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THE HETEROGENICITY OF *COWDRIA RUMINANTIUM* STOCKS: CROSS-IMMUNITY AND SEROLOGY IN SHEEP AND PATHOGENICITY TO MICE

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ABSTRACT

DU PLESSIS, J. L., VAN GAS, L., OLIVIER, J. A. & BEZUIDENHOUT, J. D., 1989. The heterogenicity of *Cowdria ruminantium* stocks: Cross-immunity and serology in sheep and pathogenicity to mice. *Onderstepoort Journal of Veterinary Research*, 56, 195–202 (1989).

Ten stocks of *Cowdria ruminantium* (Ball 3, Breed, Comoro, Germishuys, Kümm, Kwanyanga, Mali, Mara, Nonile and Welgevonden) were compared from a cross-immunity, serological and mouse pathogenicity point of view. They were found to differ in varying degrees. Except for the Ball 3, Comoro and Germishuys stocks that were similar but not identical, there was no pattern in the antigenic diversity of the 10 stocks. The Welgevonden stock emerged as the stock that elicits an immunity against most of the South African stocks. The inability of the reference Ball 3 stock to protect sheep against no fewer than 6 other stocks questions the advisability of retaining this stock as the vaccine stock. The antigenic diversity of the 10 stocks could not be correlated with the antibody levels detected with the indirect fluorescent antibody test, since the sera against all 10 stocks reacted positively to the Kümm stock antigen and the variation in titres was not stock-related.

INTRODUCTION

The existence of immunologically different stocks of *Cowdria ruminantium* has been a controversial issue for a long time. While on one hand Alexander (1931) found that 13 out of 37 sheep immune to an unspecified stock challenged with a heterologous stock either reacted or died, Neitz (1939) on the other hand concluded from cross-immunity experiments with 10 stocks that there was almost total protection between them.

Uncertainty prevailed until the Kümm stock was discovered (Du Plessis & Kümm, 1971), when it was found that sheep immune to the reference Ball 3 stock invariably succumbed to challenge with the newly isolated stock and vice versa (Du Plessis, 1982). For a while this seemed to be an isolated finding, since cross-immunity studies on stocks of *C. ruminantium* from geographically widely separated areas, such as Nigeria, Sudan, Sao Tomé, Guadeloupe, Mali and South Africa, indicated that these stocks were completely cross-protective (Van Winkelhoff & Uilenberg, 1981; Uilenberg, Zivkovic, Dwinger, Ter Huurne & Peri, 1983; Uilenberg, Camus & Barré, 1985; Logan, Birnie, Endris & Mebus, 1985).

However, it has subsequently been shown that antigenic differences, between stocks of the heartwater agent do exist. A stock from Senegal caused fatal heartwater in goats immune to Ball 3 (Jongejan, Uilenberg, Franssen, Gueye & Nieuwenhuijs, 1988) and in a subsequent study the Senegal stock again and 3 others proved to be antigenically different (Jongejan, unpublished observation, 1989). A similar finding involving the Gardel, Kümm, Kwanyanga and Mali stocks was reported by Logan, Birnie & Mebus (1987).

Stocks of *C. ruminantium* differ markedly in their murinotropism, i.e. their ability to infect laboratory mice (Du Plessis, 1985) and a correlation between their murinotropism and their antigenic differences with Ball 3 has even been suggested (Jongejan, Uilenberg & Franssen, 1988). Both the Kümm and Welgevonden stocks certainly are highly pathogenic to mice and appear to be antigenically different not only from one another but also from Ball 3. Whether this is true, when stocks over the entire spectrum of murinotropism are compared in significant numbers of animals, remains to be seen.

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The antigenic diversity of *Cowdria* stocks is important for several reasons. Since several stocks have been reported to cause reactions and even death in sheep and goats immune to Ball 3 (Jongejan *et al.*, 1988), which also serves as antigen in the production of a vaccine (Oberem & Bezuidenhout, 1987), the question arises whether this stock should not be replaced by another with a wider range of protection. Complaints by farmers that vaccinated cattle and small stock still succumb to natural tick challenge may likewise be ascribed to the inability of Ball 3 to adequately protect against local field stocks.

It has been suggested that the antigenic differences between stocks might complicate the interpretation of serological results obtained in epidemiological surveys (Jongejan, Wassink, Thielemans, Perie & Uilenberg, 1989). This would be the case if the antibodies to a particular field stock were not detected by the stock used as antigen in the serological test concerned. One of the objects of this study was therefore to ascertain to what extent the Kümm stock, used as antigen in the indirect fluorescent antibody (IFA) test (Du Plessis & Malan, 1987a), detects antibody to other stocks.

