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INFECTIVITY VIRULENCE AND IMMUNOGENICITY OF *ANAPLASMA CENTRALE* LIVE BLOOD VACCINE

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ABSTRACT

POTGIETER, F. T. & VAN RENSBURG, L., 1983. Infectivity virulence and immunogenicity of *Anaplasma centrale* live blood vaccine. *Onderstepoort Journal of Veterinary Research*, 50, 29–31 (1983).

Cross-bred *Bos taurus* calves, aged between 6 and 8 months, were inoculated with the Onderstepoort *Anaplasma centrale* live blood vaccine. One group of 15 calves were inoculated once only, while a 2nd group of 15 were revaccinated 6 months later. All the animals were challenged with approximately 1×10^{10} *Anaplasma marginale* parasites of a known virulent strain 8 months after the first vaccination. The results of blood smear examination and the card agglutination test indicated that only 20 out of 30 animals vaccinated contracted *A. centrale* infections after the first attempt, and 3 out of 5 after the second. The vaccine conferred only partial immunity to challenge with a virulent *A. marginale* strain.

INTRODUCTION

The use of live *Anaplasma centrale* blood vaccine to protect cattle against natural *Anaplasma marginale* infections in South Africa has been reviewed by Potgieter (1979). Local field reports suggested that the use of this vaccine in 6-month-old calves, as recommended, is not very effective, as so-called breakdowns in immunity occur amongst vaccinated cattle.

Two aspects have been suspect for some time, namely, the infectivity of the vaccine, mainly because of its limited shelf life, and the immunogenic properties of the *A. centrale* strain used in this vaccine since 1912.

The present study was undertaken to investigate these aspects, simulating the application of the vaccine in the field under laboratory conditions.

MATERIALS AND METHODS

Experimental animals

Cross-bred *Bos taurus* calves, aged 6–8 months, were obtained from the Cedara Experimental Farm in Natal. Upon their arrival at this Institute they were placed on a weekly dipping programme using a mixture of dioxathion and chlorfenvinphos*.

Thirty-five animals which were susceptible to anaplasmosis, as determined by blood smear examination and the complement fixation test, were selected from this group. These animals were kept in tick-free stables for 7 weeks prior to as well as during this investigation.

Anaplasma *strains*

The Onderstepoort live *A. centrale* blood vaccine described by Potgieter (1979) was used to vaccinate the experimental animals. The origin of the *A. marginale* BW-strain, used to challenge these vaccinated animals, was described by Potgieter, Sutherland & Biggs (1981).

Parameters for the Anaplasma *infections*

Parasitaemia: Thin blood smears were prepared 3 times weekly from the tip of the tail, stained with Giemsa's stain, and examined for parasites. After the detection of the first parasites, smears were examined daily to determine peak parasitaemias. Five hundred erythrocytes were differentially quantitated at the tip of the smear and the parasitaemia was expressed as a percentage of the infected erythrocytes.

Anaemia: The haematocrit (Ht) was determined with a microhaematocrit machine twice weekly for 2 weeks before infection, to determine the normal Ht, once a week after infection until the first parasites were detected in thin blood smears and daily thereafter until recovery.

* Supamix Cattle Dip, Coopers SA

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Serology

Complement fixation test: The complement fixation test (CFT) (Anonymous, 1958) was used to screen the animals for possible *Anaplasma* antibodies before the first inoculation with *A. centrale*. The animals were tested upon their arrival at this Institute and again after approximately 7 weeks after entering tick-free facilities.

Card agglutination test

A card agglutination test (CAT), adapted from the test described by Amerault, Rose & Roby (1972), was performed to detect the serological response in cattle vaccinated with *A. centrale* and challenged with *A. marginale*. *A. marginale* antigen was used.

Antigen production for the CAT: After the initial infection of a fully susceptible splenectomized ox with a blood stabilate of *A. marginale*, the infection was passaged through a further 3 splenectomized animals during rapidly rising parasitaemias. This procedure resulted in an 88% parasitaemia in the 4th animal within 4 days. Blood was collected from this animal in 12% sodium citrate, centrifuged and then, after the plasma and buffy coat had been removed, it was resuspended and washed 3 times in normal saline. The erythrocytes were then haemolyzed with sterile, filtered tap water in a ratio of 1:3,5. The released parasites and erythrocyte debris were concentrated by centrifugation at 48 400 × g for 20 min, the supernatant removed, resuspended in water, then centrifuged, as mentioned above. Two more washes in saline were done before the pellets were finally resuspended in fresh saline in the ratio 1:5. Sonication of the material at an amplitude of 15 microns for 15 s followed, using a MSE model 200 sonifier. After centrifugation of the sonicated material a jelly-like layer, visible on top of the pellets, was carefully sucked off and the pellet suspended in sterile saline, centrifuged, and finally reconstituted in an acetate buffer (pH 5) as a 4% solution. This was used as antigen. Fast green dye (1%) was added to the solution at the rate of 1 mℓ per 25 mℓ of antigen, mixed by shaking and followed by 3 bursts of sonication at an amplitude of 18 microns for 10 s each time. The antigen was centrifuged again at 17 400 × g for 30 min and suspended in fresh acetate buffer. Sonication was repeated as above. Finally, the antigen was stored as a 3% solution in acetate buffer at 4 °C.

