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## Seroconversion to *Cowdria ruminantium* of Malawi zebu calves, reared under different tick control strategies

SOLDAN (A.W.), NORMAN (T.L.), MASAKA (S.), PAXTON (E.A.), EDELSTEN (R.M.), SUMPTION (K.J.). Séroconversion à *Cowdria ruminantium* de veaux zébus au Malawi, soumis à des stratégies différentes de lutte contre les tiques. *Revue Elev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 171-177

L'ELISA indirect a été utilisé au Malawi pour comparer la séroconversion à *Cowdria ruminantium* jusqu'à l'âge d'un an de 66 veaux zébus locaux nés dans des groupes passés au bain détiqueur 17 fois par an, à celle de 32 veaux nés dans des groupes non détiqués. Le nombre d'*Amblyomma variegatum* et les cas cliniques de la maladie ont été enregistrés dans chaque groupe de bovins. Aucun cas de cowdriose n'a été observé chez les veaux des 2 groupes pendant les 22 premiers mois de leur vie. Un seul cas fut enregistré chez une vache de 8 ans chez les 1 800 bovins suivis de façon intensive pendant la même période. Presque tous les veaux non détiqués étaient devenus séropositifs à l'âge d'un an et 50 p. 100 des séroconversions ont été attribuées à des infections par des nymphes. Par ailleurs, seulement 41 p. 100 des veaux dans les groupes passés au bain étaient devenus positifs à l'âge d'un an. Ce régime de détiquage a donc diminué le taux de séroconversion de façon significative chez ces veaux. Soixante quatorze pour cent des veaux avaient des anticorps maternels contre *C. ruminantium* pendant les quatre premières semaines de leur vie. Chez tous les veaux des groupes détiqués, le taux de ces anticorps était tombé sous le seuil considéré comme positif dans l'ELISA à l'âge de 8 à 12 semaines et il n'y avait pas de conversion de séronégatif à séropositif pendant cette période chez ces veaux. La proportion de séropositifs détectés par un seul test ELISA à l'âge de 12 mois n'était pas significativement différente de celle déterminée par les résultats cumulatifs des 9 mois précédents et semble donc utilisable comme indication de l'état de l'immunité du troupeau. Les auteurs concluent qu'une stabilité enzootique à *C. ruminantium* existe chez les zébus locaux non détiqués, caractérisée par une résistance à l'infection, innée et élevée, et une séroconversion entre 3 et 9 mois d'âge de la plupart des veaux nés en mai-juin.

Mots-clés : Zébu - Cowdriose - *Cowdria ruminantium* - *Amblyomma variegatum* - Tique - Lutte antiacarien - Infestation - Test ELISA - Anticorps - Malawi.

### INTRODUCTION

The vector of *Cowdria ruminantium* in Malawi is considered to be *Amblyomma variegatum*, which has a widespread distribution in the country, except for lowland areas of southern Malawi such as the Lower Shire Valley (2). Heartwater has been frequently reported in unprotected

*Bos taurus* cattle, but the disease is seldom reported in indigenous Malawi Zebu cattle (*Bos indicus*). The number of taurine cattle in Malawi is very low (less than 20,000) in comparison with numbers of Malawi Zebu in the national herd of 800,000 (10). The status of traditionally managed Malawi Zebu to *Cowdria ruminantium* is not known, although a state of enzootic stability and/or genetic resistance in indigenous ruminants to the infection, is considered to be present in *Amblyomma variegatum* infested areas. The occurrence and timing of seroconversion to *C. ruminantium* in relation to *Amblyomma* infestations in cattle is important to understanding the nature of infection in both undipped and dipped cattle populations.