MATERIALS AND METHODS

C. ruminantium *stocks*

The 10 stocks compared in this study were the Ball 3 stock (Haig, 1952), considered a reference stock (Jongejan *et al.*, 1988) and for a long time used as a vaccine (Oberem & Bezuidenhout, 1987) and in a wide range of experiments.

The Breed stock was isolated from an Angora goat from the Messina district of the Northern Transvaal, where serious mortalities caused by heartwater were experienced in Angora goats introduced from the Eastern Cape (Du Plessis, Jansen & Prozesky, 1983).

The Comoro stock was isolated in sheep from *Amblyomma variegatum* ticks collected on the Comoro islands between Africa and Madagascar (J. D. Bezuidenhout, J. A. Olivier, J. S. Kruger, P. E. Lombard & J. L. du Plessis, unpublished observations, 1987).

The Germishuys stock was isolated in 1984 from a sheep originating from the same district in the North-eastern Transvaal where the Kümm stock had been isolated (Du Plessis & Kümm, 1971), in an

attempt to repeat the isolation of the latter (J. L. du Plessis, unpublished observation, 1984).

The Kümm stock was isolated in mice inoculated intraperitoneally with lymph node homogenate of a goat suffering from natural heartwater (Du Plessis & Kümm, 1971; Du Plessis, 1982). The stock has subsequently been passaged for more than 100 times in mice without any loss in pathogenicity to mice, sheep and goats. The peritoneal macrophages of mice infected with this stock serve as antigen in the IFA test (Du Plessis & Malan, 1987a).

The Kwanyanga (MacKenzie & Van Rooyen, 1981) and Nonile (MacKenzie & McHardy, 1984) stocks were isolated from tick infected sheep on pasture in the Eastern Cape and Natal respectively.

The Mali stock was isolated from an *A. variegatum* tick from the African state of Mali (Logan, Endris, Birnie & Mebus, 1985).

The Mara stock was isolated in 1987 from an *A. hebraeum* tick collected on the government experimental farm, Mara, not far from the farm where the Ball 3 stock had been isolated some 50 years earlier (J. L. du Plessis, unpublished observation, 1987).

The Welgevonden stock was isolated in mice inoculated intraperitoneally with the homogenate of a male *A. hebraeum* tick collected in the Northern Transvaal not far from where the Kümm and Germishuys stocks were isolated (Du Plessis, 1985).

The stocks were deep-frozen in liquid nitrogen, either as homogenates of mouse tissues in buffered lactose peptone (BLP) (liver and spleen in the case of Kümm and lung, myocardium and spleen in the case of Welgevonden), or as infected sheep blood to which equal volumes of citrated BLP had been added.

All homologous and heterologous challenge procedures were carried out with standardized infected sheep blood. Heartwater-susceptible sheep were infected i.v. with each of the 10 stocks and their blood collected in heparinized BLP solution at the height of the febrile reaction. The blood was deep-frozen in aliquots of 10 m*l*, its freedom from contaminating agents ascertained and its infectivity tested according to a standard procedure (Oberem & Bezuidenhout, 1987).

Infection and challenge of experimental sheep

A total number of 245 adult Merino and Dorper sheep of both sexes were infected with the 10 stocks during the course of a variety of experimental procedures, comprising the characterization of new stocks, infectivity control of ticks, tissue culture and freeze-dried specimens, current vaccine production and chemoprophylaxis trials. Daily early morning, rectal temperatures were recorded and oxytetracycline treatment given when necessary. To determine the cross-immunity between Ball 3 and Welgevonden and each of the other 8 stocks for the specific purpose of this study, some additional animals were infected with the standardized stabilates.

All the animals were subjected to a homologous challenge with 5 m*l* of standardized stabilate 1–3 months after the primary infection and to the heterologous challenge 1 month later. Daily temperatures were again recorded. The sheep were not treated during the homologous or the heterologous challenges.

Reaction index

A reaction index, reflecting the degree of immunity to challenge, was calculated for each animal at the time of the heterologous challenge. The day of onset of the febrile reaction was first ascertained and the mean daily temperature from the day of challenge to the day of onset of the reaction then determined. The total rise in °C above the average daily, pre-febrile temperature on each day of the ensuing reaction was recorded as the reaction index. An additional 10 points were added if the sheep showed clinical signs of anorexia, listlessness or dyspnoea.

