Field observations on the duration of immunity in cattle after vaccination against *Anaplasma* and *Babesia* species

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ABSTRACT

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In an outbreak of *Babesia bovis* in a large herd of Friesian x Malawi Zebu cattle, which occurred after an interruption of intensive dipping, clinical or fatal babesiosis occurred in 54/299 (18,1%) animals which had never been vaccinated, as compared to 9/153 (5,9%) vaccinated animals. Eight of the nine affected vaccinates had been vaccinated more than 27 months previously.

Sera were collected every 3–4 months from 33 Friesian x Malawi Zebu heifers maintained with intensive dipping and vaccinated with trivalent *B. bovis*, *Babesia bigemina* and *Anaplasma centrale* vaccine. After 2 years, 25% had become seronegative for *B. bovis* by indirect immunofluorescence, as compared to 97% for *B. bigemina* and 46% for *A. centrale*.

Because of the evidence that immunity following vaccination against *B. bovis* declines after 2 years in the absence of tick challenge, it is recommended that tick control should be relaxed after immunity has been established, in order to save acaricide, reinforce immunity and avoid any need for revaccination.

Keywords: *Anaplasma*, *Babesia*, cattle, duration, immunity, vaccination

INTRODUCTION

Babesiosis and anaplasmosis caused by *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* are some of the tick-borne diseases (TBD) restricting the introduction of improved cattle breeds into the tropics. In eastern and southern Africa, control of babesiosis and anaplasmosis in improved cattle is usually achieved by weekly dipping in acaricide, which also controls other TBD, notably East Coast fever (*Theileria parva* infection), whereas in Australia, control has in recent years been based largely on vaccination with a live-blood vaccine. With the increasing cost of acaricide, concern about environmental side effects and the development of acaricide resistance in ticks, vaccination as a control measure for TBD has become more relevant in south-eastern Africa.

When vaccination regimens are planned, the duration of immunity following vaccination must be known. The protective effect of *Anaplasma* and *Babesia* vaccines is generally considered to be prolonged, and producers of vaccine [e.g. Tick Fever Research Centre (TFRC), Queensland Department of Primary Industries, Wacol, Australia] usually recommend a single vaccination for cattle in endemic areas. The immunity stimulated by vaccination is reinforced by continuous challenge and persists for life, provided that the animals are exposed to ticks.

In animals kept under tick-free conditions, *B. bovis* has been shown to persist as a latent infection in *Bos taurus* cattle for 4 years after natural infection, conferring a strong immunity (Mahoney, Wright & Mirre

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1973). Mahoney, Wright & Goodger (1979) also confirmed the persistence of immunity in animals inoculated with live vaccine or field strains, but the number of seropositive animals did decrease gradually after 2 years, falling to 50–70% after 4 years. In an area with low tick exposure, Callow, Emmerson, Parker & Knott (1976) found that certain animals lost their *B. bovis* infection over a period of time and became susceptible to challenge, and 4/328 animals vaccinated more than 22 months previously, developed clinical babesiosis (Emmerson, Knott & Callow 1976). On the basis of these reports, Timms, McGregor & Dalgliesh (1981) recommended revaccination for *Babesia* after 18 months, in animals not exposed to ticks.

Parasitaemia after natural infection or inoculation with *B. bigemina* has been shown to be short-lived, though immunity to a mild-challenge strain persisted for 4 years (Mahoney *et al.* 1973). There is no published information on the persistence of antibodies and immunity after vaccination with *A. centrale*, although immunity after vaccination with *A. marginale* is known to depend on a long-lasting carrier stage (premunity) and is linked to persistence of antibodies (Ristic 1960). Potgieter (1979) expected lifelong immunity after a single vaccination with chilled *A. centrale* vaccine, and the OIE manual indicates that immunity following vaccination with frozen *A. centrale* vaccine lasts for several years (Anon. 1991).

This paper summarizes experiences on duration of immunity after vaccination with live *Anaplasma* and *Babesia* vaccines in two field studies in Malawi.

MATERIALS AND METHODS

Vaccine

The *Anaplasma* and *Babesia* vaccines used in the studies were live, frozen, blood-based vaccines, administered as a trivalent pool containing *B. bovis*, *B. bigemina* and *A. centrale*, obtained from TFRC ("Combavac"—Queensland DPI) or produced in Malawi by conventional methods (Anon. 1984). The *B. bovis* and *B. bigemina* strains used were the attenuated Ka strain and the attenuated G strain, respectively, both developed at TFRC, and the *A. centrale* strain was the Onderstepoort strain originating from South Africa. The dose administered was 2 mℓ of vaccine at a 1:10 dilution, which contained at least 6 ID_{50} (50% infectious dose) of each component, as demonstrated by titration in Friesian x Malawi Zebu cattle.

Serological tests

Indirect immunofluorescence tests (IFT) for *Babesia* spp. were carried out by conventional techniques (Anon. 1984). The test for *A. centrale* was based on that described by Goff, Johnson & Kuttler (1985) and modified by Montenegro-James, James & Ristic (1985). Antigen slides for each parasite were prepared from calves infected with vaccine strains. In each case, sera were tested at a dilution of 1/90. Eight test sera, together with a positive and a negative control serum on each slide, were allowed to absorb for 30 min. After they had been washed, the slides were allowed to absorb with a rabbit anti-bovine fluorescein isothiocyanate-conjugated serum. The slides were examined for fluorescence with a x40 objective. A Leitz microscope and incident ultraviolet light were used. For *A. centrale*, the antigen slides were washed in glycine pH 2,8 before sera were applied, and again with skimmed milk before conjugate was applied. Preliminary studies revealed that at a dilution of 1/90, the test confirmed the establishment of infection after vaccination in 97% animals vaccinated for *B. bovis* and in 100% animals vaccinated for *B. bigemina* and *A. centrale*. At dilutions of 1/90 there was no evidence of cross-reaction between the two *Babesia* species.

Observations

Information on the duration of immunity and the persistence of antibodies after vaccination, was obtained from observations of Friesian x Malawi Zebu cattle involved in two field studies.

Duration of immunity

During March–May 1993, a herd of crossbred cattle at Likasi Livestock Centre (LLC) near Lilongwe suffered an outbreak of babesiosis caused by *B. bovis*. The outbreak was preceded by a sudden breakdown in dipping caused by an interruption in the supply of acaricide. Until that time, the cattle at the centre had been dipped weekly in chlorfenvinphos (Supona; Shell Chemicals), except for 106 involved in a Strategic Dipping Trial (SDT). They had been vaccinated and were being monitored serologically. Natural transmission of TBD had been minimal, except for a minor outbreak of *B. bovis* in 1991 (Lawrence, Malika, Whiteland & Kafuwa 1993). Of the animals dipped weekly, 153 had been vaccinated against *B. bovis*, either alone or in combination with other vaccines, at varying times over the previous 4 years, while 299 had not been vaccinated.

During the outbreak, all animals that became clinically ill were treated with diminazene (Berenil; Hoechst), without the diagnosis being confirmed microscopically. Dead animals were subjected to a post-mortem examination. Animals were evaluated as having been sick with babesiosis if they had been observed to be clinically ill and had recovered after treatment with diminazene, or if post-mortem examination and blood or brain smears had revealed infection with *Babesia*. All dead animals were microscopically diagnosed as having had *B. bovis*, therefore all sick animals were

considered to have been infected with *B. bovis*, though the possibility of other TBD could not be excluded. Challenge with all three parasites, *B. bovis*, *B. bigemina* and *A. marginale*, during the period of the outbreak, was demonstrated serologically in the animals in the SDT, but *B. bovis* predominated. Twelve of 16 (75%) animals negative for *B. bovis* 3 months before the outbreak had seroconverted by the end of the outbreak, compared with 17/47 (36 %) for *B. bigemina* and 9/31 (29%) for *A. centrale*.

Persistence of antibodies

Ninety crossbred heifers on three government farms in the southern region of Malawi were vaccinated with trivalent *A. centrale*, *B. bovis* and *B. bigemina* vaccine. All animals on the farms were dipped weekly in chlorfenvinphos. The vaccinated heifers, as well as 95 unvaccinated heifers, were bled for serum before vaccination and every 3 to 4 months after that. After 24 months, 33 of the vaccinated heifers were still present on the farms and were assessed for persistence of antibodies.

RESULTS

Duration of immunity

The overall incidence of clinical babesiosis (presumed *B. bovis*) at LLC during the outbreak in vaccinated animals was 10/259 (3,9%), as compared to 54/299 (18,1%) in unvaccinated animals. Only one of the 106 strategically dipped, vaccinated animals developed clinical babesiosis and it recovered after treatment. Nine of the 153 (5,9%) weekly dipped, vaccinated animals developed clinical babesiosis (Table 1) and five died. Eight of the cases occurred amongst 27 cattle that had been vaccinated more than 27 months previously, compared to 1/117 in cattle vaccinated more recently.

Persistence of antibodies

Serological tests revealed a minimal exposure to TBD in unvaccinated animals on the three farms in the southern region for the first 2 years after vaccination. In the 33 vaccinated animals there was a steady reduction in the number showing antibodies (Fig. 1). After 24 months, 25% had become seronegative for *B. bovis*, as compared to 97% for *B. bigemina* and 46% for *A. centrale*. Analysis of the vaccination dates and serological status of the cattle in the SDT at LLC, before commencement of the trial, confirmed these findings (Table 2). There were no significant differences (P>10%) between the duration of persistence of antibodies in the animals in the SDT and those from the study in the southern region for the first 24 months after vaccination.

DISCUSSION

It is evident from the experiences at LLC that the susceptibility of previously unexposed, vaccinated animals to challenge with *B. bovis* increased with time after vaccination; 27 months seems to have been a critical point. This is in accordance with the findings of Emmerson *et al.* (1976), but not with those of Mahoney *et al.* (1973; 1979). