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RESEARCH COMMUNICATION

AN ATTEMPT TO IMPROVE THE IMMUNIZATION OF SHEEP AGAINST HEARTWATER BY USING DIFFERENT COMBINATIONS OF 3 STOCKS OF *COWDRIA RUMINANTIUM*

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ABSTRACT

DU PLESSIS, J. L., POTGIETER, F. T. & VAN GAS, L., 1990. An attempt to improve the immunization of sheep against heartwater by using different combinations of 3 stocks of *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 57, 209–212 (1990)

Neither sheep immune to the Ball 3, the Kwanyanga or the Mara stocks of *Cowdria ruminantium* nor those immunized with combinations of these 3 stocks were protected against challenge with the Mali stock. Against challenge with the Welgevonden stock, however, immunization with each of the 3 combinations and with single stocks effected a protective immunity.

INTRODUCTION

The blood of sheep infected with the Ball 3 stock of *Cowdria ruminantium* is issued at present as a vaccine against heartwater (Oberem & Bezuidenhout, 1987). A recent study on the cross-immunity between 10 different stocks of *C. ruminantium* (Du Plessis, Van Gas, Olivier & Bezuidenhout, 1989), however, has shown that sheep immune to Ball 3 were only partially, or even unprotected, against challenge with 7 other stocks.

The apparent inability of the Ball 3 stock to protect adequately against other stocks under laboratory conditions may be the reason why vaccinated small stock and even cattle still succumb to heartwater following natural tick challenge. It was suggested that the Ball 3 stock should perhaps be replaced by another with a wider range of protection (Du Plessis *et al.*, 1989). It was also clear from the same study, however, that none of the the 10 stocks investigated elicited an adequate immunity against challenge with each of the others. It was therefore decided to determine whether the concurrent infection with 2 stocks would widen the immunogenic stimulus and induce a better immunity against heterologous challenge.

MATERIALS AND METHODS

Immunizing stocks

The Ball 3 (Haig, 1952), Kwanyanga (MacKenzie & Van Rooyen, 1981) and Mara (Du Plessis *et al.*, 1989) stocks were selected to compose the 3 possible combinations. The first was selected because it is considered a reference stock (Jongejan, Uilenberg & Franssen, 1988) and because much experience has been gained in its use as a vaccine. The other 2 stocks were chosen because immunization with both conferred good immunity against challenge with Ball 3 and reasonably good protection against the virulent Welgevonden stock (Du Plessis *et al.*, 1989). Furthermore, the fact that both these stocks can be propagated in mice would greatly facilitate control of the infectivity of a possible future combination vaccine consisting of these 2 stocks.

Ten heartwater susceptible sheep were immunized with each of the 3 possible combinations, viz., Ball 3/Kwanyanga, Ball 3/Mara and Mara/Kwanyanga. Each sheep was inoculated intravenously with 5 mℓ of blood comprising 2,5 mℓ of each stock. As controls, 4 sheep were infected with 2,5 mℓ of the infective sheep blood of each of the 3 stocks. Early morning rectal temperatures were recorded and treatment with oxytetracycline at a dosage level of 10 mg/kg body mass was given intramuscularly on the 2nd or 3rd day of the febrile reaction. If considered necessary, a second treatment was given.

Challenge stocks

Six weeks after having been infected with the combined stocks, 5 sheep, immunized with each of the 3 combinations, were challenged with the Welgevonden (Du Plessis, 1985) and 5 with the Mali stock (Logan, Endris, Birnie & Mebus, 1985) at a dosage rate of 5 mℓ, given intravenously. Although the Mali stock was isolated outside the Republic of South Africa, these 2 highly virulent stocks were chosen as challenge stocks because it was reasoned that the protection conferred by 1 or more of the 3 combinations against 1 or both of these 2 challenge stocks, would also possibly protect against a wide range of field stocks. Temperatures were again recorded and a reaction index, reflecting the degree of immunity to challenge, was calculated for each animal, as previously described (Du Plessis *et al.*, 1989).

Two sheep of each of the 3 control groups infected with the single stocks were likewise challenged with the Welgevonden and 2 with the Mali stock.

An additional control sheep was inoculated with 5 mℓ of the Mali stock infected stabilate, used as challenge material, and another with the Welgevonden stabilate.

RESULTS

The febrile reactions of the 30 sheep, infected with the 3 combinations, and the 12 control animals, inoculated with the single stocks are given in Table 1. It can be seen that all the sheep reacted severely and that all the animals were treated either once or twice. One out of 4 control sheep infected with Kwanyanga died, in spite of having been treated once. There was very little variation between the average incubation period of each combination: 10, 9,5 and 10,4 days, respectively, for Ball 3/Mara, Mara/Kwanyanga and Ball 3/Kwanyanga. There was also no difference between these averages and those recorded in the case of the single infections: 10, 10,5 and 11 days, respectively, for Ball 3, Mara and Kwanyanga.

The reaction indices, reflecting the resistance to heterologous challenge of the 30 sheep immunized with the combinations, are also shown in Table 1 and summarized in Table 2.

