

IMMUNITY OF TICK-EXPOSED SERONEGATIVE AND SEROPOSITIVE SMALL STOCK CHALLENGED WITH TWO STOCKS OF *COWDRIA RUMINANTIIUM*

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ABSTRACT

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Nineteen per cent and 32 % of serologically positive sheep and goats in heartwater endemic areas of the Republic of South Africa were susceptible to challenge with, respectively, the Ball 3 and Welgevonden stocks of *Cowdria ruminantium*. There was good correlation between the results of the indirect fluorescent antibody test and immunity, since without exception all the seronegative animals reacted when they were challenged. The fact that only 74 % of the seropositive sheep and goats were immune to challenge can probably be ascribed to the poor cross-protection between stocks of *C. ruminantium* and not to false positive serological reactions.

INTRODUCTION

During recent years extensive as well as intensive farming with sheep and goats has expanded considerably in the heartwater endemic areas of the Transvaal and Eastern Cape provinces of South Africa. Heartwater has emerged as the most important infectious disease, responsible for heavy losses and as the principal limiting factor to further extension of this farming activity. Goats are kept on extensive grazing to utilize and thereby control certain bushes not eaten by cattle. The majority sheep are kept on intensive grazing on artificial pasture.

Heartwater losses occur in both species and on both types of grazing. Immunization is practised on a limited scale and the control of the disease is confined to tick control and the treatment of clinical cases. Despite intensive tick control, mortalities are by no means uncommon, even among sheep on artificial pasture where the chances of tick intrusion and survival would seem remote. In certain areas of heavy tick infestation in the Eastern Transvaal lowveld, Natal and the so-called valley-bushveld of the Eastern Cape, it is not uncommon for farmers to dip their small stock and pass them through a footbath every week and to administer tetracyclines to all the animals in a particular flock as soon as clinical cases or mortalities occur. Even then losses due to several outbreaks per year are reported to be substantial. This untenable and unscientific state of affairs indicates the urgent need for more effective control.

One possible solution to the problem would be vaccination on a large scale, but the drawbacks of the vaccine are well known (Oberem & Bezuidenhout, 1987). The infection and treatment method of vaccination (Van der Merwe, 1987) is the method of choice but is still hazardous in sheep and goats because of their higher susceptibility compared to that of cattle. If treatment is postponed unduly, the animal may be lost, and if given too early, an adequate immunity may be compromised. Treatment given too early is also undesirable in the case of the block method of vaccination (Du Plessis & Malan, 1987b).

Apart from these shortcomings in the present methods of vaccination and the fact that the vaccine must of necessity be given alive and intravenously, it has recently come to light that there is only partial or sometimes no cross-immunity between the Ball 3 stock at present used in the vaccine and several other stocks, not only from outside (Jongejan, Uilenberg & Franssen, 1988) but also in the Republic of South Africa (Du Plessis, Van Gas, Olivier & Bezuiden-

hout, 1989). It has been suggested that the Ball 3 be replaced by another stock, possibly Welgevonden, that would confer immunity against most of the stocks with which it has been compared under laboratory conditions (Du Plessis *et al.*, 1989).

To throw some light on the advisability of such a step it was decided to ascertain the extent to which sheep and goats, immune to field stocks of *Cowdria ruminantium*, are protected against challenge with the Ball 3 and Welgevonden stocks in a field situation. This would also be an opportunity to ascertain whether the indirect fluorescent antibody (IFA) test can be used to determine the immune status of small stock in epidemiological surveys.

MATERIALS AND METHODS

Experimental animals

Six farms where immunization against heartwater is not practised were selected in areas of the RSA where the disease is endemic. The animals comprised Dorper and Letelle sheep and Angora and Boerbok breeds of goats, all of them on extensive grazing. Serum was collected from adult animals on each farm in order to select seropositive and seronegative individuals for subsequent challenge. On 2 of the farms where losses amongst weaned lambs were reported to be particularly heavy, serum was also collected from 4-6-month-old lambs in order to compare their immune status with that of the adult animals. A brief account of the prevalence of heartwater and the type of tick control practised in the case of each farm was obtained.

Serology

Sera at a dilution of 1:20 of a total of 287 sheep and goats were submitted to the IFA test as previously described (Du Plessis & Malan, 1987a). To get an indication of the levels of antibody in the serum of naturally infected small stock, fourfold dilutions of 10 positive sera selected at random from those of the adult ewes on Farm 4, were also tested.