Dipping to control tick-borne disease was first made compulsory in certain areas of Malawi in the early 1920's (DE MAZA, 1925, cited in MARES (8)). Although current legislation provides for weekly dipping of cattle in arsenic trioxide it is estimated that only 20-40 % are dipped regularly. A survey of cases presented to veterinary assistants at diptanks in north and central regions showed that ECF morbidity varied between 0.5 and 1.8 % (all ages) ; clinical cases of babesiosis and anaplasmosis were rare and heartwater was not recorded. The dipping attendance over the year was only 50 % and therefore the low ECF morbidity was not attributed to the suppression of ticks through dipping (5).

A three year trial commenced in 1990, which undertook to investigate the effect of reduced intensity dipping and non-dipping in traditionally managed cattle, upon morbidity, mortality, productivity and economic indicators. Seroconversion to *C. ruminantium* in cohorts of calves born during this trial into study herds at six diptanks is reported here.

An indirect ELISA test was used to test serum samples for antibodies to detergent soluble antigens extracted from the purified elementary body of *C. ruminantium* (SUMPTION, MASAKA and PAXTON, unpublished results). The significance of positive test results in immunofluorescent antibody (IFA) tests upon sera from animals from areas where *Amblyomma* ticks are present is unclear, because positive reactions have been observed in IFA tests with sera from some *Amblyomma* free areas (3). The latter results are presumed to be the result of cross-reactions caused by antibodies to *Ehrlichia* species, because serological cross-reactions in IFA tests have been observed between antisera raised to various *Ehrlichia* species and *C. ruminantium* antigens in infected mouse macrophages (3) or neutrophils (6, 7). The

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indirect ELISA used to test sera from Malawi utilized detergent soluble elementary body antigens, because a number of cross-reactive antigens to *E. phagocytophila* and *E. ondiri* were removed during the detergent extraction process; the ELISA test consequently has a low level of detection of antibodies to these pathogens in comparison with IFA using *Cowdria* infected goat neutrophils or infected endothelial cells (SUMPTION and PAXTON, unpublished). The presence of *E. ondiri* or *E. bovis* has not been demonstrated in Malawi, and therefore antibody reactions in this study are assumed to be to *C. ruminantium*.

## MATERIALS AND METHODS

### Location of study area

Malawi is located between 9° - 17° South and 33° - 36° East in Central Africa. As part of a larger study, six dip-tanks in the same ecological zone were chosen in the Lilongwe area. This area is on the Central African Plateau with an undulating, almost flat topography about 1,100 m above sea level. Four of the tanks chosen were in good repair and had been using arsenic trioxide up to the start of the study in November 1991. Cattle at two dip-tanks which were to act as non-dipped controls had effectively not been regularly dipped as a result of tank disrepair, or because there was a large group of farmers who did not dip their cattle.

### Organization of study

Approximately 300 animals at each of the six tanks were tagged in November 1990. Each of the cattle were Malawi-Zebu and belonged to smallholders, and were communally grazed. No alteration in management was instituted and no prophylactic treatments were given during the trial. Dipping was carried out in four tanks at 2 weekly intervals in the rainy season (December 1990 to March 1991 and December 1991 to March 1992) and at 4 weekly intervals through the dry season (April 1991 to November 1991). Dipping at two tanks was in chlorfenvinphos (Supona 30, Shell Chemicals Ltd.) and at the other two in amitraz (Triatix TR, Coopers Animal Health).

Acaricide concentrations and replenishment were as recommended by the manufacturers, and the total replacement method was used for amitraz. Dipping of cattle was not carried out at the two control tanks. An active disease monitoring system was set up with the aim of identifying the specific cause of each case of death or disease in cattle at each dip tank in the trial. The routine samples collected from dead animals were faeces, blood, lymph node, spleen, and brain crush smears. These were examined by staff of the protozoology section of the

Central Veterinary Laboratory and a project veterinary officer. The veterinary assistant associated with each dip-tank visited owners with tagged cattle every week and project staff visited every 2 weeks throughout the study period.

