4), they had reservations as to the validity of

the serological response as an indicator of the protective value of the vaccine, as opposed to its ability to stimulate antibody production.

In this paper it is demonstrated that the antibody response is, indeed, an indicator of the development of protective immunity after immunization, confirming the findings of Du Plessis and Malan (2).

## MATERIALS AND METHODS

### Animals

Thirty-four Friesian cross steers, 6-12 months old, originating from farms with a history of good tick control and maintained under tick-free conditions. The animals were divided in 3 categories as follows.

Vaccinated, seropositive: 10. The animals were shown to be positive at a dilution of 1/30 or above by indirect immunofluorescence (5) at least 42 days after vaccination. Four had received vaccine alone, 6 others were selected from a group of 29 steers which had been treated with a slow-release implant of doxycycline ("Doximplant B", George Schwulst Labs. Ltd, Republic of South Africa) administered subcutaneously at the base of the ear at a rate of 5-8 mg/kg at the same time as the vaccine, of which only 16 seroconverted.

Vaccinated, seronegative: 14. Serologically negative 42 days after vaccination. One had received vaccine alone, the other 13 vaccine and doxycycline implant.

Control, seronegative : 10.

### Vaccine

2.5 ml of frozen blood vaccine (1) produced at Central Veterinary Laboratory, Lilongwe, Malawi containing 7.3 cattle ID<sub>50</sub> (50 % immunizing dose) of the Ball 3 strain of *C. ruminantium* as assessed by titration in cattle (5). The vaccine was administered by slow intravenous injection.

### Challenge isolate

The Kalota isolate of *C. ruminantium* preserved as a frozen blood stabilate prepared from a Dorper-cross ram in the terminal stages of clinical heartwater. The sheep had

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TABLE I  
Protective immunity to challenge with *Cowdria ruminantium* (Kalota) in cattle vaccinated with or without doxycycline implants, in relation to serological status

Treatment	Antibodies	Not Immune	Partially Immune	Immune	Total
Vaccinated	positive	0	1	9	10
Vaccinated	negative	4	2	8	14
Control	negative	6	2	2	10

been infected by applying 14 adult *Amblyomma variegatum* ticks which had been collected as nymphs from sheep involved in an outbreak of heartwater and had been allowed to moult. The challenge dose was 12.5 ml, administered by slow intravenous injection.

### Assessment of immunity

Cattle were examined daily for rectal temperature and clinical signs after challenge and were assigned to one of three categories:

Not immune: died, or recovered after treatment with long-acting tetracycline (Vetamycin LA®, C-Vet, UK) at the onset of clinical signs, namely anorexia, ataxia, hyperaesthesia. One animal was treated after three days of fever exceeding 40.5°C, without other clinical signs.

Partially immune: fever exceeding 40.5°C for 1-2 days, without other clinical signs, recovery without treatment.

Immune: no fever, or fever not exceeding 40.5°C, no clinical signs.

## RESULTS

A difference was seen in the response of the three groups of animals to challenge (table I). All 10 seropositive animals were immune or partially immune, while 4/14 seronegative vaccinates and 6/10 controls were considered not immune and three died. The difference between the seropositive and the seronegative animals was statistically significant (Fisher's Exact Test,  $p = 0.030$ ). The apparent difference between the vaccinated seronegative group and the control group was not significant ( $p > 0.20$ ).

## DISCUSSION

The Ball 3 vaccine strain was found to stimulate a very good protective immunity in cattle against the Kalota isolate of *C. ruminantium*. A similar degree of protection was also demonstrated in sheep (unpublished observations).

The results demonstrate a close correlation between the development of antibodies after vaccination and the establishment of a protective immunity, as previously reported by Du Plessis and Malan (2), using immunofluorescence

with a mouse macrophage antigen. They thus validate the use of immunofluorescence of vaccinated cattle as an indicator of the immunizing quality of the vaccine. However, even in the absence of vaccination, 2 of 10 control animals did not react to challenge, confirming previous reports that a significant proportion of cattle have an innate resistance to infection unrelated to previous exposure and specific immunity (3).

Lawrence, Whiteland, Malika, Kafuwa and Jongejan (5) have shown previously that the application of doxycycline implants at the same time as vaccination results in a marked reduction in immunogenicity of the vaccine, as assessed by the antibody response. In the present trial, 13 animals of the 29 which were vaccinated together with doxycycline failed to seroconvert. Four of the 13 were completely susceptible to challenge, confirming that the product also inhibits the protective immune response in cross-bred cattle in the Malawi environment. Subsequent studies (unpublished observations) revealed no improvement in the proportion of animals seroconverting after vaccination when the dose of doxycycline was reduced to 2.5-4.0 mg/kg, nor when the implant was administered at the standard dosage rate seven days after vaccination.

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A significant correlation was demonstrated in Friesian-cross steers between the serological response to previous vaccination with the Ball 3 strain of *Cowdria ruminantium* and the development of protective immunity against the Kalota isolate from Malawi. Of 10 animals which seroconverted after vaccination, all were completely or partially immune to challenge. Ten of the 14 animals which failed to seroconvert were immune but the proportion was not significantly different from that in the unvaccinated controls (4/10). Of 29 animals vaccinated and treated simultaneously with a slow-release doxycycline implant, 13 failed to seroconvert, and of these, four were completely susceptible to challenge.

*Key words:* Cattle - Heartwater - *Cowdria ruminantium* - Immunology - Vaccine - Antibiotics.

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**LAWRENCE (J.A.), TJORNEHOJ (K.), WHITELAND (A.P.), KAFUWA (P.T.).** Respuesta serológica a la inmunización contra cowdriosis en ganado, como índice de inmunidad de protección. *Revue Élev. Méd. vét. Pays trop.*, 1995, 48 (1) : 63-65

Se demostró una correlación significativa en toretes cruzados Friesian, entre la respuesta serológica a una vacunación previa con la cepa Ball 3 de *Cowdria ruminantium* y el desarrollo de inmunidad de protección contra aislamientos Kalota de Malawi. De 10 animales que mostraron seroconversión después de la vacunación, todos presentaron inmunidad completa o parcial al test. Diez de los 14 animales que no mostraron seroconversión, fueron inmunizados pero la proporción no fue significativamente diferente de la de los controles no vacunados (4/10). De los 29 animales vacunados y tratados simultáneamente con un implante de doxiciclina de liberación lenta, 13 no presentaron seroconversión, cuatro de los cuales fueron completamente susceptibles al test.

*Palabras clave :* Bovino - Cowdriosis - *Cowdria ruminantium* - Inmunología - Vacun - Antibiótico.