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VIRULENCE AND HETEROLOGOUS STRAIN IMMUNITY OF SOUTH AFRICAN AND AUSTRALIAN *BABESIA BOVIS* STRAINS WITH REDUCED PATHOGENICITY

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ABSTRACT

DE VOS, A. J., BESSENGER, R. & FOURIE, C. G., 1982. Virulence and heterologous strain immunity of South African and Australian *Babesia bovis* strains with reduced pathogenicity. *Onderstepoort Journal of Veterinary Research*, 49, 133–136 (1982).

A South African *Babesia bovis* strain showed loss of virulence after 10 rapid passages in splenectomized calves. The virulence was comparable with that of an attenuated Australian vaccine strain. Vaccination of cross-bred *Bos indicus* cattle with the local strain resulted in a solid immunity to heterologous challenge. The degree of protection afforded by the Australian vaccine strain was adequate for controlling challenge with a virulent South African strain, but somewhat less than the degree conferred by the local vaccine strain. Serological observations with the indirect fluorescent antibody test confirmed a close relationship between the 2 modified strains.

INTRODUCTION

A babesiosis vaccine, consisting of infected blood harbouring virulent strains of both *Babesia bovis* and *Babesia bigemina*, has been issued from this Institute for many years. This vaccine has not been entirely satisfactory because, amongst other reasons, severe reactions were seen in some vaccinated animals (De Vos, 1978).

Recently, Callow, Mellors & McGregor (1979) reported reduced virulence of Australian *B. bovis* strains after rapid passage in splenectomized calves. The purpose of the present study was to compare the virulence and degree of resistance afforded by a South African strain passaged as described by Callow *et al.* (1979) with that of an attenuated strain used for vaccine production in Australia.

MATERIALS AND METHODS

Animals

The animals used in this study were obtained from a private farm in southern South-West Africa/Namibia where *Boophilus* spp., and therefore *B. bovis* and *B. bigemina*, were absent. Only animals 1–2 years old and serologically negative for both *Babesia* spp. were used. The animals, all cross-bred *Bos indicus* cattle, were alloted to the different experimental groups based on body mass.

Strains of B. bovis

1. The South African unmodified F strain was isolated from experimentally produced, tick-transmitted cases (De Vos, 1978) and preserved as stabilate in the gas phase of a liquid nitrogen refrigerator. ACD (citric acid, sodium citrate, dextrose solution) was used as anticoagulant and dimethyl sulphoxide as cryo-protectant (De Vos, Combrink & Bessenger, 1982).

2. In an attempt to reduce its virulence, the South African S strain (De Vos, 1978) was passaged rapidly 10 times in splenectomized calves as described by Callow *et al.* (1979). After passage, this strain was stored as stabilate in a liquid nitrogen refrigerator. It has been the only *B. bovis* strain used since 1978 in the Onderstepoort babesiosis vaccine and is produced from blood of splenectomized calves inoculated with stabilate of Passage 10.

3. The Australian attenuated vaccine K strain (Dalgliesh, Stewart & Duncalfe, 1981) was obtained as Passage 23 in the form of infected unfrozen blood from the Tick Fever Research Centre, Wacol, Queensland. On receipt, it was passaged once in a splenectomized calf before being stored in a liquid nitrogen refrigerator as stabilate of Passage 24.

Method of infection

Before use, all the *Babesia* strains were passaged once in splenectomized calves and blood with suitable parasitaemias collected in ACD. The number of parasites/mℓ of infected blood was calculated from the percentage of infected erythrocytes, as determined in stained thin smears of jugular blood, and the red cell count obtained with the aid of a Coulter Counter*. The parasitaemia was adjusted with the addition of uninfected whole blood to ensure approximately 1×10^7 parasitized erythrocytes/2 mℓ inoculum. In all the experiments the animals were each inoculated subcutaneously with about 1×10^7 parasites.

Quantification of reactions

Maximum parasitaemia (based on a score ranging from 1–8), maximum PCV (packed cell volume) depression (expressed as a percentage of the norm) and mean total temperature rise were the parameters used to monitor reactions in the experimental groups as outlined by De Vos (1978). Student's 't' test was used to determine the significance of differences between the means of the different sets of figures.

Experimental procedure

A total of 40 animals were used as follows: 15 were vaccinated with the South African S and 5 with the Australian K strains. Primary vaccine reactions were monitored and, 26 days after vaccination, the animals were challenged with the F strain. As controls, 15 susceptible animals were infected with the F strain. To obtain the normal body temperature for every day, a further 5 animals acted as uninfected controls throughout.

Indirect fluorescent antibody test

The technique used for the serological comparisons has been described by Gray & De Vos (1981) and is essentially a modification of the technique of Joyner, Donnelly, Payne & Brocklesby (1972). Each antigen slide contained 2 rows of 6 wells and thick blood smears in the wells were used as antigen. Smears, prepared of both S and K strains, using blood of infected splenectomized animals with parasitaemias of 2–3%, were stored at −20°C and fixed in cold acetone before use.

Both groups of animals infected with the S and K strains were bled for serum 26 days after infection and again 28 days after challenge with the F strain. The sera were stored at −20°C before being tested.

One well on each slide was used for a negative control serum (giving no specific fluorescence at 1:40 dilution) and 1 well for a weak positive control serum (S strain at 1:40 dilution). Five pre- and post-challenge antisera of

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the S strain were compared with comparable antisera of the K strain in a series of 5 doubling dilutions, starting at 1:40. For control purposes 1 serum of each of the S and K strains was titrated in parallel on the same slide as outlined by Callow, Quiroga & McCosker (1976). If the end-point was not reached, a further series of 5 doubling dilutions was performed on a 2nd slide, starting at 1:640. The titre was taken as the reciprocal of the highest dilution giving specific fluorescence.

RESULTS

Virulence of 2 vaccine strains of B. bovis

The reactions of *Bos indicus*-cross cattle following vaccination with the S (Passage 11) and K (Passage 25) strains are summarized in Table 1 and compared with the reactions induced by the unmodified F strain. The means of the parameters observed did not differ significantly in the 2 vaccinated groups. However, the mean maximum PCV depression and mean total temperature rise of the vaccinated groups were significantly lower than the means of the group infected with the F strain. There was little difference between the parasitaemias of the different groups. None of the vaccinated animals were clinically affected, while 5 of the 15 animals infected with the F strain, though showing obvious clinical signs of disease, recovered without therapy.

Challenge of vaccinated cattle

When the group vaccinated with the S strain was challenged 26 days later with the unmodified F strain, the reactions were significantly less severe than those of the unvaccinated control group with regard to all 3 parameters recorded (Table 2). The group vaccinated with the K strain also had less severe reactions than the controls, but only the differences in PCV depression and total temperature were statistically significant (Table 2).

Of the 2 vaccine strains, the S strain had a greater ability to control a F strain challenge: the mean maximum parasitaemia and mean PCV depression of the challenge reactions were significantly lower than those of the group vaccinated with the K strain. Four of the 15 animals vaccinated with the S strain had no detectable parasitaemia, while all 5 vaccinated with the K strain developed parasitaemias comparable with those seen in the unvaccinated controls. None of the animals in the 2 vaccinated groups developed clinical signs of disease when challenged. As stated above, 5 of the 15 unvaccinated controls developed obvious signs of disease.

Serology

Reciprocal titrations of S and K strain antisera against homologous and heterologous strain antigens are given in Table 3. The sera were collected 26 days after infection. In both strains the titres against the homologous antigen were generally slightly higher than those against the heterologous antigen.

TABLE 3 Reciprocal titres of *B. bovis* post-vaccination antisera in the IFA test using South African (S) and Australian (K) vaccine strains as antigens

Antiserum		Antigen	
Vaccine strain	Animal No.	S Strain	K Strain
S	1842	1280	640
	2313	1280	1280
	2439	640	320
	2860	160	320
	3335	320	320
K	1621	320	640
	1711	160	640
	2583	160	320
	2986	320	640
	3374	160	640

Sera of the same animals were also collected 28 days after challenge with the virulent F strain and tested against S and K strain antigens. In the homologous test 2 out of the 5 S strain antisera had increased titres (1–2 dilutions), 1 remained the same and the titres of 2 were lower (1 dilution). In the heterologous test, 1 serum had a higher titre (1 dilution), 1 remained the same, and 3 were lower (1–2 dilutions). Two out of the 5 K strain antisera remained the same and 3 were lower (1 dilution) in the homologous test, while 4 had increased titres (1–3 dilutions) against the heterologous antigen, and 1 remained the same.

DISCUSSION

From the results it is evident that the virulence of the South African and Australian vaccine strains of *B. bovis* used in this study did not differ significantly and both strains were less virulent than the unmodified F strain.

TABLE 1 Primary reactions of South African (S) and Australian (K) vaccine strains and the unmodified F strain of *B. bovis*

Strain	Passage No.	Number of animals	Reaction		
			Mean maximum parasitaemia*	Mean maximum PCV depression**	Mean total temperature rise (°C)
S	11	15	$4,9^a \pm 1,58$	$15,9^a \pm 7,39$	$2,4^a \pm 2,74$
K	25	5	$4,8^a \pm 2,05$	$15,9^a \pm 4,54$	$2,0^a \pm 1,68$
F	—	15	$5,6^a \pm 1,64$	$31,7^b \pm 12,62$	$7,4^b \pm 2,12$

* Based on score ranging from 1–8 ± S.D.

** Depression given as a percentage of normal

[a, b] Means significantly ($P < 0,01$) different

TABLE 2 Reactions in vaccinated cattle when challenged 26 days later with the unmodified F strain of *B. bovis*

Vaccine strain	Number of animals	Reaction		
		Mean maximum parasitaemia*	Mean maximum PCV depression**	Mean total temperature rise (°C)
S	15	$2,0^b \pm 1,40$	$3,8^b \pm 3,35$	$1,2^a \pm 1,64$
K	5	$4,8^a \pm 2,49$	$9,2^a \pm 5,93$	$2,2^a \pm 1,72$
Unvaccinated controls	15	$5,6^a \pm 1,64$	$31,7^c \pm 12,62$	$7,4^b \pm 2,12$

* Based on score ranging from 1–8 ± S.D.

** Depression given as percentage of normal

[a, b, c.] Means significantly ($P < 0,01$) different

The results obtained here with Passage 11 of the S strain are in marked contrast to the results published by De Vos (1978) on the virulence of the unmodified form of the strain. Using parameters similar to those used in this study, he found the virulence of the unmodified S strain did not differ significantly from that of the virulent F strain. The observations made above on the pathogenicity of the F strain are in accordance with the results of De Vos (1978). Although the unmodified form of the S strain was not used for comparative purposes in the present study, it is evident that the rapid passage of this strain in splenectomized calves resulted in a reduction in its virulence within 11 passages.

These results are consistent with observations made by Callow *et al.* (1979). In work on *B. bovis* in Australia, these workers found a marked reduction in the virulence of 2 strains for intact cattle after 11 rapid passages in splenectomized calves. Response to infection in intact cattle was minimal, while splenectomized animals were severely affected. Callow *et al.* (1979) observed that, as virulence decreased with passage for intact animals, parasites became more plentiful in arterial and venous blood of splenectomized calves, possibly because of a decreasing predilection of *B. bovis* for the capillaries. This is shown by a decrease in the ratio of parasite numbers between jugular and capillary blood. Although the reasons for the reduction in virulence for intact cattle is unknown, Callow *et al.* (1979) surmised that modified *B. bovis,* by virtue of a greater tendency to circulate freely, may be more vulnerable than the unmodified parasite to immune effector mechanisms.