Challenge of seropositive and seronegative animals

More or less equal numbers of adult seropositive ewes on each farm were challenged with an intravenous injection of 5 ml of sheep blood, infected either with the Ball 3 (Haig, 1952) or the Welgevonden (Du Plessis, 1985) stock of *C. ruminantium*. The blood was drawn from deep-frozen batches of standardized stabilates, recently used in a cross-immunity study (Du Plessis *et al.*, 1989). To prevent the *Amblyomma hebraeum* population on the farms from being infected with the Welgevonden stock, the challenged animals were treated with flumethrin

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TABLE 1 Serology of 287 sheep and goats from heartwater endemic regions in South Africa

Farm No. and locality	Breed and age	No. of sera tested	No. of seropositives	Immune status of flock (%)	Prevalence of heartwater disease	Tick control total strategic
1 North-Western Transvaal	Dorper ewes	28	26	93	+ ²	+
2 Eastern Transvaal	Boerbok ewes	29	24	83	+	+
3 Eastern Transvaal	Dorper ewes	40	24	60	++	+
4 Northern Transvaal	Dorper ewes Dorper lambs	36 ¹ 35	36 15	100 43	+ +++	+
5 Natal	Angora ewes Letelle ewes Letelle lambs	27 ¹ 15 20	27 10 5	100 67 25	+ +++ +++	+
6 Eastern Cape	Angora ewes Boerbok ewes Dorper ewes	18 ¹ 25 ¹ 14 ¹	12 17 8	67 68 57	+++ ++ ++	+

¹ Group of animals from which seropositives were challenged
² Low (+), moderate (++) and high (+++) prevalence of disease

TABLE 2 Immunity of naturally infected seropositive sheep and goats to challenge with the Ball 3 and Welgevonden stocks of *C. ruminantium*

Farm No.	No. challenged			No. that reacted to challenge				% susceptible to challenge
	Ball 3 stock	Welgevonden stock	Died	Reaction index			No reaction	
				>20	>10	<10		
1	12	16	0 0	0 0	0 2	4 1	8 13	0 12,5
2	10	10	0 5	3 3	2 1	5 0	0 1	50 90
3	12	8	0 0	0 0	4 2	3 2	5 4	33 25
4	9	10	0 0	0 0	2 1	4 2	3 7	22 10
5	10	10	0 0	0 2	0 1	4 2	6 5	0 30
6	10	11	0 1	0 2	1 1	2 2	7 5	10 36
Total	63	65	0 6	3 7	9 8	22 9	29 35	19 (13) ¹ 32 (22) ²

¹ (13) = total % susceptible to Ball 3 if Farm 2 is excluded
² (22) = total % susceptible to Welgevonden if Farm 2 is excluded

pour-on¹ on the day when they were challenged and fortnightly thereafter for 2 months.

Seronegative lambs and ewes on 4 of the farms were also challenged with 5 ml of Welgevonden-infected sheep blood.

The early morning rectal temperatures of all the challenged animals were taken and a reaction index calculated for each animal, as previously described (Du Plessis *et al.*, 1989). Animals that reacted were treated with oxy-tetracyclines on the 3rd day of the febrile reaction on condition that the temperature on the 1st day was at least 39,5 °C and progressively higher on the 2 subsequent days. If a high tempera-

ture persisted on the 2nd day after the previous treatment, a 2nd and sometimes a 3rd treatment was given. Ten additional reaction points were added for each time the animals were treated.

RESULTS

Serology

The number of sera from the animals on each of the 6 farms that reacted positively to the IFA test are given in Table 1. It can be seen that 57-100 % of the adult ewes were serologically positive. Only 43 % and 25 % of the lambs on Farms 4 and 5 respectively were seropositive.

It is noteworthy that, firstly, the seropositivity of the adult animals on Farms 1, 2 and 4, where strategic tick control was practised, was considerably

¹ Bayer SA (Pty) Ltd

TABLE 3 Correlation between serology and immunity: Challenge of 38 seronegative animals with the Welgevonden stock of *C. ruminantium*

Farm No.	No. challenged		No. that reacted to challenge				% positive correlation	
	Adults	Lambs	Died	Reaction index				No. reaction
				>20	>10	<10		
3	4			1	3		100	
4		11	2	9			100	
5		17	1	16			100	
6	6		3	2	1		100	
Total	38		6	28	4	0	0	

higher than on Farms 3, 5 and 6, where tick control was intensive. The Angora ewes on Farm 5 were the only exception. Secondly, the percentage of serologically positive lambs on Farms 4 and 5 was significantly lower than that of the adult animals on these farms.

