Observations on the use of *Anaplasma centrale* for immunization of cattle against anaplasmosis in Zimbabwe

J.A. TURTON[1,2], T.C. KATSANDE[1], M.B. MATINGO[1]*, W.K. JORGENSEN[3], U. USHEWOKUNZE-OBATOLU[1] and R.J. DALGLIESH[3]

ABSTRACT

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A total of 93 *Bos taurus* cattle was used in pen trials to compare vaccine stocks of *Anaplasma centrale* from South Africa and Australia (which stock came from South Africa in 1934) in protecting against three virulent field isolates from clinical *Anaplasma marginale* infections. In addition, field observations were made on the use of a vaccine, prepared from the Australian stock, in over 9553 cattle of mixed age and breeds on 16 co-operator farms and at one communal dip.

The results of the pen trials indicated that the two vaccine stocks were comparable and that neither provided adequate protection against two of the three isolates of *A. marginale*. The field observations indicated that the vaccine was highly infective and produced mild reactions in most recipient cattle, and that users were generally satisfied with the vaccine.

These somewhat conflicting results are discussed in the context of observations in Australia and future vaccination against anaplasmosis in Zimbabwe.

Keywords: Anaplasmosis, *Anaplasma centrale, Anaplasma marginale,* vaccine

INTRODUCTION

Bovine anaplasmosis, caused by the blood rickettsia, *Anaplasma marginale,* occurs in many countries where the tick vectors (*Boophilus, Hyalomma* & *Rhipicephalus* spp.) are endemic. Cattle can be protected against clinical anaplasmosis by prior infection with the less virulent organism, *Anaplasma centrale*. This cross-protection was first recognized by Theiler in 1911 (Potgieter & Stoltsz 1994) and Theiler's original isolate of *A. centrale* has since been used in vaccines in several countries, including South Africa, Australia, Israel and Uruguay. The immunity afforded by *A. centrale* is, however, not absolute and reports of unsatisfactory protection are recorded from experimental studies (Rogers & Shiels 1979; Wilson, Parker & Trueman 1980; Potgieter & Van Rensburg 1983).

In Zimbabwe and other countries in southern Africa, anaplasmosis and other tick-borne diseases have been largely suppressed by intensive (approximately weekly) dipping of cattle in acaricides, a practice begun in about 1910 to control theileriosis (Lawrence 1992). Arguments against intensive dipping in Zimbabwe have been developed, based on economic and environmental considerations (Norval 1983; Perry, Mukhebi, Norval & Barrett 1990) and the country is now moving towards reliance on strategic dipping and vaccines for the control of tick-borne diseases.

* Author to whom correspondence is to be directed

[1] Central Veterinary Laboratory, Department of Veterinary Services, P.O. Box CY 551, Causeway, Harare, Zimbabwe

[2] Present address: Unit 2/3 Munro Avenue, Mt Waverley, Victoria, Australia

[4] Animal Research Institute, Department of Primary Industries, Locked Mail Bag 4, Moorooka, Brisbane, Australia

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To accelerate this process, Zimbabwe has imported proven vaccine stock of certain tick-borne parasites and is testing these against local isolates of the target organisms. This paper describes our assessment in pen trials of the efficacy of a blood vaccine containing *A. centrale* against three Zimbabwean isolates of *A. marginale,* and our observations on the performance of this vaccine in field trials.

MATERIALS AND METHODS

Pen trials

Experimental cattle

A total of 93 *Bos taurus* cattle, approximately two years of age, was used in the three pen trials performed. All of the cattle were negative in the modified card agglutination test (CAT) for antibodies to *Anaplasma* (Amerault & Roby 1971) before use. Sixty-nine of the cattle had been vaccinated with *A. centrale* as part of our assessment of the infectivity and virulence of vaccine prepared from imported stocks. The vaccine used was either in chilled form (Wright & Leatch 1996) or frozen form (Dalgliesh, Jorgensen & De Vos 1990) depending on the assessment being made. For the challenge trials described here, we considered that the form of vaccine used had no bearing on immunity at challenge as long as the recipient was shown to have become infected by the vaccine. Thus, all cattle used in the present work were shown to have become infected by both blood smear examination and seroconversion following vaccination (Turton, Katsande & Matingo, unpublished data 1996). The remainder of the experimental cattle were age- and breed-matched cattle added to the trials as non-infected controls to assess the effects of challenge with *A. marginale.*

Cattle in trials 1 and 2 were maintained at Mazowe field station, about 35 km from Harare, and cattle in trial 3, at the Central Veterinary Laboratory, Causeway, Harare. All cattle were housed indoors. Fortnightly applications of a synthetic pyrethroid preparation (Drastic Deadline, Bayer) precluded natural tick infestation. The cattle were fed concentrates and hay which had been fumigated with methyl bromide to kill ticks.

Parasites and challenge procedures

A. centrale used for vaccination of experimental cattle was derived from two separate stocks, one imported from the Onderstepoort Veterinary Institute, South Africa (hereafter referred to as OVI origin) and the other imported from the Tick Fever Research Centre, Wacol, Australia (TFRC origin). An objective of the present work was to compare the protection provided by these stocks.

A. marginale was obtained from three geographically separated farms (coded U, M and V) in Zimbabwe during 1994–1995. Isolate M was obtained from a cow in a group that had been vaccinated with *A. centrale* about ten weeks previously but was nevertheless experiencing a severe *A. marginale* infection. Thus this isolate was considered to be a "breakthrough" isolate. Isolates U and V were also obtained from cattle experiencing clinical anaplasmosis but in these cases the cattle had never been vaccinated. Blood from the cattle was brought to the laboratory and inoculated into susceptible splenectomized calves. When these became parasitaemic, blood from them was stored in liquid nitrogen, using polyvinylpyrrolidine as a cryoprotectant (Standfast & Jorgensen 1997).

To prepare a challenge inoculum for each trial, blood infected with *A. marginale* was thawed at 37 °C and 10 mℓ inoculated intravenously into a splenectomized calf previously shown to be free of antibodies to *Anaplasma.* Its rectal temperature and packed-cell volume (PCV) were measured daily and parasitaemia was monitored by examining thin films of blood from the jugular vein. When the parasitaemia was 10% or more of infected erythrocytes, approximately 500 mℓ of blood was collected from the jugular vein into heparin. The parasitaemia and PCV of the collected blood were then measured to calculate the volume of blood required for each challenge dose.

Challenge infections were monitored, beginning ten days after the challenge dose was given, by daily measurements of PCV, parasitaemia (% infected erythrocytes), rectal temperature and inspection for other clinical signs. Percentage PCV depression was calculated by the following formula:

$$\frac{\text{(PCV at time of challenge inoculation – minimum PCV recorded after challenge) x 100}}{\text{PCV at time of challenge inoculation}}$$

Parasitaemias of ≥ 33,3 % infected RBC were recorded as 33,3 %. Cattle meeting any of the following criteria were treated with either a long-acting tetracycline or imidocarb:

- Obvious clinical disease
- PCV equal to or less than 15%
- PCV less than 20% and parasitaemia greater than 5%

If clinical signs persisted, cattle were treated again.

Statistical methods

Analysis of variance was used to test the effects of treatments using an error term estimated from animal to animal variation. Data for animals treated were coded as 0 (not treated) and 100 (treated). Treatment means were compared using the protected least significant difference (l.s.d.) procedure operating at the 5% level of significance. Due to the non normality of

the data for % parasitaemia and % animals treated and the small number of animals in some groups, tests of significance for these two are approximate.

Experimental design

TRIAL 1—to determine the protection of TFRC *A. centrale* vaccine and compare responses of Senepol and Hereford breeds to *A. marginale* challenge

In this trial, conducted in February 1995, five Herefords vaccinated with *A. centrale* TFRC origin in July 1994, and 13 nonvaccinated cattle (six Herefords and seven Senepols) were inoculated intravenously with 10^8 *A. marginale* (U isolate). The two breeds of cattle were included to compare any inherent resistance to anaplasmosis.

TRIAL 2—to compare protection of vaccines from TFRC and OVI from challenge with M isolate of *A. marginale*

This trial, conducted in January 1996, was designed to compare the efficacies of *A. centrale* vaccines from TFRC and from OVI. All of the 34 cattle used were Herefords and experimental groups and vaccination inoculations are outlined in Table 1. All of the cattle were inoculated intravenously with 10^{10} *A. marginale* (M isolate).

TRIAL 3—to compare protection of vaccines from TFRC and OVI from challenge with V or M isolates of *A. marginale*

In this trial, conducted in June 1996, vaccine efficacy was tested against two challenge strains. All of the 41 cattle were Friesians and all but the control group were vaccinated in February 1996. The experimental groups and source of *Anaplasma* inoculations are outlined in Table 2. All of the cattle were inoculated intravenously with 10^8 *A. marginale* parasites of the respective challenge isolates.

TABLE 1 Showing the experimental groups of Hereford cattle used in trial 2 and their history of inoculation with *A. centrale*

Number of cattle per group	Vaccination month in 1995	Origin of *A. centrale* vaccine
7	January	OVI
7	June	OVI
4	June	TFRC
9	October	TFRC
7	nil vaccine	–

TABLE 2 Showing the different experimental groups of Friesian cattle used in trial 3 and the respective *A. centrale* and *A. marginale* challenge inoculations that they received

Number of cattle per group	Origin of *A. centrale*	Challenge isolate
10	OVI	V
10	TFRC	V
8	OVI	M
9	TFRC	M
4	nil vaccine	V

Field observations

Vaccine

The vaccine used for all field trials was derived from *A. centrale* TFRC origin and was in frozen form. It was manufactured using methods described in detail by Katsande & Turton (1995) and Dalgliesh *et al.* (1990). The frozen vaccine was prepared for use as described by Dalgliesh *et al.* (1990). For nearby farms it was thawed at the laboratory by immersion of the 5 mℓ cryovials containing vaccine in water at approximately 37°C, diluted and transported on ice to the crush-side where it was inoculated intramuscularly within 4 h of thawing. For two more distant sites, the frozen vaccine was transported to the crush-side in dry ice and then thawed, diluted and inoculated as described above.

Vaccinated herds

Co-operator farms were selected on the basis that the owners wished to have their cattle vaccinated against gallsickness (anaplasmosis), and agreed to monitor the cattle post-vaccination for severe reactions and provide appropriate care and attention if these were needed. Co-operators were advised to provide specific therapy if severe reactions were apparent and, if sick cattle were observed subsequently, to contact either the Central Veterinary Laboratory, local government veterinarians or private veterinarians so that pre-treatment blood smears could be made and autopsies performed if any were found dead. Co-operators were also advised to prevent tick-infestation for at least 2 months after vaccination to allow immunity to develop before possible natural infection with *A. marginale* could occur.

During the period November 1994 to August 1996, 9,553 cattle in 28 separate groups, comprising six to 1,488 cattle, were vaccinated on 16 farms and at one communal dip. Herds on 11 farms and the communal herd had a history of gallsickness prior to vaccination. The cattle were of mixed breed and type (including Friesian, Hereford, Limousin, Senepol, Sussex, Brahman and Afrikander), of both sexes and mixed age although the field teams vaccinated animals predominantly under one year of age.

On the first eight farms, 10% of the cattle were bled to provide pre-vaccination serum. Those that were negative in the CAT were bled again 56–60 days after vaccination to assess infectivity of the vaccine as indicated by seroconversion. The practice was discontinued after sufficient data were collected to minimise the inconvenience that the additional mustering of the cattle caused co-operator farmers.

TABLE 3 Responses to infection with 10^8 *A. marginale* (U isolate) in cattle that were either vaccinated with TFRC origin *A. centrale* (Herefords) or not vaccinated before infection (Herefords and Senepols)

Groups	Number	% PCV depression	Max. parasitaemia %	% treated (proportion treated)
Vaccinated Herefords	5	49,2[b]	11,5[b]	20 (1/5)[b]
Nonvaccinated Herefords	6	64,0[a]	30,6[a]	83 (5/6)[a]
Nonvaccinated Senepols	7	70,2[a]	33,3[a]	100 (7/7)[a]
Average LSD ($P = 0,05$)		13,7	9,3	41

Within columns, means with common superscripts are not significantly different ($P = 0,05$)

TABLE 4 Responses to infection with 10^{10} *A. marginale* (M isolate) in Hereford cattle either vaccinated with TFRC or OVI origin *A. centrale* or not vaccinated before infection

Groups	Number	% PCV depression	Max. parasitaemia %	% treated (proportion treated)
Challenged 12 months after vaccination (OVI origin)	7	60,1	27,1	100 (7/7)
Challenged 7 months after vaccination (OVI origin)	7	62,4	24,3	86 (6/7)
Challenged 7 months after vaccination (TFRC origin)	4	60,6	26,3	100 (4/4)
Challenged 3 months after vaccination (TFRC origin)	9	61,4	21,4	67 (6/9)
Nonvaccinated	7	64,4	33,3	100 (7/7)
Average LSD ($P = 0,05$)		14,7	13,4	41

No significant differences ($P > 0,05$) between treatments for any of the three variables

TABLE 5 Responses to infection with 10^8 *A. marginale* (either V or M isolate) of Friesian cattle either vaccinated with TFRC or OVI origin *A. centrale* or not vaccinated before infection

Groups	Number	% PCV depression	Max. parasitaemia %	% treated (proportion treated)
OVI origin vaccine, challenged with V isolate	10	44,7[a]	16,2	70 (7/10)
TFRC origin vaccine, challenged with V isolate	10	42,1[a]	13,2	50 (5/10)
OVI origin vaccine, challenged with M isolate	8	49,3[a]	22,3	88 (7/8)
TFRC origin vaccine, challenged with M isolate	9	46,8[a]	22,1	89 (8/9)
Nonvaccinated, challenged with V isolate	4	24,2[b]	11,7	75 (3/4)
Average LSD ($P = 0,05$)		8,9	9,7	47

Means within % PCV column with common superscripts are not significantly different ($P = 0,05$)
No significant differences ($P > 0,05$) between treatments for % parasitaemia and animals treated

RESULTS

Pen trials

Trial 1

The responses of the group of Herefords vaccinated with *A. centrale* were significantly different to the nonvaccinated group of Herefords in terms of % PCV depression and % maximum parasitaemia upon challenge with 10^8 U isolate *A. marginale* (Table 3). Only one animal in the vaccinated group and all but one in the nonvaccinated groups required treatment. Responses of nonvaccinated Herefords and Senepols were not significantly different upon challenge.

Trial 2

The results of this trial (Table 4) indicated that none of the groups vaccinated with *A. centrale* of TFRC or OVI origin was significantly protected against challenge with M isolate of *A. marginale,* with more than 50 % of animals in each group requiring treatment. There were no significant differences in % PCV depression, % maximum parasitaemia or number treated between the nonvaccinated group and vaccinated groups.

Trial 3

The results of trial 3 (Table 5) indicated that none of the vaccinated groups was protected against challenge

with V or M isolates of *A. marginale,* with a minimum of 50% of animals in each group requiring treatment. There were no significant differences in % PCV depression, maximum parasitaemia or number treated between vaccinated groups receiving TFRC or OVI origin *A. centrale* upon challenge with V or M isolates. Responses of the nonvaccinated group challenged with V isolate were not significantly different in maximum parasitaemia and % animals treated from vaccinated groups but the parameter of % PCV depression was significantly less. This result was interpreted as an experimental anomaly due to the small group number (4) and unusually low PCVs in all four animals at the start of challenge.

Field observations

The majority of the 9,553 cattle apparently showed little or no evidence of clinical signs during the period when reactions to vaccination might be expected (30–50 days post-vaccination). In one group of 131 closely-monitored bulls (*Bos taurus*), 12 were treated for severe reactions. One of the bulls developed clinical *A. marginale* infection 1–2 months later, suggesting that the herd was experiencing a field challenge. On another farm the cattle apparently experienced a field challenge within 3–4 weeks of vaccination, with three of 220 animals (*Bos taurus*) being treated for gallsickness (unconfirmed). On yet another farm where several hundred cattle (mix of *Bos taurus* and *B. indicus* breeds) were vaccinated, two died and six were treated for unconfirmed anaplasmosis during the "reaction period"; several weeks later (about ten weeks after vaccination) at least two cattle died and two were clinically affected as a result of confirmed *A. marginale* infections. It was from this farm that the M isolate of *A. marginale* was obtained for the pen trials. On a further farm a group of vaccinated cattle was treated, perhaps prematurely, because of clinical signs in some of the group.

Of 167 cattle that were negative in the initial CAT for antibodies, 159 (95%) were positive in the second test indicating a high seroconversion rate following vaccination. All farms but one followed recommendations to continue dipping for at least 2 months indicating that seroconversion was largely a result of vaccination.