More recently, Wright, Goodger & Mahoney (1981) reported that virulent strains of *B. bovis* contained high levels of proteases, while substrains, rendered avirulent by rapid passage in splenectomized calves or by irradiation, contained insignificant levels of these enzymes. The virulence of *B. bovis* is dependent on the ability of the parasite to induce kinin formation and hypotensive shock—an ability dependent on the level of the proteases it contains (Wright *et al.*, 1981). Loss of virulence of a strain of *B. bovis* may therefore be due to the loss of these substances in the organisms.

The attenuation of *B. bovis* obtained by rapid passage can be reversed, since Callow *et al.* (1979) reported that an attenuated strain passaged 34 times in splenectomized calves reverted to full virulence after 1 passage in intact cattle. This, however, has not proved to be important in practical vaccination.

Results obtained with the S strain indicated that vaccination with this strain afforded strong protection to challenge with the unmodified F strain. The same applied, although to a lesser extent, to vaccination with the K strain. Despite the fact that the latter strain failed to contain the peak parasitaemia of the challenge reaction to the same extent as the S strain, this was not reflected in the PCV depression or fever reaction. It must therefore be concluded that protection provided by the K strain is adequate to control challenge with strains of the nature of the F strain.

Two reasons can be suggested for the observed differences in the degree of protection afforded by the 2 strains. Firstly, it is suspected that vaccine strains of *B. bovis* may protect against decreasing numbers of field strains as the number of passages increases (De Vos, 1978; Callow *et al.*, 1979). As the K strain had been rapidly passaged 23 times as against 10 times of the S strain, it is conceivable that there is a difference, albeit slight, in the protective properties of the 2 strains. Mahoney, Wright & Goodger (1979), however, found the immunity conferred by the commercial Australian tick fever vaccine to be comparable in strength with that conferred by a tick-induced infection.

Secondly, and probably more importantly, immunogenic differences are known to exist between strains of *B. bovis (=argentina)* (Callow, 1968). The differences between the Australian K strain and local strains, however, were not such that it materially affected the ability of the K strain to protect animals against a virulent South African strain. This finding is in agreement with observations made by McCosker (1975) and Callow *et al.* (1976) on the use of Australian *B. bovis (=argentina)* vaccine strains in Bolivia. These strains were shown to confer adequate immunity and prevent acute natural infections.

This finding also confirms the recent suggestion of Callow, Kanhai & Vandenberghe (1981) that, based on the close serological relationship between *B. bovis* in Mozambique and Australia, the Australian vaccine by implication should protect cattle introduced into southern Africa from *B. bovis*-free environments.

Serological observations made in this study confirmed a close relationship between the attenuated South African and Australian vaccine strains of *B. bovis* even though titres against homologous antigens were slightly higher than against heterologous antigens. Despite the mild reactions caused by the 2 strains, titres obtained in this study were comparable with or only slightly lower than those observed by Gray & De Vos (1981) with sera of unmodified South African *B. bovis,* and by Goldman & Rosenberg (1974) with similar sera tested against *B. bovis (=argentina)* antigen.

Challenge of the vaccinated animals with the F strain had little effect on the titres against S and K strain antigens, except that 4 out of 5 K strain antisera had higher titres against the heterologous antigen after challenge than before. It is conceivable that this antibody response after challenge was a consequence of the marked parasitaemic reaction seen in these animals after challenge. It is interesting to note that this antibody response was not detectable with the K strain antigen.

Dalgliesh *et al.* (1981) made observations on 2 modified strains of *B. bovis,* one of them, the K strain, passaged 27–33 times in splenectomized calves. Except for reduced virulence for cattle, these strains were also found to have low pathogenicity for replete female *Boophilus microplus.* In comparison with an unmodified strain of *B. bovis,* the modified strains infected higher proportions of ticks but killed significantly fewer of them. In preliminary observations, however, Wright & Mahoney (1980) found a strain of *B. bovis,* rendered avirulent by irradiation, not only to be immunogenic but also non-transmissible by ticks immediately after irradiation. Thus irradiation may be a rapid way of producing an avirulent parasite population which is also not vector-transmissible.

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