Murinotropism of stocks

Seven of the stocks, the murinotropism and antibody response of which were poorly known, were inoculated into 6-week-old conventional inbred BALB/c mice. Three mice per stock were each inoculated i.v. with 0,3 m*l* of standardized blood stabilate. Mice that died were autopsied and histological sections were prepared from their lungs and myocardium. Those that survived were killed by ether inhalation 1 month after inoculation. Blood was collected for serology, and the spleens, lungs and myocardium of all the mice that had shown clinical signs were deep-frozen *in toto* in liquid nitrogen for future sub-inoculation into sheep. Except for Ball 3, whose pathogenicity to mice is known (Haig, 1952), the tissues of those mice that did not show clinical signs and were seropositive were homogenized in BLP and inoculated into susceptible sheep.

Infection of sheep and goats for serology

To determine the levels of antibody detectable with the IFA test in animals infected with 8 of the stocks, 2 seronegative sheep or goats per stock were inoculated i.v. with 5 m*l* of the standardized blood stabilates. They were given a homologous challenge a month later and a heterologous challenge a month after that. Blood samples for serology were collected on the day of infection, 2 and 3 weeks thereafter, on the day of the homologous as well as on that of the heterologous challenge, and 4 weeks after the latter. The reaction index for each animal was calculated when they were infected and on the days of both the homologous and heterologous challenges. In the case of the primary infection, the animals were treated before the development of clinical signs, for which 10 points were added to the reaction index scores of these animals. No treatment was given at the homologous challenge, nor at the heterologous challenge, except for 3 animals to prevent them from succumbing to the challenge.

Serology

The sera of the mice, sheep and goats were subjected to the IFA test as previously described (Du Plessis & Malan, 1987a). Serum dilutions of 1:20 and 5 further four-fold dilutions were tested. All the animals were seronegative on the day of infection.

Results

Reactions to infection and homologous challenge

All 245 sheep reacted to the primary infection and some of them were treated once or twice. Seventy-two per cent of the animals showed a mild to moderate reaction to the homologous challenge. There was no difference between the proportion of the 72 % sheep that subsequently reacted to the heterologous challenge and that of the animals that did not react to the homologous challenge. Twenty-two per cent of the sheep that had reacted to the homologous challenge had a reaction index of 10 or higher when they were subjected to the heterologous challenge, against 26 % of the sheep that failed to react to the homologous challenge.

TABLE 1 Resistance to heterologous challenge of 245 sheep immune to 10 stocks of *C. ruminantium*

Stock to which immune	No. of sheep	Heterologous stock challenge	No. reacting to heterologous stock				
			Died	Reaction index			Failed to react
				>20	>10	<10	
Ball 3	9	Breed			2	4	3
Ball 3	5	Comoro				3	2
Ball 3	5	Germishuys				3	2
Ball 3	9	Kümm	5	4			
Ball 3	8	Kwanyanga			3	4	1
Ball 3	4	Mali	2	2			
Ball 3	10	Mara		1	3	4	2
Ball 3	8	Nonile		1	1	6	
Ball 3	9	Welgevonden		1	4	3	1
Breed	4	Ball 3					4
Breed	2	Kümm		2			
Breed	1	Welgevonden		1			
Comoro	2	Ball 3					2
Comoro	6	Welgevonden	1		4	1	
Germishuys	6	Ball 3				1	5
Germishuys	1	Kümm		1			
Germishuys	6	Welgevonden			1	1	4
Kümm	10	Ball 3			4	6	
Kümm	2	Breed	2				
Kümm	2	Comoro	2				
Kümm	3	Germishuys	3				
Kümm	3	Welgevonden	2			1	
Kwanyanga	3	Ball 3				1	2
Kwanyanga	5	Welgevonden			1	2	2
Mali	1	Ball 3				1	
Mali	1	Kümm			1		
Mali	2	Welgevonden	1			1	
Mara	5	Ball 3			1		4
Mara	8	Welgevonden	2			3	3
Nonile	5	Ball 3				1	4
Nonile	6	Welgevonden			1	1	4
Welgevonden	14	Ball 3				7	7
Welgevonden	8	Breed	1			6	1
Welgevonden	19	Comoro	3		4	8	4
Welgevonden	15	Germishuys			4	6	5
Welgevonden	1	Kümm		1			
Welgevonden	8	Kwanyanga				3	5
Welgevonden	16	Mali	6	2	1	7	
Welgevonden	4	Mara				2	2
Welgevonden	9	Nonile				4	5
Total	245		30	16	35	90	74
%			12	21		37	30

TABLE 2 Cross protection between 10 stocks of *C. ruminantium*

Stocks to which sheep were immune	Challenge stock	
	Ball 3	Welgevonden
Ball 3	—	5/9[1] (0/14)[2]
Breed	0/ 4 (2/ 9)	1/1 (1/ 8)
Comoro	0/ 2 (0/ 5)	5/6 (7/19)
Germishuys	0/ 6 (0/ 5)	1/6 (4/15)
Kümm	4/10 (9/ 9)	2/3 (1/1)
Kwanyanga	0/ 3 (3/ 8)	1/5 (0/ 8)
Mali	0/ 1 (4/ 4)	1/2 (9/16)
Mara	1/ 5 (4/10)	2/8 (0/ 4)
Nonile	0/ 5 (2/ 8)	1/6 (0/ 9)
Welgevonden	0/14 (5/ 9)	—

[1] 5/9 = 5 out of 9 sheep immune to Ball 3 were susceptible to challenge with Welgevonden

[2] (0/14) = in the reverse challenge not one out of 14 sheep immune to Welgevonden was susceptible to challenge with Ball 3

Reactions to cross-challenge

Seventy-four (30 %) out of 245 animals challenged with heterologous stocks failed to react. A mild reaction (reaction index <10) was recorded in 90 (37 %) of them, 51 (21 %) had moderate to severe reactions and 30 (12 %) died (Table 1). The 81 sheep in the last 2 categories were considered not to have been protected.

The number of sheep protected against challenge with either Ball 3 or Welgevonden after having been immunized with 10 stocks of *C. ruminantium*, are given in Table 2. In each instance, the result of the reverse challenge, i.e. the number of sheep immune to either Ball 3 or Welgevonden cross-challenged with the other stocks, are shown in brackets. In transferring the results recorded in Table 1 to Table 2, a reaction index of 10 or higher was considered failure of protection. This was based on the observation that the mean reaction of the sheep that died from the heterologous challenge, taking into account the febrile reaction only, was 9,4.

It can be seen that, on one hand, only 5 out of 50 (10 %) sheep immune to 9 of the stocks were susceptible to challenge with Ball 3, 4 of which had been immune to Kümm. On the other hand, 20 out of 46 (43 %) sheep were susceptible to challenge with Welgevonden. Although in the case of each of the 9 stocks one or more sheep failed to resist challenge with Welgevonden, those immune to Ball 3, Comoro and Kümm in particular showed little or no protection.

A second feature of the cross-protection between Ball 3 and the other stocks is that in the case of no fewer than 7 of them many more sheep immune to Ball 3 were susceptible to challenge with these 7 stocks than in the case of the reverse challenge.

TABLE 3 Susceptibility of BALB/c mice to 7 stocks of *Cowdria*

Stock	Clinical signs	Treated	No. that survived	Reaction of sheep inoculated with mouse tissue	Reciprocals of IFA test titres Mouse No.		
					1	2	3
Ball 3	No	No	3	Not done	80	80	80
Breed	No	No	3	Not done	−ive	−ive	−ive
Comoro	No	No	3	Reacted & died	−ive	320	−ive
Germishuys	No	No	3	No reaction	20	20	20
Kwanyanga	Yes	Yes	1	Not done	320	—	—
Mali	Yes	Yes	1	Not done	80	—	—
Mara	Yes	Yes	3	Not done	320	320	320

TABLE 4 Details of isolation and infectivity to mice of *C. ruminantium* stocks

Stock	Origin	Year of isolation	Original host	Infectivity to mice				
				Inoculation routes i.v. & i.p.*	Inoculation route i.v.	Asymptomatic carrier	Antibody response only	Antibody not detectable
Ball 3	Northern Transvaal, RSA	1952	Bovine			+		
Breed	Northern Transvaal, RSA	1983	Goat					+
Comoro	Comoro islands	1987	*A. variegatum* tick			+		
Germishuys	North-eastern Transvaal, RSA	1984	Sheep				+	
Kümm	North-eastern Transvaal, RSA	1971	Goat	+				
Kwanyanga	Eastern Cape, RSA	1981	Sheep		+			
Mali	Moribabougou, Mali	1985	*A. variegatum* tick		+			
Mara	Northern Transvaal, RSA	1987	*A. hebraeum* tick		+			
Nonile	Natal, RSA	1984	Sheep		+			
Welgevonden	Northern Transvaal, RSA	1985	*A. hebraeum* tick		+			

* i.v. = intravenous; i.p. = intraperitoneal

Noteworthy exceptions were the Comoro and Germishuys stocks, where 5 out of 5 sheep immune to Ball 3 were fully resistant to these 2 stocks. This contrasted sharply with the reactions of 19 and 15 sheep immune to Welgevonden of which 7 and 4 respectively were susceptible to challenge with Comoro and Germishuys.