In 1997, 12 months after the last vaccination, 16 cooperator farms which had had problems with anaplasmosis were surveyed by mail to determine the level of satisfaction with the *A. centrale* vaccine. Twelve replies were received (seven very good; four good; one satisfactory). Of these, one property had three unconfirmed deaths from anaplasmosis (satisfactory rating) and one had a confirmed vaccine breakdown (breakthrough) confirmed by the Central Veterinary Laboratory (good rating).

DISCUSSION

In the pen trials, *A. centrale* provided variable protection, ranging from moderate to poor, against field isolates of *A. marginale* obtained from different areas of Zimbabwe. The degree of protection was reasonable against isolate U, but much less against the other two isolates. The poor protection provided against isolate M was predictable as this isolate had caused overt disease in vaccinated cattle in the field, but the similar results obtained with isolate V were surprising. Overall, our results support findings of modest protection being provided by *A. centrale* against its more virulent relative under experimental conditions (Potgieter & Van Rensburg 1983).

The origin of the *A. centrale* stock used to vaccinate cattle in the pen trials had no apparent effect on the results. Although both the TFRC and OVI stocks came from the same isolate many years ago, *A. centrale* was taken to Australia from South Africa in 1934 (Seddon 1952) and we suspected that significant immunogenic differences between the stocks may have arisen during the long period of disassociation. No such difference was apparent in these experiments.

The size of the challenge dose (10^8 compared with 10^{10}) also had no obvious effects on the results. After the second trial, it was suggested that 10^{10} *A. marginale* may be an excessive challenge dose (Potgieter, Onderstepoort Veterinary Institute, South Africa, personal communication 1996), but reversion to a dose of 10^8 for the third trial did not markedly reduce the severity of challenge infections. It is possible that either dose, when given intravenously, is a far greater test of the hosts' immunity than the challenge encountered by vaccinated cattle under field conditions. In a previous study, the severity of primary infections with *A. marginale* given intravenously was not dose dependent when doses of 10^{10}, 10^8 and 10^6 were administered (Gale, Leatch, De Vos & Jorgensen 1996) and the same may apply with challenge infections.

Field observations on use of the vaccine were by no means complete but some conclusions can be drawn from our own observations and from information received from farmers. Seroconversion rates following vaccination indicated high infectivity of the vaccine, though on some properties tick-borne infection may have confounded the data. In general, response of recipients to the vaccine was apparently mild but reactions requiring treatment occurred in certain classes of cattle, namely mature *Bos taurus* bulls and cows. Certain management practices apparently exacerbated some post-vaccination problems. Thus, there were indications of severe tick-borne challenge in some herds too soon after vaccination to allow a reasonable expectation that the vaccine would be protective. Also, cattle on some properties were subjected to procedures, such as walking long distances,

which were likely to provoke a vaccination reaction. These and other observations during the course of this work influenced the formulation of recommendations for future use of the vaccine under Zimbabwean conditions (Katsande & Turton 1995).

What implications arise from this work for future control of anaplasmosis in Zimbabwe? A confirmed or suspected history of gallsickness in 12 of the 17 study herds is indicative of high prevalence of the disease and suggests that control measures such as vaccination will be needed increasingly as acaricide usage decreases. The pen trials indicate that *A. centrale* can be expected to provide only partial protection against *A. marginale*, with the level of protection provided probably being poor in some locations. Nevertheless, the general response of co-operators to use of the vaccine in field trials has been very positive, with the majority being satisfied that vaccination was a manageable, cost-effective and beneficial procedure. The Australian experience with *A. centrale* vaccine has been similar, in that the vaccine appears to be useful under field conditions (Callow & Dalgliesh 1980; Callow 1984) but provides variable, sometimes poor, protection against experimental challenge (Rogers & Shiels 1979; Wilson, Parker & Trueman 1980). A mild strain of *A. marginale* could be considered as an alternative to *A. centrale* (Tbele & Palmer 1991) for incorporation into a vaccine. In field use, however, live *A. centrale* vaccine is less likely to cause severe reactions than live *A. marginale* vaccines and provides more protection than killed *A. marginale* vaccines currently available (reviewed by Pipano, Frank & Shkap 1991). On balance, the use of *A. centrale* vaccine appears to be justified until a more effective alternative becomes available.

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