### Calf cohort study

The peak calving season in the Lilongwe area occurs between May and July each year. As part of a productivity study 15 calves born in May/June 1991 had serum samples collected at 8 week intervals with the first sample being collected at the first visit after birth. Samples were frozen at -20 °C and aliquots for *Cowdria* serology were forwarded on ice by airfreight to the Centre for Tropical Veterinary Medicine, Edinburgh, where they were tested by indirect ELISA. Chi squared tests were used to compare proportions.

### Indirect ELISA for the detection of antibodies to *Cowdria ruminantium*

An indirect ELISA developed at the CTVM was used to test sera at a dilution of 1 in 50. The ELISA uses soluble antigens extracted from the elementary body (EB) stage of *C. ruminantium* (Welgevonden stock) following release from cell cultures, and has an extremely low reactivity to antibodies present in antisera raised to *Ehrlichia phagocytophila*, in comparison with immuno-fluorescent antibody tests (IFA) using Welgevonden infected neutrophils or infected endothelial cells. It also has an excellent sensitivity in the detection of experimentally infected animals (SUMPTION and PAXTON, unpublished results). Cross-reactions with *Ehrlichia* spp. present considerable difficulties in the interpretation of IFA tests for heartwater (3, 4). The indirect ELISA was developed using detergent soluble fractions of the *Cowdria* elementary body because these fractions have a reduced number of antigens with cross-reactivity to *E. phagocytophila* and *E. ondiri* sera, than is found in whole EB or infected cell preparations (SUMPTION and PAXTON, manuscript in preparation). Soluble antigen is prepared from EB's semipurified from culture medium by centrifugation for 20 min at 1,000 g for the pelleting of endothelial cells, followed by centrifugation at 10,000 g for the pelleting of EB's. Pellets were washed in sterile phosphate buffered saline (PBS, pH 7.4), and recentrifuged at 10,000 g for 20 min. The procedure was repeated two times, followed by detergent lysis of the elementary body in 0.5 % nonidet NP40 and 0.5 % sodium deoxycholate in 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, 150 mM sodium chloride and 1 mM phenylmethylsulfonylfluoride for 2 min at room temperature followed by rapid passage through a 26 g needle to disaggregate elementary bodies, and incubation at 37 °C for 30 min. After a further round of needle passage, insoluble antigen was removed by centrifugation at 4 °C for 30 min at 16,000 g. Soluble antigen extracts were then stored at -20 °C until

used in ELISA. Antigen was diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6) and 100  $\mu$ l added to each well of 9 well immunoplates (Immulon II, Dynatech Laboratories) and incubated overnight at 4 °C. Plates were then washed 5 times in PBS diluted 1:4 in distilled, de-ionized water which contained 0.05 % Tween 20. Serum samples were diluted to 1 in 50 in PBS containing 0.05 % Tween 20 (PBST) and 100  $\mu$ l added to duplicate wells and incubated for 60 min at 37 °C in a shaking incubator. Plates were then washed as before and 100  $\mu$ l of rabbit anti-bovine IgG horse radish peroxidase conjugate (Sigma Chemical Company) added to each well and incubated for 60 min at 37 °C. Plates were then washed and 100  $\mu$ l orthophenylene diamine (OPD, 0.4 mg/ml) and hydrogen peroxide (0.015 %) added to each well and incubated for 6 min at 20 °C. 100  $\mu$ l of IM sulphuric acid was then added to each well to stop the reaction and the optical densities recorded at 492 nm in a Titertek Multiscan Spectrophotometer. Four positive controls, 2 negative controls and two blank wells (no serum) were used per plate.

## Tick counts

Half body counts of *Amblyomma variegatum* adults and nymphs were conducted every 4 weeks at each diptank on 5 animals between the ages of 6 and 12 months. Individual animals were not counted more than once in the study period and each animal, on any one date, came from different farmers. In dipped cattle, tick counts represent maximal burdens as they were carried out immediately prior to dipping.