Murinotropism of stocks

It can be seen from Table 3 that the mice inoculated with Kwanyanga, Mali and Mara showed clinical signs and were treated. In spite of having been treated, only 1 out of 3 mice survived in the case of both Kwanyanga and Mali. Colonies of *C. ruminantium* were demonstrable in histological sections prepared from the lungs and myocardium of the mice that died. Both mice that survived and the 3 infected with Mara were seropositive. None of the mice inoculated with the other 4 stocks showed any clinical signs.

The sheep, inoculated with the tissues of the mice infected with Comoro, showed a severe febrile reaction that commenced 12 days after inoculation, lasted for 6 days and attained a maximum of 41,6 °C. The sheep died, and at autopsy its brain smear was positive for heartwater. One of the 3 mice, the tissues of which was injected into the sheep, was seropositive, but the other 2 negative.

The sheep, inoculated with the tissues of the mice infected with Germishuys, failed to show a febrile reaction, but low levels of antibody were detected in the serum of all 3 mice. All 3 mice infected with Ball 3 were seropositive, but those inoculated with Breed infected sheep blood were seronegative.

The murinotropism of the 10 stocks is summarized in Table 4.

Serological response to Cowdria *stocks*

The reaction indices at the time of the primary infection and both the homologous and heterologous challenges of 13 sheep and 3 goats infected with 8 stocks are given in Table 5. It can be seen that 6 of the animals reacted to the homologous and 10 to the heterologous challenge. Mortalities or reaction indices of 10 or higher were recorded in 6 of these 10, 2 of which were cross-challenged with Welgevonden, 2 with Kümm and 1 each with Germishuys and Mali.

The antibody titres detected with the IFA test in the sera of these animals are also shown in Table 5. It is evident that of 9 animals tested, in all except 2 animals (Sheep 2 and 8), low levels of antibody were detectable 2 weeks after infection. At 3 weeks post-infection (p.i.), titres were generally higher and even at their maximum in the case of 5 animals (Sheep 1, 2, 3 and 9 and Goat 2). At 4 weeks p.i., on the day when the homologous challenge was given, titres varied from as low as 1/80 for Sheep 2 (Comoro) and 9 (Mali) to 1/1280 in the case of Goat 1 and Sheep 3 (Comoro), 5 (Kümm) and 12 and 13 (Welgevonden) and even 1/5120 for Goat 2 (Germishuys) and Sheep 11 (Mara). Sheep 6, infected with Kümm, was inadvertently treated before a distinct febrile reaction had commenced. Hence the comparatively low reaction index of 15,2 and the low antibody level of 1/20.

The titres, recorded on the day of the heterologous challenge, reflecting the influence of the homologous challenge on the antibody response, were in the majority of cases lower than those recorded before this challenge. The only exceptions were Sheep 2, 3, 9 and 10, where antibody levels remained unchanged, and Sheep 6, that had a higher titre for the reason already explained.

TABLE 5 Reaction indices and serological response of sheep and goats infected with 8 stocks of *C. ruminantium* and subsequently cross-challenged