Collection of serum samples for the CAT: The blood was collected and allowed to clot overnight, centrifuged and the serum left at 22–25 °C for 48 hours before being tested. Known positive and negative sera as well as the Bovine Serum Factor (BSF) were also collected weekly from cattle kept under tick-free conditions.

Test procedures for the CAT: The test was performed essentially as described by Amerault & Roby (1968) and Amerault *et al.* (1972). The only modification was that the cards supplied in the Brewer Diagnostic Kit were substituted with white perspex plates and the test was read with the aid of a strong cold light source which facilitated the reading of weak agglutination.

Experimental design

Thirty susceptible calves were injected subcutaneously with the Onderstepoort *A. centrale* vaccine (Potgieter, 1979) 4 days after issue. Blood smears were examined and the Ht determined of all animals as described above. Serum samples of the animals were tested with the CAT on a weekly basis from the 7th week after inoculation with *A. centrale*.

Six months later the 30 animals were divided into 2 groups (Groups 1 and 2). Each group consisted of 10 animals known to have become infected after vaccination and 5 that failed to react. The 15 animals in Group 2 received a second dose of vaccine.

All 30 animals in Groups 1 and 2 as well as 5 susceptible controls (Group 3) were challenged with approximately 1×10^{10} *A. marginale* parasites 8 months after the first inoculation with *A. centrale*. The challenge reactions were monitored as before.

RESULTS

Infectivity of A. centrale *vaccine*

Out of the 30 animals inoculated with *A. centrale* vaccine, only 20 contracted the infection as demonstrated by blood smear examination and the CAT (Table 1). The second inoculation of 15 animals, 5 of which failed to react to the 1st inoculation, resulted in an equally poor infection rate, as only 3 out of a possible 5 became infected (Table 1).

The results of the CAT and blood smear examination showed a 100% correlation.

Virulence of A. centrale *vaccine*

Mild vaccine reactions were seen in the majority of cases. The virulence of the *A. centrale* vaccine compared with that of the *A. marginale* strain used to challenge the vaccinated animals is indicated in Table 2.

The 10 animals which reacted on the 1st vaccination were not noticeably affected by the revaccination in terms of increased parasitaemia or lowered Ht.

Protection afforded by A. centrale *vaccine*

The *A. marginale* challenge reactions of the vaccinated animals are compared with those of the unvaccinated controls in Table 3. The level of protection is clearly rather low (see also Table 5).

The animals that failed to react to inoculation with *A. centrale* vaccine were regarded as fully susceptible. Their reactions to *A. marginale* challenge are given in Table 4 and compared with those of the 5 susceptible control animals in Group 3.

It was surprising to find that 3 out of the 10 animals in Group 1 that showed the highest *A. centrale* parasitaemia and also the highest Ht depression reacted very severely upon the *A. marginale* challenge (Table 5).

TABLE 1 Infectivity of the *A. centrale* vaccine

Vaccination	No. of animals	No. of reactors	Mean prepatent period (days)	Reaction	
				Blood-smear positive (No.)	CAT positive (No.)
1st vaccination	30	20	47,7	20	20
2nd vaccination	15*	3	49,6	3	3

* Including 5 non-reactors from the 1st vaccination.

TABLE 2 Virulence of *Anaplasma* spp. used

	No. of animals	Mean maximum parasitaemia (%)	Mean Ht depression (%)
A. centrale	23	2,9*	19,1
A. marginale (Unvaccinated controls)	5	32,0	60,8

* *A. centrale* parasitaemia < 1% taken as $\bar{x}$ 0,2%

TABLE 3 Reactions in vaccinated animals when challenged with *A. marginale*

Group	No. of animals	Mean maximum parasitaemia (%)	Mean Ht depression (%)
1 Vaccinated 1×	13*	16,7	51
2 Vaccinated 2×	10	14,7	46,4
3 Unvaccinated controls	5	32,0	60,8

* Including 3 animals from Group 2 that were only infected once successfully

TABLE 4 *A. marginale* reactions in animals that failed to react to *A. centrale* or were uninfected