## RESULTS

### Disease

A laboratory confirmation of diagnosis was reached in over 80 % of deaths occurring in tagged cattle during this study. East Coast Fever was the only tick borne disease observed in both dipped and undipped cattle. No cases of heartwater were observed between May 91 and June 92. A significant difference was not observed in disease mortality and tick numbers between cattle in herds in which a regime of chlorfenvinphos or amitraz was used. Dipped cattle are therefore compared to non-dipped cattle populations for the comparison of seroconversion.

### Dipping percentages of cattle

For tagged cattle the average dipping percentage was 83 % and for untagged cattle attending the same tanks it was 41 %. Tagged cattle made up between 10 and 22 % of the cattle population for each tank.

## Tick counts

Figure 1 shows the relationship between *A. variegatum* adults and nymphs in undipped cattle. Infestations of cattle showed a highly seasonal distribution, with peak numbers of nymphs in August and September, followed by peak numbers of adults in November and December. Nymphal activity was from March to November with adults present from October to February. Table I shows mean adult and nymph *A. variegatum* half body counts for various periods of the study.

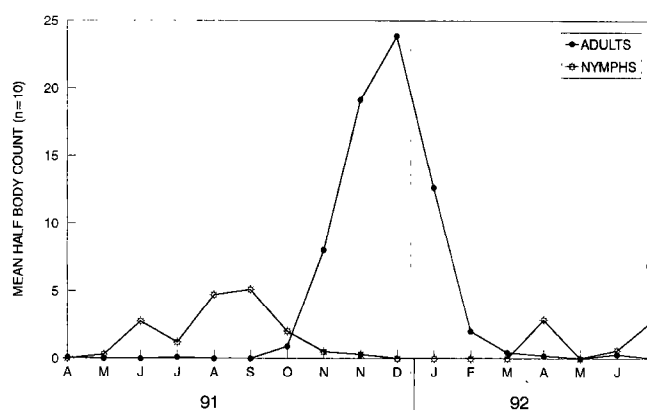


Figure 1: *Amblyomma variegatum* adults and nymphs undipped cattle.

TABLE I *Amblyomma variegatum* counts on 6 to 12 month calves for three periods of the year.

	May-October 1991		November 1991-February 1992		March-June 1992	
	non-dipped	dipped	non-dipped	dipped	non-dipped	dipped
Adults	0.2	0.1	13.1	3.3	0.2	0.0
Nymphs	2.7	1.0	0.2	0.1	0.9	0.9

Figures are mean half body tick counts for the three periods indicated, for 20 calves in the four dipped groups and 10 calves in the two non-dipped groups.

## Determination of cut-off values for ELISA

Sera collected from calves in October-November 1991 and May-June 1992, from dipped and non-dipped groups, were tested in ELISA at a dilution of 1/50 and the frequency distributions of OD values was plotted (figures 2, 3, 4, 5). The dipped calves in October-

November had OD values which were characterized by a bimodal frequency distribution with a group of values less than 0.25 OD units, and a small number of sera with values greater than this value. A similar distribution was also observed for the same calves in May-June 1992 (figure 4), whereas non-dipped calves in October-November (figure 3) had a comparatively high

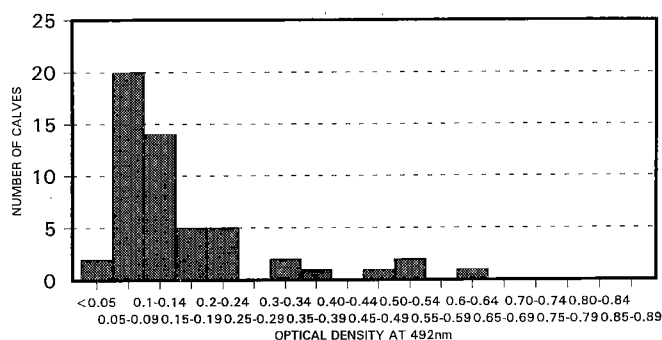


Figure 2 : Indirect ELISA for antibodies to Cowdria OD values frequency distribution dipped calves samples October or November 1991.