Animal No.	*Cowdria* stock	Febrile reaction to infection			Reaction indices to		Reciprocals of IFA test titres				Heterologous challenge stocks
		Day of onset	Days p.i. treated	Reaction index	Homologous challenge	Heterologous challenge	2 weeks p.i.	3 weeks p.i.	Day of homologous challenge	Day of heterologous challenge	4 weeks after heterologous challenge	
Sheep 1(D)[1]	Ball 3	8	11	24,5	0	Died	20	320	320	80	—[3]	Kümm
Goat 1	Ball 3	10	12	18,1	0	19,1[2]	20	320	1 280	320	20 480	Mali
Sheep 2(D)	Comoro	11	14+18	24,7	0	0	–ive	80	80	80	80	Ball 3
Sheep 3(D)	Comoro	11	14	22,8	0	8,1	20	1 280	1 280	1 280	80	Ball 3
Sheep 4(D)	Germishuys	8	10	21,7	1,9	0	—	320	320	80	—	Welgevonden
Goat 2	Germishuys	10	12	27,7	0	12,8	20	5 120	5 120	1 280	1 280	Kümm
Sheep 5(D)	Kümm	12	18	23,3	0	16	20	80	1 280	320	1 280	Welgevonden
Sheep 6(M)[1]	Kümm	12	15	15,2	9,5	Died	—	—	20	1 280	—	Welgevonden
Sheep 7(D)	Kwanyanga	8	10	22,9	7	0	20	80	320	20	20	Welgevonden
Sheep 8(D)	Kwanyanga	10	12	22,5	17,8	3,1	–ive	20	320	80	20	Welgevonden
Sheep 9(D)	Mali	10	12	25,1	3,3	8,6	20	80	80	80	80	Ball 3
Goat 3	Mali	Died in spite of treatment on day 3 of febrile reaction										
Sheep 10(M)	Mara	9	11	22,8	0	0	—	—	320	320	—	Welgevonden
Sheep 11(M)	Mara	10	13	23,9	0	0	—	—	5 120	320	—	Welgevonden
Sheep 12(M)	Welgevonden	8	13	23	0	8,8	—	—	1 280	320	—	Comoro
Sheep 13(M)	Welgevonden	8	13	24,6	5	11,5	—	—	1 280	320	—	Germishuys

[1] (D) = Dorper; (M) = Merino

[2] Animal treated during heterologous challenge

[3] — = not done

Titres of sera collected 4 weeks after heterologous challenge were either unchanged or lower than on the day of cross-challenge. Noteworthy exceptions were Sheep 5 and Goat 1 where titres had risen.

It is interesting to note that in general, and irrespective of the stock with which they were infected, antibody levels recorded in the 2 goats and the Merino sheep were considerably higher than those detected in the Dorper sheep. The only Dorper sheep with a high antibody titre was Sheep 3.

Discussion

This study suggests that the antigenic diversity of *C. ruminantium* stocks is perhaps even more marked than might have been suspected from earlier reports (Jongejan *et al.*, 1988). Out of the 10 stocks investigated, only 3, Ball 3, Comoro and Germishuys, appear to be closely related, since both Comoro and Germishuys gave full protection against Ball 3 and in the reverse challenge sheep immune to the latter were solidly immune against Comoro and Germishuys. The other stocks, however, all differed to varying degrees. Some were more divergent than others so that there was a spectrum of antigenic diversity. There were several stocks—Breed, Kwanyanga, Mali, Nonile and Welgevonden—that protected against Ball 3, but 22 % (Breed) to 100 % (Mali) sheep immune to Ball 3 were susceptible to the reverse challenge. This was also the case with a stock isolated from cattle in Senegal (Jongejan *et al.*, 1988).

In this respect, Welgevonden differed markedly from Ball 3. While not 1 of the 9 stocks protected all the sheep against challenge with Welgevonden, all the animals immune to Welgevonden were also fully immune against no fewer than 4 stocks: Ball 3, Kwanyanga, Mara and Nonile. Welgevonden protected poorly, however, against Comoro (37 % susceptible), Germishuys (27 % susceptible) and Mali (56 % susceptible).

Although Ball 3, Comoro and Germishuys are antigenically closely related, they do not appear to be identical, if one compares their relationship to Welgevonden. Comoro and Germishuys may be identical in that a significant number of sheep immune to Welgevonden were susceptible to challenge with these 2 stocks, but Welgevonden protects against Ball 3 in every case.

Opposite Ball 3, Comoro and Germishuys, the Kümm stock occupies the other extremity of the antigenic spectrum in that it is antigenically different from all other stocks to which it has so far been compared. It does not protect at all against Breed, Comoro, Germishuys or Welgevonden and in 60 % of cases against Ball 3. There is also no cross-protection between Kümm and Kwanyanga (Logan, Birnie & Mebus, 1987). In the reverse situation, sheep, immune to Ball 3, Breed, Germishuys, Mali and Welgevonden, were fully susceptible to challenge with Kümm. Furthermore, Logan, Birnie and Mebus (1987) found that neither Kwanyanga nor Mali, nor the Gardel stock, isolated on the Caribbean island of Guadeloupe (Uilenberg *et al.*, 1985), protect against Kümm.

Although not as radically different from other stocks as Kümm, Welgevonden also broke through the immunity of at least some of the sheep that had recovered from infection with each of the other 9 stocks. Inversely, itself did not protect against Breed, Comoro, Germishuys, Kümm and Mali.

The finding that not a single sheep immune to Ball 3 and only 44 % of those immune to Welgevonden were protected against challenge with the Mali stock, suggests that this stock also differs markedly from the others. It has, however, been compared with too few of the other stocks to place it with certainty in this region of the spectrum. The Breed, Kwanyanga, Mara and Nonile stocks probably occupy an intermediate position, but they too have not been sufficiently investigated.

It has been suggested that the pathogenicity of *Cowdria* stocks to mice may be linked to antigenic differences with the reference Ball 3 stock (Jongejan, Uilenberg & Franssen, 1988). The fact that the Senegal stock, antigenically different from Ball 3, can be serially passaged in mice is advanced in support of this theory (Jongejan, unpublished observation, 1989), but in the present study the observation that there is total cross-immunity between Ball 3 and Comoro, both of which like Senegal cause a subclinical infection in mice, does not support this hypothesis. Since the murinotropism of *Cowdria* stocks is relative and stretches across a spectrum, it is questionable whether pathogenicity to mice can be used as a criterion.

Furthermore, because the Germishuys stock elicits antibodies in mice, it is close to Ball 3, Comoro and Senegal in the murinotropism spectrum. In its cross-immunity with Ball 3, though, it differs from Senegal and therefore also questions the validity of relating pathogenicity to mice to antigenic differences with Ball 3 or for that matter with any other stock. In our view there is no clear-cut relationship between the murinotropism of *Cowdria* stocks and their antigenic diversity.

The murinotropism of the 10 stocks of *C. ruminantium* investigated in this study is classified into 5 categories in Table 4. In order of pathogenicity to mice, it can be seen that, whereas the Germishuys stock elicits an antibody response without establishing a carrier status, Breed failed to do so. Ball 3 (Haig, 1952) and Comoro induce an asymptomatic carrier status as well as an antibody response in mice. The majority of the stocks—Kwanyanga, Mali, Mara, Nonile and Welgevonden—administered along the i.v. route, cause fatal infection in mice. The observation that the Mali stock is pathogenic to mice differs from that of Logan, Endris, Birnie & Mebus (1985). Kümm is the most pathogenic stock to mice, irrespective of whether injected i.v. or i.p.

This classification differs somewhat from that of MacKenzie & McHardy (1987) in that the use of the IFA test made it possible to differentiate the Ball 3 and Comoro stocks that infect mice sub-clinically, from Germishuys on one hand, which is unable to do so but which elicits an antibody response, and on the other from the Breed stock which fails to elicit antibody levels detectable with the IFA test. The 2 classifications are supplementary and together record the murinotropism of some 20 stocks, thereby illustrating the wide variation in pathogenicity. It must be pointed out that the Mara stock (Haig, 1952) in the classification by MacKenzie & McHardy (1987) was isolated some 35 years earlier than the stock by the same name referred to in the present study and that was isolated on the same farm.

Serology contributed little towards a better understanding of the complexity of the antigenic differences between *Cowdria* stocks. The serum of the sheep and goats infected with 8 stocks all reacted positively in the IFA test in which Kümm stock infected mouse peritoneal macrophages are used as antigen. This finding differs from that of Jongejan, Wassink, Thielemans, Perie & Uilenberg (1989), who found that with the IFA test, developed by Logan, Whyard, Quintero & Mebus (1987), the sera of an unspecified number of goats immune to Kwanyanga and Kümm did not react with Senegal antigen and some or all of the goats immune to the Senegal, Ball 3 and Kwanyanga stocks were seronegative against Welgevonden antigen.

Although antibody titres varied from as low as 1/80 for the Comoro and Mali stocks to 1/1280 for Ball 3, Comoro, Kümm and Welgevonden and even 1/5120 for Germishuys and Mara, variation in titres for several reasons cannot be used to differentiate between antigenically different stocks. First, it depends on whether titres recorded on sera collected before or after the homologous challenge are considered in this respect, since we found that in the case of sera against several stocks there was a drop in the IFA test titre after the homologous challenge. Second, in more than 1 instance the antibody response in this study varied in the 2 animals immunized with the same stock, possibly due to species or breed differences, since higher antibody titres were recorded in goats and Merino sheep than in Dorper sheep. It can therefore not be concluded for example that Mara and Welgevonden are antigenically closer to Kümm than Comoro is to Kümm because higher titres were recorded for the former 2 stocks than for Comoro. This would also be inconsistent with the total absence of cross-immunity between Kümm and the other 3 stocks.