Group	No. of animals	Mean maximum parasitaemia (%)	Mean Ht depression (%)
1 Vaccinated 1×	5	23	58
2 Vaccinated 2×	2	22,5	56,1
3 Unvaccinated controls	5	32	60,8

TABLE 5 Vaccine and challenge reactions of the animals in Group 1 that showed the highest *A. centrale* parasitaemia compared with those of the controls

	No. of animals	Mean maximum parasitaemia (%)	Mean Ht depression (%)
Vaccine *A. centrale*	3	13,7	45,9
Challenge *A. marginale*	3	20	54,6
Controls *A. marginale*	5	32	60,8

DISCUSSION

Contrary to observations made on the infectivity of the Onderstepoort *A. centrale* vaccine in a similar laboratory study (Bigalke, 1980), the results of this investigation indicate that the current *A. centrale* vaccine has a poor infection rate. The animals used in this investigation were selected from a group of field animals on the basis of negative CF tests. Others in the group proved positive. There is therefore a possibility both that the animals that showed no vaccine reactions could have had some resistance and that we were unable to demonstrate it with the CF test prior to vaccination. This could possibly then be reflected in the poor infectivity of the vaccine. However, after vaccination, no parasites could be detected in their blood smears and they remained negative in the CAT. The fact that their reactions to the virulent *A. marginale* challenge can be compared with those of the unvaccinated controls indicates that they were probably susceptible at the time of vaccination.

At present, the Onderstepoort *A. centrale* vaccine contains approximately 5×10^6 parasites on the day the blood is collected (Potgieter, 1979). In this trial the prepatent periods of the *A. centrale* infections were 6–7 weeks, whereas the prepatent period of the Australian *A. centrale* vaccine, containing 1×10^7 parasites per dose, used in a similar study, was reported to be in the region of 4 weeks (Wilson, Parker & Trueman, 1980). A drop in viability of the parasites in a live-blood vaccine is to be expected, and this aspect should be investigated in the case of the Onderstepoort vaccine which in this study was applied 6 days after collection (4 days after issue) and 2 days before the expiry date. It will not be possible at present to increase the number of parasites per dose or standardize the dose because the blood is collected weekly from long-standing, splenectomized *A. centrale* carrier animals, mostly showing parasitaemias of <1%.

The infectivity of the vaccine applied as a subcutaneous injection, compared with its infectivity by alternative routes, should also receive attention.

The Onderstepoort *A. centrale* strain, originally isolated by Theiler (1912), normally produces a mild clinical response, the exception being severe reactions sometimes seen in older adult cattle (Potgieter, 1979; Bigalke, 1980). Kuttler (1966), using the same *A. centrale* isolate from South Africa in a study of the comparative virulence of *A. marginale* and *A. centrale*, observed only minor differences in virulence of 2 American isolates of *A. marginale* and that of *A. centrale*. A buffalo (*Syncerus caffer*) isolate of *A. marginale* made in South Africa proved to be even less virulent than *A. centrale* (Potgieter, 1980, unpublished observations). Preliminary investigations have shown that this mild organism unfortunately affords even less protection against virulent *A. marginale* challenge than *A. centrale*.

We believe that strain differences of *A. marginale* may play an important role in the epidemiology of anaplasmosis in South Africa. This aspect should be investigated, as it may identify mild *A. marginale* strains with better immunogenic qualities than *A. centrale*. The latter could then possibly be used in a vaccine, as also suggested by Kuttler (1967).

The degree of protection afforded by the vaccine against *A. marginale* challenge was equally disappointing. Even a 2nd vaccination, as suggested by Wilson *et al.* (1980), given 6 months later to 10 animals in this study, did not improve their immune status to the extent that it could be regarded as an effective procedure.

Although these laboratory observations may not represent the natural course of the disease, especially as far as the artificial challenge of 1×10^{10} *A. marginale* parasites and the virulence of the strain are concerned, it is still disturbing to see that vaccination with *A. centrale* under these conditions did not prevent severe *A. marginale* reactions. Wilson *et al.* (1980) also found that vaccination of 6-month-old calves with *A. centrale* left more than 50% of the animals without "adequate immunity".

One would expect, and Wilson *et al.* (1980) have shown, that a good antibody response, as measured by a serological test, a marked reduction in Ht and a high parasitaemia, would result in resistance to a subsequent challenge with *A. marginale*. However, in the present study, the 3 animals that showed the highest *A. centrale* parasitaemia and also the lowest Ht did not show a higher level of resistance to challenge than the rest of the vaccinated animals.

These results indicate that the *A. centrale* vaccine, if applied as recommended, would probably not protect animals against natural challenge with *A. marginale* strains as virulent as the one used in this study.

Field trials would be the obvious extension of this experimental work to study the effect of natural challenge of vaccinated animals of different ages.

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