Onderstepoort J. vet. Res., 49, 155–158 (1982)

BABESIA BIGEMINA VACCINE: COMPARISON OF THE EFFICACY AND SAFETY OF AUSTRALIAN AND SOUTH AFRICAN STRAINS UNDER EXPERIMENTAL CONDITIONS IN SOUTH AFRICA

A. J. DE VOS, M. P. COMBRINK and R. BESSENGER, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

DE VOS, A. J., COMBRINK, M. P. & BESSENGER, R., 1982. *Babesia bigemina* vaccine: Comparison of the efficacy and safety of Australian and South African strains under experimental conditions in South Africa. *Onderstepoort Journal of Veterinary Research*, 155–158 (1982).

A *Babesia bigemina* vaccine strain (G strain) of reduced virulence was obtained from Australia and tested experimentally for efficacy and virulence. The strain caused mild reactions in 10 animals and afforded good protection to challenge with a virulent South African strain. The virulence of the local vaccine strain was not noticeably reduced after 3 slow passages in intact calves and it was consequently replaced by the Australian strain in the Onderstepoort babesiosis vaccine.

INTRODUCTION

A babesiosis vaccine, consisting of both *Babesia bigemina* and *Babesia bovis*, has been issued from the Veterinary Research Institute for many years (De Vos, 1978). One major drawback of the vaccine until now has been its virulence. This often necessitated preventive therapy and must have been a major factor limiting the demand for this vaccine to only 120 000 doses annually.

Attempts to attenuate a local strain of *B. bovis* by passaging it rapidly through a series of splenectomized calves as described by Callow, Mellors & McGregor (1979) have succeeded in reducing its virulence to a satisfactory level (De Vos, Bessenger & Fourie, 1982). It is known, however, that the virulence of *B. bigemina* is not appreciably reduced when it is passaged in this way and laboratory-maintained strains tend to be even more virulent than most strains occurring in nature (Callow, 1977). Virtually all known cases of severe or fatal vaccine reactions in South Africa are caused by the *B. bigemina* component in the vaccine (De Vos, unpublished observations, 1980).

Recently, Dalgliesh, Callow, Mellors & McGregor (1981) described the development of a highly effective *B. bigemina* vaccine of reduced virulence. The reduction in the virulence of the strain used was achieved by "slow" passaging in intact calves. The calves were splenectomized 1–14 weeks after inoculation and the ensuing relapse parasitaemias were used for passaging. The purpose of this paper is to record observations made on the virulence of this strain under experimental conditions in South Africa and the immunity conferred by it to challenge with an unmodified local strain. In addition, an attempt was made to reduce the virulence of the South African vaccine strain of *B. bigemina* using the technique of Dalgliesh *et al.* (1981).

MATERIALS AND METHODS

Animals

Bos taurus calves born and bred at this Institute under strict, tick-free conditions, were used for the passaging of the *Babesia* strains and for the preparation of stabilates. Cross-bred *Bos indicus* cattle, originating from a government experimental farm in the northern Cape Province where *Boophilus* spp. are not well established, were used in the comparative study. The animals were 1–2 years of age when used and were serologically negative in the indirect fluorescent antibody test for *B. bigemina* and *B. bovis*. All of them, however, gave positive reactions for *Babesia occultans* (Gray & De Vos, 1981). The animals were sorted into experimental groups in such a way that the mean mass of each group was approximately the same.

Cryopreservation

Stabilates of the different strains (Lumsden & Hardy, 1965) were prepared using a technique which incorporated the principles outlined by Dalgliesh (1972) for the successful freezing of parasitic protozoa.

Received 3 May 1982—Editor

Dimethyl sulphoxide (DMSO) was added to phosphate buffered saline (PBS), pH 7,4, on a magnetic stirrer (final concentration 20% DMSO v/v) and cooled to 4 °C. The PBS was the same as that described by Dalgliesh (1969). At the same time, infected blood was collected into an anticoagulant citrate dextrose solution (ACD) at a ratio of 4 parts blood to 1 part ACD and also cooled to 4 °C. Once refrigerated, the diluted DMSO was added slowly to the blood in equal volumes on a magnetic stirrer to give final concentrations of 40% whole blood and 10% DMSO v/v. The diluted blood was then dispensed in 2 mℓ quantities in screwcap polypropylene vials* and stored in the gas phase of a liquid nitrogen refrigerator. The volumes used were limited to enable dispensing and storing procedures to be performed within 10 min of mixing.

The number of parasites/mℓ of infected blood at the time of collection 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**.