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TABLE 1 Reactions of sheep at infection and when they were challenged

Sheep No.	Immunizing stock(s)	Febrile reaction at infection			Treatment	Challenge stock	Reaction index at challenge
		Day of onset	Duration in days	Maximum temp. °C			
1	Ball 3/Mara	11	6	41,4	2,6*	Welgevonden	2,3
2	Ball 3/Mara	9	7	41,2	3,5	Welgevonden	4,5
3	Ball 3/Mara	10	6	41,2	2	Welgevonden	3
4	Ball 3/Mara	9	6	41,9	3	Welgevonden	7,6
5	Ball 3/Mara	11	7	41,5	2	Welgevonden	0
6	Ball 3/Mara	9	9	41,4	3	Mali	24,9 Died
7	Ball 3/Mara	10	8	41,6	3,7	Mali	26,6 Died
8	Ball 3/Mara	11	8	41,9	3,5	Mali	28,5 Died
9	Ball 3/Mara	10	9	41,5	3	Mali	28,4 Died
10	Ball 3/Mara	10	8	41,4	2,7	Mali	8,8
11	Mara/Kwanyanga	9	4	41,2	3	Welgevonden	0
12	Mara/Kwanyanga	9	5	41,8	3	Welgevonden	9,4
13	Mara/Kwanyanga	9	5	41,7	3	Welgevonden	6,6
14	Mara/Kwanyanga	9	5	42,0	3	Welgevonden	2,2
15	Mara/Kwanyanga	9	7	41,7	3	Welgevonden	3,1
16	Mara/Kwanyanga	9	8	41,5	3	Mali	28 Died
17	Mara/Kwanyanga	10	6	41,7	3	Mali	29,2 Died
18	Mara/Kwanyanga	10	6	41,6	2	Mali	25,5 Died
19	Mara/Kwanyanga	11	6	41,4	3	Mali	28,7 Died
20	Mara/Kwanyanga	10	5	41,7	3,4	Mali	32,1 Died
21	Ball 3/Kwanyanga	11	7	41,5	2	Welgevonden	12,4
22	Ball 3/Kwanyanga	10	7	41,4	3	Welgevonden	0
23	Ball 3/Kwanyanga	10	7	41,4	3	Welgevonden	5
24	Ball 3/Kwanyanga	10	7	41,4	2	Welgevonden	0
25	Ball 3/Kwanyanga	10	8	41,3	3	Welgevonden	1,6
26	Ball 3/Kwanyanga	11	6	41,4	2,4	Mali	30,3 Died
27	Ball 3/Kwanyanga	10	6	41,9	2,4	Mali	27,6 Died
28	Ball 3/Kwanyanga	11	7	42,1	2	Mali	2,6
29	Ball 3/Kwanyanga	11	5	41,8	2	Mali	26,5 Died
30	Ball 3/Kwanyanga	10	6	41,5	3,5	Mali	6,1
31	Ball 3 control	10	6	41,5	4,5	Welgevonden	4,6
32	Ball 3 control	10	8*	41,4	3,7	Welgevonden	7
33	Ball 3 control	10	6	40,9	3	Mali	4,7
34	Ball 3 control	10	6	41,6	2	Mali	34,2 Died
35	Mara control	10	5	41,5	3	Welgevonden	7,2
36	Mara control	12	6	41,4	2,3	Welgevonden	0
37	Mara control	10	11	41,3	3,7	Mali	26 Died
38	Mara control	9	8	41,5	3,5	Mali	28,8 Died
39	Kwanyanga control	11	7	41,8	3	Died from immunizing infection	
40	Kwanyanga control	10	5*	40,6	3,5	Welgevonden	0
41	Kwanyanga control	11	5	41,9	3	Mali	31,7 Died
42	Kwanyanga control	12	4	42,0	3	Mali	7,2
43	Challenge control	—	—	—	—	Welgevonden	Died
44	Challenge control	—	—	—	—	Mali	Died

* Sheep 1 was treated on Days 2 and 6 of the febrile reaction

TABLE 2 Resistance to challenge with the Welgevonden and Mali stocks of sheep infected with different combinations of 3 stocks and singly as controls

		Challenge stock			
		Welgevonden		Mali	
		Susceptible[1]	Resistant	Susceptible	Resistant
Immunization stock combinations	Ball 3 & Mara (10) [2]	0	5	4	1
	Mara & Kwanyanga (10)	0	5	5	0
	Ball 3 & Kwanyanga (10)	1	4	3	2
Controls	Ball 3 (4)	0	2	1	1
	Kwanyanga (3)	0	1	1	1
	Mara (4)	0	2	2	0

[1] Sheep with a reaction index of 10 or higher were considered susceptible

[2] (10) = 10 sheep immunized with the Ball 3/Mara combination were challenged with Welgevonden and Mali

In transferring the reaction indices recorded in Table 1 to Table 2, a reaction index of 10 or higher was considered as failure of protection and that the sheep was therefore susceptible. This criterium was used and motivated in an earlier study (Du Plessis *et al.*, 1989).