Thirdly, variation in antibody levels detectable with the Kümm-infected peritoneal macrophage IFA test is also related to the severity of the reaction to infection with *C. ruminantium* (Du Plessis & Malan, 1987b) and to the degree to which the heartwater agent replicates (Du Plessis & Malan, 1987a). Further support for this conclusion in the present study is the observation that only in the case where high reaction indices to the heterologous challenge were recorded, was there a substantial rise in the antibody levels of sera collected 4 weeks after the heterologous challenge. This is also consistent with the high titres recorded in sheep challenged with Ball 3 after having been immunized against Kümm and vice versa (Du Plessis, 1982).

Until recently the Kümm stock was considered atypical in its antigenic differences from other stocks, its exceptional pathogenicity to mice and its absence of pathogenicity to cattle. It was even suggested that it might be justifiable to consider it to be a 2nd species of *Cowdria* (Du Plessis, 1982). In view of the subsequent isolation of the Kwanyanga, Mali, Mara, Nonile and, particularly, the Welgevonden stocks, all of them pathogenic to mice and antigenically different from one another, Kümm no longer appears to be so atypical. The conclusion that Kümm is just one of the continually growing number of stocks of *C. ruminantium* rather than a species of *Cowdria* seems justified. The fact that Kümm antigen can be used to detect appreciable levels of antibody in the serum of sheep immune to 8 different stocks is further justification. The practical implication of this is that the Kümm-infected antigen used at present in the IFA test is suitable to detect antibodies to natural infection by ticks infected with a wide range of stocks. It must be added, however, that in rare instances ticks infected with stocks that fail to elicit an antibody response in mice detectable

with the IFA test, like the Breed stock, may escape detection with the method used at present to determine the *C. ruminantium* infection rate of ticks (Du Plessis, 1985).

From a cross-immunity, serological and mouse pathogenicity point of view, there does not seem to be any pattern in the antigenic diversity of *Cowdria* stocks. There are no 2 stocks that are identical, because even though Ball 3, Comoro and Germishuys are mutually cross-protective, they differ in their relationship to Welgevonden which protects against Ball 3 but not against Comoro and Germishuys. There is also no geographic relationship, since on one hand the above 3 cross-protective stocks were isolated far apart, and, on the other, stocks that were isolated from the same region such as Kümm and Germishuys from north-eastern and Ball 3, Breed and Mara from the far northern Transvaal, are antigenically different.

Alexander (1931) once remarked, and the present study seems to confirm his conclusion, that there does not appear to be a master stock that protects against all other stocks and thereby enable more efficient vaccination. In view of the inadequate protection that Ball 3 gives against no fewer than 5 other stocks apart from Kümm, and considering stubborn complaints by farmers over many years of losses in vaccinated cattle and small stock, the replacement of Ball 3 by Welgevonden in the vaccine deserves consideration. The inability of the latter to protect against Comoro, Germishuys and Mali is, on one hand, compensated for by the fact that 2 of these originate from outside the Republic of South Africa. On the other it is now evident that there are probably many more stocks in nature, against some of which Welgevonden may not protect. Although apparently limited in distribution, Kümm stock for one is neither controlled by immunization with Welgevonden nor by any other stock. There are, however, several practical considerations, outside the scope of this discussion, that would necessitate prudence in a change of vaccine stock. The known high pathogenicity of Welgevonden coupled to its countrywide distribution in a vaccine are important in this respect.

The heterogenicity of *Cowdria* stocks renders effective vaccination against heartwater even more difficult. The fact that so few stocks elicit a solid immunity against even only 1 other stock indicates the extremely narrow specificity of protective immunity in heartwater; narrow, in the sense that only a particular antigen or a specific combination of antigens specific to each stock elicits a solid immunity against it. Not only does it seem that a particular inoculum of *Cowdria* must be pathogenic and capable of causing a reaction to elicit protective immunity (Du Plessis & Malan, 1987b), but its antigenic composition must also be very specific. This makes effective vaccination all the more exacting and is one good reason why the best possible use should be made of natural immunization through ticks infected with the local field stock, which implicates the practice of strategic tick control (Bezuidenhout & Bigalke, 1987).

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