Strains of B. bigemina

The attenuated Australian G strain (Dalgliesh *et al.*, 1981) was obtained from the Tick Fever Research Centre, Wacol, Queensland, as unfrozen infected blood. On receipt, the blood was inoculated into an intact calf. Splenectomy 12 weeks later was followed by a very mild parasitaemic relapse and the number of parasites/mℓ was considered to be too low (< 1%) for the preparation of stabilate. Consequently, the parasite was passaged once only in a splenectomized calf and stabilate prepared when it reacted. There were approximately 8×10^7 parasites/2 mℓ vial when frozen.

The South African P strain was unmodified and stored in a liquid nitrogen refrigerator as stabilate of a tick-transmitted infection. The number of parasites per 2 mℓ was about 4×10^7 when frozen.

In an attempt to attenuate the P strain, it was "slow passaged" 3 times following the technique described by Dalgliesh *et al.* (1981). An intact calf was infected with the blood stabilate described above. Twelve weeks later the calf was splenectomized and the ensuing relapse parasitaemia utilized to infect a 2nd intact calf. The procedure was repeated to infect a 3rd intact calf. Fourteen weeks after infection this calf was also splenectomized and, during the ensuing relapse, blood was collected and stored in a liquid nitrogen refrigerator. The number of parasites per 2 mℓ vial was approximately 20×10^7 before freezing. At the time of the experiments outlined below this stabilate was used to infect splenectomized calves at this laboratory for production of the vaccine. For the purpose of this study it will be designated the passaged P strain.

* Nunc cryotubes

** Coulter Electronics Inc.

Method of infection

Frozen 2 mℓ vials were transported on dry ice and thawed on site by agitation in a water-bath at approximately 40 °C. Each animal was inoculated subcutaneously with the contents of 1 vial within 3 min of the blood being thawed.

Evaluation of reactions

Maximum parasitaemias, based on a score ranging from 1–8, maximum packed cell volume (PCV) depression expressed as a percentage of the normal and mean total temperature rise above normal, were used to monitor the reactions as outlined by De Vos (1978). Student's 't' test was used to determine the significance of differences between means of the various sets of figures. To determine the prepatent periods, stained thick blood smears (Mahoney & Saal, 1961) were prepared daily from the peripheral blood of every animal and examined for a maximum period of 5 min.

Experimental procedures

Two groups of 10 animals each were inoculated respectively with the G and passaged P strains. The reactions were evaluated and, 28 days after infection, the animals were challenged with the unmodified P strain. Eight unvaccinated controls were infected simultaneously with the unmodified P strain, while a further 5 animals acted as uninfected controls throughout.

Indirect fluorescent antibody test

The technique used routinely at this laboratory was that described by Gray & De Vos (1981). The antigen smears were prepared of both the G and the passaged P strains, using blood of acutely infected splenectomized calves showing a parasitaemia of 2–3%. Antisera of both strains were collected 28 days after infection, and titrated. A comparison of the titres of the sera was made as outlined by De Vos *et al.* (1981).

RESULTS

Infectivity of stabilates

Subcutaneous inoculation of passaged P strain stabilate (20×10^7 parasites/2 mℓ prior to freezing) resulted in the infection of all the animals with a mean prepatent period of 3,8 ± 0,45 days. The unmodified P strain (4×10^7 parasites/2 mℓ) had a mean prepatent period of 5,4 ± 1,52 days, while the G strain (8×10^7 parasites/2 mℓ) had one of 7,1 ± 0,35 days.

Observations on virulence

The reactions following infection with the G strain and both forms of the P strain are summarized in Table 1.

The G strain had a considerably reduced virulence, as the significant differences recorded in all 3 parameters show. Parasites were detected in the smears of all the animals but only reached a level of 0,2% in 2 of the 10 animals; the remaining animals all had peak parasitaemias of less than 0,1%. None of the animals showed any signs of disease. A splenectomized calf, infected initially to obtain blood with a parasitaemia suitable for the production of stabilate, recovered without therapy after developing a peak parasitaemia of 4,8%.

The passaged P strain was significantly more virulent than the G strain (Table 1). Although it does appear from the table that this stabilate was slightly less virulent than the unmodified one, the differences observed were not statistically significant. In addition, 4 out of the 10 animals infected with the passaged strain were severely affected and showed signs of listlessness, anorexia and haemoglobinuria, while 3 out of the 8 animals infected with the unmodified strain were affected clinically. Treatment (diminazene*) of one of the former 4 animals was deemed necessary to prevent death.

Challenge of vaccinated cattle

The 10 cattle vaccinated with the G strain resisted challenge, as the differences between their reactions and those of the unvaccinated controls indicate (Table 2). Apart from the parasitaemia, the reactions to challenge seen in this group were similar to those of the group vaccinated with the passaged P strain. Parasites were present in detectable numbers in thin blood smears of 4 out of 10 of the animals for 1–2 days (parasitaemia < 0,1%) and in thick blood smears of the remaining 6. In contrast, 4 of the 8 unvaccinated controls had parasitaemias in excess of 2%.

* Berenil Hoechst

TABLE 1 Primary reactions of the Australian G and South African P strains of *B. bigemina*

Strain	No. of animals	Reaction		
		Mean maximum parasitaemia** ± S.D.	Mean maximum PCV depression***	Mean total temperature rise (°C)
G	10	$3,8^a$ ± 2,15	$15,2^a$ ± 9,5	$0,3^a$ ± 0,34
P (passaged)	9*	$6,9^b$ ± 1,37	$34,5^b$ ± 19,45	$2,6^b$ ± 2,30
P (unmodified)	8	$7,7^b$ ± 0,93	$41,4^b$ ± 19,05	$2,9^b$ ± 2,45

* One of the original 10 animals was treated with diminazene

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

*** Depression given as a percentage of the norm

Means with different superscripts are significantly ($P < 0,01$) different

TABLE 2 Reactions in vaccinated cattle when challenged 28 days later with P strain (unmodified)

Vaccination strain	No. of animals	Challenge reactions		
		Mean maximum parasitaemia*	Mean maximum PCV depression**	Mean total temperature rise (°C)
G	10	$2,8^a$ ± 2,47	$8,5^a$ ± 5,91	$0,1^a$ ± 0,15
P (passaged)	9	$1,7^b$ ± 1,35	$8,1^a$ ± 4,37	$0,1^a$ ± 0,11
Unvaccinated controls	8	$7,7^c$ ± 0,93	$41,4^b$ ± 19,05	$2,9^b$ ± 2,45

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

** Depression given as a percentage of the norm

Means with different superscripts are significantly ($P < 0,01$) different

Serology

Reciprocal titres of sera titrated against homologous and heterologous strain antigens are given in Table 3. Although the sera produced titres slightly higher against the homologous antigen than against the heterologous antigen, the results indicate cross-reactivity between the attenuated G strain from Australia and the local passaged P strain.

TABLE 3 Reciprocal titres of sera of animals inoculated with the G and P strains of *B. bigemina* as measured with the IFA technique

Sera		Antigens	
Strain	Animal No.	G strain	P strain (passaged)
G	1924	160	80
	1923	160	160
	1921	320	320
	1893	320	320
	3981	640	160
	7125	640	160
	1952	640	320
	7135	640	320
P (passaged)	1947	160	320
	1919	320	320
	7139	640	320
	2922	320	640
	3326	320	640
	1911	320	640
	1945	320	640
	1931	640	640
	3323	1280	640
	4060	640	1280

DISCUSSION

Dalgliesh *et al.* (1981) succeeded in markedly reducing the virulence of the G strain after 7 "slow" passages. They found the parasitaemia in 19 infected splenectomized calves ranged from 1–10% and all but 3 recovered without treatment. In the present study we infected only one splenectomized calf and it recovered spontaneously after showing a parasitaemia of almost 5%. Dalgliesh *et al.* (1981) also infected 23 intact cattle, all of which controlled the reactions without obvious clinical signs of disease. They detected parasites in low numbers in thin blood smears of 16 of these animals and in thick blood smears of the remaining 7. In our experiment no signs of disease were seen in 10 recipients of the G strain and only 2 reached a parasitaemia of 0,1%. This strain thereby fulfils one of the important requirements of a good vaccine, namely, low virulence.

Immunization with the G strain provided almost total protection against harmful effects of challenge with the unmodified South African P strain. It thus fulfils a second major requirement for a good vaccine, namely, solid protection to local challenge. A parasitaemia detectable in thin blood films from some of the animals following challenge may have been due to an antigenic difference between the vaccinating and challenge strains. Such antigenic variations between strains of *B. bigemina* have been documented before (Callow 1964; 1967; Curnow, 1973). That the parasitaemias after challenge persisted for only 1–2 days indicates that the animals' immune mechanisms rapidly controlled the infections.