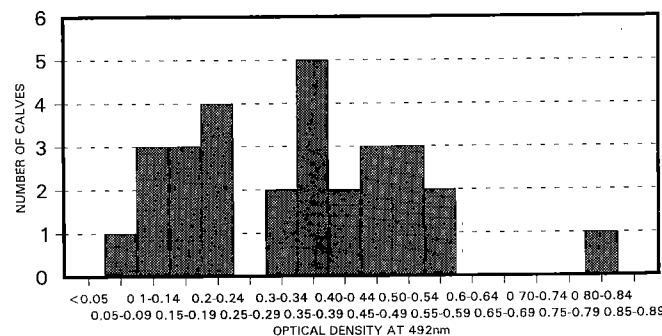


Figure 3 : Indirect ELISA for antibodies to Cowdria OD values frequency distribution non-dipped calves October or November 1991.

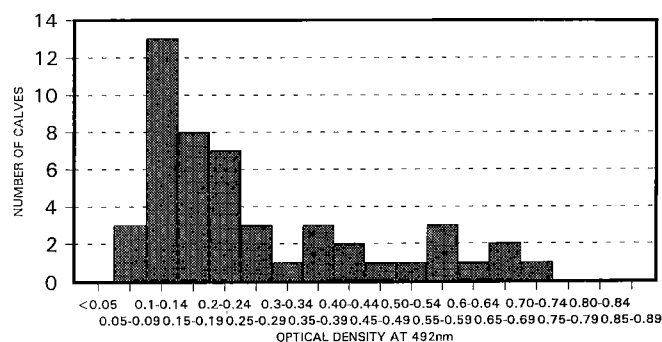


Figure 4 : Indirect ELISA for antibodies to Cowdria OD values frequency distribution dipped calves samples May or June 1992.

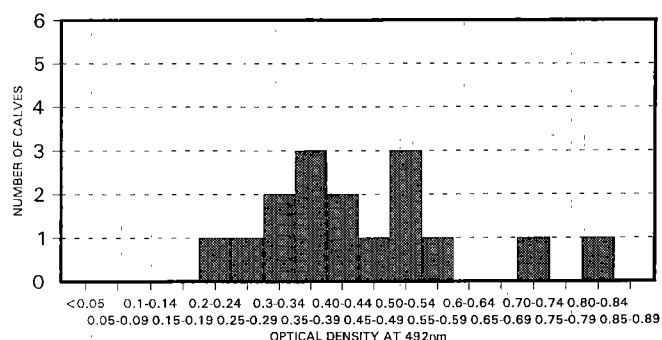


Figure 5 : Indirect ELISA for antibodies to Cowdria OD values frequency distribution non-dipped calves May/June 1992.

proportion of values (18/29) greater than 0.25 OD and a single peak of values for sera collected in May-June (figure 5) of which a high proportion (15/16) were greater than 0.25 OD units. The low values (less than 0.25 OD units) were assumed to represent a population of calves which had not seroconverted to heartwater, because dipped calves had very low tick counts from birth to the time of sampling in October-November. The presumed negative population had an expected skewed distribution which was characterized by a tail of high OD values ; the latter was also observed for sera from heartwater free areas of Europe and the Caribbean (unpublished results). The chosen cut-off value of 0.25 was similar to that determined for sera from other parts of the world, and was chosen on the basis of OD values which separated the high and low OD value distributions, and from the baseline values observed following the decline in maternal derived antibody in dip trial calves.

### Maternally derived antibody levels in calves

Twenty-nine of the forty calves (72.5 %) which were sampled in the first four weeks of life were seropositive (table II). This was considered to be the result of maternal antibody. The number of calves with detectable maternal antibody was slightly higher in the undipped group (10/12, 80 %) than in the dipped group (19/30, 63 %) but the difference was not significant ( $P > 0.05$ ). No calves in dipped herds had a positive result in samples taken when they were aged between 8 and 12 weeks. The chosen cut-off value of 0.25 was higher than the maximum value (0.247) observed (median = 0.113,  $n = 42$ ) in calves 8 to 12 weeks of age in dipped groups. Evidence of seroconversion was therefore considered to be the finding of an OD value greater than 0.25 in calves over 12 weeks of age.

**TABLE II** Proportion of seropositive serum samples from cohort calves less than 16 weeks of age at the six diptanks in the trial.

Diptank	Regime	0 to 4 weeks	4 to 8 weeks	8 to 12 weeks	12 to 16 weeks
Likuni Tonde	non-dipped	1/2	3/5	2/10	3/4
	non-dipped	9/10	6/6	0/1	3/6
	Total non-dipped	10/12	9/11	2/11	6/10
Namaguya Dickson Mbabzi Sinyala	dipped	2/3	1/13	0/8	0/11
	dipped	8/11	0/7	0/11	1/14
	dipped	6/9	2/15	0/5	1/10
	dipped	3/5	2/12	0/3	1/12
	Total dipped	19/28	5/47	0/27	3/47
	Total all tanks	29/40 (73 %)	14/58 (24 %)	2/38 (5 %)	*9/57 (16 %)

Serum considered seropositive if the OD value exceeded 0.25 OD units relative to a positive control (Nyaga 2) at 1.053 OD units.

\* 8 of the positives were from probable seroconversions.

### Seroconversion to *C. ruminantium* in cohort calves

The proportion of calves considered seropositive by ELISA in samples collected in October 1991, and February and June 1992 (or in samples collected in the preceding month if no sample was taken in that month) is shown in table III. There was a significant difference ( $P < 0.01$ ) between seroconversion in dipped and undipped calves at all three periods of the year. After 12 months of the trial 96 % (23/24) of undipped calves had seroconverted compared to only 41 % (18/44) of dipped calves. Tagged cattle at these dip tanks were monitored for a further year but clinical cases of heartwater were not observed in cohort calves or other tagged cattle at the diptank except for a single confirmed case in an 8 year old cow.

**TABLE III** Seroconversion to *Cowdria ruminantium* in calves born May/June 1991.

	October 1991		February 1992		June 1992	
	Spot	Cumulative	Spot	Cumulative	Spot	Cumulative
Dipped	3/55 (5 %)	5/56 (9 %)	17/49 (35 %)	19/51 (37 %)	15/42 (36 %)	18/44 (41 %)
Non-dipped	15/29 (52 %)	15/29 (52 %)	21/26 (81 %)	23/26 (88 %)	17/18 (94 %)	23/24 (96 %)

Results are given of single sample tests (spot tests) and cumulative seroconversion of the groups (including samples collected in the month for which results are stated).

The seroconversion of cohort calves was also determined from the results of the serial collection of serum samples, and the results are given as a cumulative seroconversion for the cohort (table III). The proportion of calves considered seropositive was very similar by both methods ( $P > 0.05$ ).

The highest OD value observed for serum samples from calves which seroconverted varied from 0.261 to 0.993 OD units, with a median value for 58 calves of 0.501. Optical density values for serum samples from ten of the 58 calves declined to below the cut-off value, between 8 and 24 weeks after seroconversion. However, a decline to below the cut-off value was observed in only 3 of the 31 calves which seroconverted in the non-dipped groups which had received a higher tick challenge.