Two, 2, 5 and 1 of the 10 seropositive adult ewes on Farm 4 were positive to titres of 1:20, 1:80, 1:320 and 1:1280 respectively.

Challenge of seropositive animals

The number of sheep and goats that reacted to challenge with the Ball 3 and Welgevonden stocks are given in Table 2. In calculating the percentage of animals that were susceptible to challenge, the same criterion as that applied to a laboratory cross-immunity study (Du Plessis *et al.*, 1989), was used. Animals that died or that had reaction indices of 10 or higher were considered to be susceptible to the challenge.

It is evident that in the case of 4 flocks (Farms 1, 2, 5 and 6) a higher percentage of animals were not protected or had only a partial protection against the Welgevonden stock than against Ball 3. The observation that the reverse situation was true for Farms 3 and 4 was noteworthy. On the whole, 32 % of the 65 ewes were susceptible to challenge with Welgevonden against 19 % of the 63 challenged with Ball 3.

The 5 goats that died on Farm 2, all of which had been challenged with Welgevonden, were autopsied. Extensive acute to sub-acute fibrino-purulent bronchopneumonia, characteristic of *Pasteurella* infection, was present in all 5 of them. Their brain smears were positive for heartwater. One Angora ewe on Farm 6 died from uncomplicated heartwater.

Challenge of seronegative animals

All 38 (100 %) seronegative ewes and lambs reacted to challenge with the Welgevonden stock (Table 3). Thirty-four animals either died or showed severe reactions in spite of having been treated and only 4 had moderate febrile reactions. Compared to the 9 challenged, seropositive ewes that had mild reactions (index <10, Table 2), not one seronegative animal fell into this category. It is noteworthy that all 28 Dorper lambs had to be treated 2 or 3 times, some of which as early as on Day 9 after infection. Despite the intensive treatment, 3 of them died. This illustrates the marked pathogenicity of the Welgevonden stock to sheep.

DISCUSSION

Compared to the 43 % of sheep experimentally immunized against 10 stocks of *C. ruminantium* that were susceptible to challenge with the Welgevonden stock (Du Plessis *et al.*, 1989), only 32 % of naturally

infected sheep and goats were not protected against this stock. If the results of the goats on Farm 2, where sub-clinical pasteurellosis most probably complicated the challenge procedure, are excluded, the 22 % that proved to be susceptible is surprising. If one considers that, under laboratory conditions, as many as 56 % of sheep immune to Ball 3 and 83 % immune to a stock isolated on the Comoro Islands (J. D. Bezuidenhout, J. A. Olivier, J. S. Kruger, P. E. Lombard & J. L. du Plessis, unpublished observation, 1987), reacted to challenge with Welgevonden (Du Plessis *et al.*, 1989), there was no large scale collapse of immunity with a disturbing number of deaths or clinical cases amongst the field animals as might have been expected.

Although the brain smears of the 5 goats on Farm 2, in which outspoken lesions typical for pasteurellosis were observed at autopsy, were positive, their death cannot be attributed solely to heartwater. It is interesting to note that specific pathogen-free lambs infected with *Pasteurella haemolytica* during a reaction to *Cytoecetes phagocytophila*, a rickettsial agent close to *C. ruminantium*, showed more severe pasteurellosis than control lambs given *P. haemolytica* alone (Brodie, Holmes & Urquhart, 1986). Although the situation was probably reversed in the present study in that goats sub-clinically infected with *Pasteurella* were super-infected with *C. ruminantium*, there can be little doubt that these animals were more susceptible to fatal heartwater.

As for the Ball 3 stock, the findings in the field situation were also somewhat different from those in experimentally immunized sheep. It was to be expected that a smaller percentage of naturally immune ewes would react to challenge with Ball 3 than with Welgevonden, but it was nevertheless surprising that as many as 19 % reacted to Ball 3 at all. What was even more surprising was that in the case of 2 flocks (Farms 3 and 4), more sheep reacted to Ball 3 than to Welgevonden.