The stabilate prepared of the G strain proved to be highly infective when inoculated subcutaneously, but the prepatent period was noticeably longer than that of the passaged and unmodified P strains. The small standard deviation indicates that the animals all reacted at virtually the same time and thus a deficiency in the freezing procedure of the G strain is discounted. Experience at this laboratory indicates that prepatent periods of stabilates with a low infectivity are not only longer but often also more variable than those of highly infective stabilates (De Vos, unpublished observations, 1981). The longer prepatent period of the G strain stabilate may therefore be an indication that this strain has a longer doubling time in erythrocytes than the P strain. This work will have to be repeated before any firm conclusions can be drawn.

As was to be expected, the immunity afforded by the passaged P strain to challenge with the original isolate was almost complete. This work was not controlled with observations on homologous challenge of the passaged strain, but the results indicate that the effect of the 3 passages on the immunogenicity of the strain was negligible. Basing their conclusions on serology and reactions to challenge, Thompson, Todorovic & Hidalgo (1977; 1978) recorded antigenic variations between blood-inoculated and tick-transmitted forms of *B. bigemina*.

The serological observations made in this study indicate a virtually complete cross-reaction between the P and G strains, despite the marked modification of the latter. To the best of our knowledge this is the first report proving the serological identity of *B. bigemina* from the African and Australian continents.

Attempts to reduce the virulence of the P strain by passaging it 3 times in intact calves over a period of almost 9 months had very little effect on the virulence of this strain. The virulence of the Onderstepoort babesiosis vaccine has always been regarded as a major disadvantage and the situation has therefore been reassessed. A decision was made to continue the attempt to attenuate the P strain but, as this could take a long time, also to incorporate the G strain in the interim in the vaccine. As a result, the G strain is now used in the Onderstepoort babesiosis vaccine.

ACKNOWLEDGEMENT

We wish to thank Mr R. J. Dalgliesh, Tick Fever Research Centre, Wacol, Queensland, Australia for making available the G strain of *B. bigemina* used in this study.

REFERENCES

CALLOW, L. L., 1964. Strain immunity in babesiosis. *Nature*, 204, 1 213–1 214.

CALLOW, L. L., 1967. Sterile immunity, coinfectious immunity and strain differences in *Babesia bigemina* infections. *Parasitology*, 57, 455–465.

CALLOW, L. L., 1977. Vaccination against bovine babesiosis. *In:* MILLER, L. H., PINO, J. A. & McKELVEY, J. J. (eds.). Immunity to blood parasites of man and animals. pp. 121–149. New York: Plenum Press.

CALLOW, L. L., MELLORS, L. T. & McGREGOR, W., 1979. Reduction in virulence of *Babesia bovis* due to rapid passage in splenectomized cattle. *International Journal for Parasitology*, 9, 333–338.

CURNOW, J. A., 1973. The use of a slide agglutination test to demonstrate antigenic differences between *Babesia bigemina* parasites. *Australian Veterinary Journal*, 49, 290–293.

DALGLIESH, R. J., 1969. Permeation of bovine erythrocytes with glycerol and their protection during rapid freezing and thawing. *Research in Veterinary Science*, 10, 351–354.

DALGLIESH, R. J., 1972. Theoretical and practical aspects of freezing parasitic protozoa. *Australian Veterinary Journal*, 48, 233–239.

DALGLIESH, R. J., CALLOW, L. L., MELLORS, L. T. & McGREGOR, W., 1981. Development of a highly infective *Babesia bigemina* vaccine of reduced virulence. *Australian Veterinary Journal*, 57, 8–11.

DE VOS, A. J., 1978. Immunogenicity and pathogenicity of three South African strains of *Babesia bovis* in *Bos indicus* cattle. *Onderstepoort Journal of Veterinary Research*, 45, 119–124.

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.

GRAY, J. S. & DE VOS, A. J., 1981. Studies on a bovine *Babesia* transmitted by *Hyalomma marginatum rufipes* Koch, 1844. *Onderstepoort Journal of Veterinary Research*, 48, 215–223.

LUMSDEN, W. H. R. & HARDY, G. J. C., 1965. Nomenclature of living animal material. *Nature*, 205, 1 032.

MAHONEY, D. F. & SAAL, J. R., 1961. Bovine babesiosis: Thick blood films for the detection of parasitaemia. *Australian Veterinary Journal*, 37, 44–47.

THOMPSON, K. C., TODOROVIC, R. A. & HIDALGO, R. J., 1977. Antigenic variation of *Babesia bigemina*. *Research in Veterinary Science*, 23, 51–54.

THOMPSON, K. C., TODOROVIC, R. A. & HIDALGO, R. J., 1978. The immune responses to antigenic variants of *Babesia bigemina* in the bovine. *Research in Veterinary Science*, 24, 234–237.

Printed by and obtainable from the Government Printer, Bosman Street, Private Bag X85, Pretoria, 0001