### DISCUSSION

The undipped calves in this study had an almost continuous *Amblyomma* challenge through the first year of life (figure 1). For the first 5 months this was almost entirely of nymphae (table I), and approximately 50 % of the calves seroconverted to *C. ruminantium* in this period (table II). The remaining calves seroconverted during the months of adult *Amblyomma* activity. At the end of one year of life almost 100 % of undipped calves had seroconverted to *C. ruminantium*. In contrast the dipped calves had a significantly reduced level of seroconversion throughout the study period, and therefore the reduced intensity dipping had significantly affected exposure of calves to the agent of heartwater. Dipped cattle carried significantly lower tick burdens than undipped cattle and this was reflected in significantly lower seroconversion rates in dipped calves, which reached only 41 % at 12 months of age. In the subsequent 10 months (June 1992-April 1993), heartwater was not observed in any of the dipped calves which had not seroconverted by June 1992, despite a suspension in dipping from April 1992 to November 1992. Seroconversion in the absence of clinical cases of heartwater was observed among the calves aged between 5 and 12 months in the non-dipped groups, between October 1991 and June 1992. Approximately 50 % of the non-dipped calves seroconverted in this period. The results therefore suggest that Malawi Zebu are resistant to heartwater until at least one year of age. The observation of a case of heartwater in an 8 year old cow is surprising, because of the high seroconversion in non-dipped herds and the low dip attendance prior to the onset of the dip trial which would be expected to result in a very high herd immunity of animals older than 2 or 3 years. Cases of heartwater in this age group have been attributed to relapse as a result of stress (9). In addition, an increased susceptibility to immunization with the Ball3 vaccine stock in animals older than 8 years has been observed (11). The apparently high innate resistan-

ce of Malawi Zebu calves to the development of clinical heartwater suggests that a combination of factors may have been associated with clinical heartwater on this occasion.

The suppression of *Babesia bovis* seroconversion in the same calves by dipping during the dry season was considered to create an enzootically unstable situation and therefore at the end of the rains in March 1992, dipping was discontinued in favour of a strategy of non-dipping in the dry season. This strategy may be expected to increase nymphal *Amblyomma* infestations and seroconversion to *C. ruminantium* at 6 months of age in calves born in May-June 1992.

The finding that there was no significant difference between the proportion of dipped and undipped calves which had maternal antibodies is probably the result of similar exposure of cattle in these groups to *Amblyomma* ticks despite the statutory requirements which existed for dipping. This suggests that the dipping in arsenic trioxide which occurred before the trial in 4 of the groups resulted in a similar proportion of seropositive cows to the non-dipped groups; this may have occurred because of insufficient dip attendance or acaricide activity. The observation that maternal antibody to *C. ruminantium* was not detected after 8-12 weeks of age is similar to that of DU PLESSIS *et al.* (4) who found that in 18 out of 21 calves born to naturally exposed dams, maternal derived antibody was not detected after 12 weeks of age, in IFA tests. Comparison of spot seroconversion rates and cumulative seroconversion rates (table II) showed that there was no difference between the two methods. This suggests that this ELISA test may be of value in herd studies for the investigation of comparative seroconversion rates in the field, with serum samples collected at a single point in time. In this study, seropositive status continued for at least 8-10 months after seroconversion in the majority of calves which seroconverted, in the presence of intermittent or continuous tick challenge, and only 3 out of 31 calves exposed to high tick numbers in the non-dipped groups underwent a temporary reversion to a seronegative status.

## CONCLUSION

In undipped Malawi Zebu cattle in the study area, *C. ruminantium* appears to be in a state of enzootic stability with high seroconversion rates in cattle and the absence of clinical disease. Malawi Zebu seem to have some degree of innate resistance to heartwater because calves not challenged in their first year showed no clinical disease under tick challenge in their second. The ELISA proved useful in determining comparative seroconversion rates in herds kept under different management regimes, and results of single sampling were not significantly different from serial sampling in the determination of seroconversion rates at a given point in time.

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SOLDAN (A.W.), NORMAN (T.L.), MASAKA (S.), PAXTON (E.A.), EDELSTEN (R.M.), SUMPTION (K.J.). Seroconversion to *Cowdria ruminantium* of Malawi zebu calves, reared under different tick control strategies. *Revue Élev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 171-177

The seroconversion by indirect ELISA to *Cowdria ruminantium* over the first year of life of sixty-six Malawi zebu calves born into groups which were dipped 17 times per year was compared to seroconversion of 32 calves born into non-dipped groups. *Amblyomma variegatum* tick counts and clinical disease in each group of cattle were monitored throughout the study period. No cases of heartwater were seen in either group of calves over the first 22 months of life. Only one case of heartwater was observed, in an 8 year old cow, in the 1,800 intensively monitored cattle over the same period. By 12 months of age almost all undipped calves had seroconverted and 50 % of seroconversions were attributed to nymphal challenge. In contrast, only 41 % of calves had become seropositive by 12 months of age in the dipped groups. The dipping regime used therefore significantly decreased seroconversion rates to *C. ruminantium* in these calves. 73 % of calves had detectable levels of maternal antibodies to *C. ruminantium* in the first 4 weeks of life. Antibody levels in each of the calves in dipped groups had waned to below the cut off point for the ELISA by 8-12 weeks. Seroconversion did not occur in the first 8-12 weeks of life in dipped herds. The indirect ELISA test results were not significantly different in the proportion positive in single tests at 12 months of age, or by cumulative test results of the previous 9 months, and therefore the test may be of value as a test of herd immunity. It is concluded that a state of enzootic stability exists to *C. ruminantium* in undipped Malawi zebu cattle in the study area, which is characterized by a high innate resistance to the infection and seroconversion of the majority of the calves born in May-June to the agent between 3 and 9 months of age.

*Key words* : Zebu - Cattle - Heartwater - *Cowdria ruminantium* - *Amblyomma variegatum* - Tick - Tick control - Reinfection - Elisa test - Antibody - Malawi.

SOLDAN (A.W.), NORMAN (T.L.), MASAKA (S.), PAXTON (E.A.), EDELSTEN (R.M.), SUMPTION (K.J.). Seroconversión a *Cowdria ruminantium* en terneros cebú Malawi, criados con diferentes estrategias de control de garrapatas. *Revue Élev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 171-177

Mediante el ELISA indirecto, se compara la seroconversión a *Cowdria ruminantium*, durante el primer año de vida, de sesenta y seis terneros cebú Malawi, nacidos de grupos bañados 17 veces al año, contra la seroconversión de 32 terneros nacidos en grupos no tratados. El número de *Amblyomma variegatum* y la enfermedad clínica de cada grupo fueron seguidos durante el estudio. Durante los primeros 22 meses de vida, no se observaron casos de cowdriosis en ninguno de los grupos de terneros. De los 1 800 animales seguidos durante el período de estudio solamente se observó un caso de cowdriosis, en una vaca de 8 años. La seroconversión se demostró a los 12 meses de edad, en casi todos los animales no tratados. Cincuenta por ciento de las seroconversiones se atribuyen a los estadios ninfales. Solamente 41 p. 100 de los terneros en el grupo tratado mostró una seropositividad a los 12 meses de edad. El sistema de baños disminuyó consecuentemente la tasa de conversión a *C. ruminantium* en estos terneros. Setenta y tres por ciento de los terneros mostraron niveles detectables de anticuerpos maternos contra *C. ruminantium* durante las primeras 4 semanas de vida. Hacia 8 o 12 semanas, los títulos de anticuerpos en los terneros tratados bajaron más allá de los niveles detectables mediante el ELISA. En las primeras 8 a 12 semanas de vida no hubo seroconversión en los terneros tratados. El resultado del ELISA indirecto entre los tests individuales a 12 meses y los resultados de los tests acumulados de los 9 meses anteriores, no fue significativamente diferente, lo que sugiere que el test podría ser de utilidad como examen de la inmunidad del hato. Se concluye que un estado de estabilidad enzootica de *C. ruminantium* existe en los hatos no tratados de ganado cebú Malawi, caracterizado por una resistencia innata a la infección y una seroconversión de la mayoría de los terneros nacidos entre mayo y junio, entre 3 y 9 meses de edad.

*Palabras claves* : Cebú - Cowdriosis - *Cowdria ruminantium* - *Amblyomma variegatum* - Garrapata - Control de garrapata - Infestación - Test ELISA - Anticuerpo - Malawi