However, in spite of these mitigating observations in favour of the Welgevonden stock, it cannot be concluded from the findings of this cross-immunity field study that it would not be more dangerous to spread Welgevonden as vaccine stock than would be the case with Ball 3. Although Welgevonden protects against a much wider spectrum of stocks, its dangerously high pathogenicity, once again clearly shown by the challenge of the seronegative Dorper lambs, outweighs this advantage.

There was good correlation between the serology of the naturally infected small stock and their susceptibility to challenge. Without exception, all the seronegative animals reacted when they were challenged. This differs from the situation in cattle, where substantial numbers of seronegative animals may be partially, or totally, resistant to artificial

challenge (Camus & Barré, 1987; Du Plessis & Malan, 1987a).

It may be argued that in the reverse situation the correlation was not so good, since only 74 % of the seropositive sheep and goats were immune to challenge with either the Ball 3 or the Welgevonden stock. In view of the poor, or only partial, cross-protection between many stocks of *C. ruminantium* (Alexander, 1931; Du Plessis *et al.*, 1989; Jongejan *et al.*, 1988; Logan, Birnie & Mebus, 1987), however, the susceptibility to challenge of the remaining 26 % of the animals can in all probability be ascribed to a lack of cross-protection and not to false positive serological reactions.

In the case of seropositive animals, the relationship between serology and immunity is once again different in cattle, because seropositive cattle have consistently been found to be resistant to artificial challenge with the Ball 3 stock of *C. ruminantium* (Du Plessis & Malan, 1987a). This discrepancy between small stock and cattle can be attributed to the greater resistance to heartwater that cattle have in general. Because of this higher resistance of cattle, reactions to cross-challenge with heterologous stocks, the phenomenon that accounts for the fact that seropositive sheep may prove to be susceptible to challenge with heterologous stocks, would be less likely to occur in the case of cattle.

The failure of seronegative cattle to react to challenge can on one hand be attributed to their non-specific resistance, a factor which does not play a role in sheep older than 2 weeks, hence the observation that 100 % of the serologically negative sheep reacted. On the other hand, failure of seronegative cattle to react may be due to a specific immunity in which antibody levels, if present at all, are too low to be detected by the IFA test. The observation that the antibody levels of cattle drop after being re-infected and that they even become seronegative after a 2nd re-infection (Du Plessis & Malan, 1987a) and do not persist for much longer than 5 months in naturally infected calves (Camus & Barré, 1987), explains this phenomenon. This factor apparently does not play a role in sheep, since 83–100 % of positive serological reactions to titres as high as 1:320 and even 1:1280 of sheep and goats suggest frequent re-infection through ticks on Farms 1, 2 and 4, where strategic tick control was practised. Furthermore, antibodies are detectable with the IFA test in the serum of sheep for much longer after experimental infection than in the case of cattle (Du Plessis & Malan, 1987a).

It must be added, though, that the above observations on the antibody response in cattle have been made on animals infected with Ball 3 stock. Because of the higher pathogenicity of the Welgevonden stock also to cattle (J. L. du Plessis, unpublished observation, 1988), it may well be that on one hand seropositive cattle immune to an immunologically different stock of *C. ruminantium* and on the other a greater percentage of serologically negative animals may react to challenge with the Welgevonden than with the Ball 3 stock.

Considering earlier findings in conjunction with those of the present study, however, the conclusion seems justified that the IFA test, applied to epidemiological surveys in small stock, is a good indication of their true state of immunity, much more so than in the case of cattle. The percentage of seropo-

sitive animals would seem accurately to reflect the proportion of a flock infected through ticks.

The low percentage of seropositivity of weaned lambs compared to that of the adult animals on Farms 4 and 5 reflect a challenging epidemiological situation which is probably by no means uncommon amongst small stock in heartwater endemic areas. The heavy losses amongst weaned lambs reported in these 2 flocks is consistent with the practice, reported not only on these 2 farms but also in other areas where heartwater losses are severe, of administering tetracyclines not only to the clinical cases in an outbreak but also to the rest of the flock. In addition to the subclinical cases in such a flock, not fully immune from an earlier infection because treated too early in the incubation period, the treatment, several times per year, of clinical cases and a large portion of those late in the incubation period, probably accounts for the high percentages of seropositivity reported in this study.

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