It can be seen that all the sheep, except 1 infected with the Ball 3/Kwanyanga combination, were resistant to challenge with the Welgevonden stock, whereas the reverse situation prevailed in the case of the 15 sheep challenged with the Mali stock. Twelve sheep immune to the 3 combinations were fully susceptible and succumbed to the challenge. Only 1 animal, immune to the Ball 3/Mara combination, and 2, immune to the Ball 3/Kwanyanga combination, were resistant to Mali.

The resistance to challenge with either Welgevonden or Mali of the singly infected control animals

was no different from that elicited by the combinations. All 5 sheep challenged with Welgevonden were resistant and 4 out of 6 challenged with Mali had no immunity.

Both control sheep infected with the 2 challenge stocks reacted and died, their brain smears being positive for heartwater.

DISCUSSION

It would appear that, irrespective of the different combinations, the immunization of sheep against heartwater by the concurrent infection of 2 stocks of *C. ruminantium* gives no better protection than when given singly. With the exception of 2 out of 5 sheep immunized with the Ball 3/Kwanyanga combination, all the animals infected with the 3 possible combinations used in this study were fully susceptible to challenge with the Mali stock. This is disappointing, since in the case of the controls immunized with single infections of Ball 3 and Kwanyanga, 1 out of 2 sheep was also resistant to this challenge. In an earlier study (Du Plessis *et al.,* 1989), 4 sheep infected and re-infected with Ball 3 had no protection against challenge with Mali. But then, only 7 out of 16 sheep immune to the Welgevonden stock were resistant to challenge with Mali. It is therefore evident that, while the Ball 3/Kwanyanga combination protected 2 out of 5 sheep against challenge with this virulent stock, the other 2 combinations had no beneficial effect.

Perhaps Mali was an unfortunate choice as a challenge stock, but its use has at least shown that not only is its origin geographically widely different from that of the 3 South African stocks, it is also antigenically totally different from them.

Against challenge with another virulent stock, the Welgevonden stock, the 3 combinations protected almost equally well. But here again, the immunity elicited by the 3 stocks as single infections was as good as that given by the combinations. Although only 2 sheep per stock were challenged with Welgevonden, the observations in the present study differ somewhat from those recorded earlier, when larger numbers of sheep immune to the 3 stocks in question were challenged with Welgevonden (Du Plessis *et al.,*1989). Particularly in the case of the Ball 3 immune sheep, 56 % of the animals then were susceptible to challenge with Welgevonden. One out of 5 sheep immune to Kwanyanga and 2 out of 8 immune to Mara were also not protected against Welgevonden. This is difficult to explain, particularly if one bears in mind that in this earlier study the sheep had been given a homologous challenge before they were subjected to the Welgevonden challenge.

Because the aim of the present trial was to simulate the immunization of sheep in a field situation, where a successful combination vaccine would be administered only once, the sheep were not given a homologous challenge. It is doubtful whether this procedure would have enhanced their immunity against the Mali challenge, since it has previously been found that there was no difference between the percentage of animals that reacted to the heterologous challenge with several stocks after having shown mild to moderate reactions to the homologous challenge and that of the sheep that had failed to react to the homologous challenge (Du Plessis *et al.,* 1989).

Because it is known that in experimentally induced heartwater pathogenicity parallels immunogenicity and that the severer the reaction to infection the better the immunity to subsequent homologous challenge (Du Plessis & Malan, 1987), care was taken in this study not to treat the sheep unnecessarily early. Despite treatment, all the sheep showed severe reactions. This and the fact that all the sheep infected with the combined stocks were immune to challenge with the Welgevonden stock, suggest that the absence of immunity against the Mali stock was not attributable to poor or unduly hampered reactions to the concurrent infections.

Of crucial importance in this respect is whether both stocks in each combination had developed and replicated in parallel with one another, so that the immunogens of both stocks participated to the same extent in the expression of the immune response. The observation that there was no real difference between the average incubation period of each combination on one hand and between these averages and those recorded in case of the control single infections on the other, suggests that both stocks contained in the combinations probably participated in parallel. It may be argued that this could have been verified by immunizing a further 2 control sheep with each of the 3 combinations and subsequently submitting 1 sheep to challenge with one of the stocks, comprising the combination, and the 2nd sheep with the other stock. If, however, it should e.g. be found that the sheep immunized with the Ball 3/Mara combination is immune against challenge with Ball 3, this would not necessarily prove that the Ball 3 moiety of the combination had participated in the immune response, since it has previously been found that 4 out of 5 sheep immune to Mara were also immune to Ball 3 (Du Plessis *et al.,* 1989). Much the same applies to the Ball 3/Kwanyanga combination.

It has been stated that there does not seem to be any pattern in the antigenic diversity of *Cowdria* stocks (Du Plessis *et al.,* 1989). The present study confirmed this and has shown that there is sometimes also a lack of consistency in the cross-immunity between stocks, as evidenced by the singly immunized controls challenged with the Welgevonden stock. Despite the small number of these controls, and well aware of the shortcoming in this experiment that it could not be proved beyond doubt that both stocks in each of the combinations had participated in the immune response, it is nevertheless suggested that there is no justification to replace the Ball 3 stock in the present vaccine with any one of the 3 combinations examined in this study.

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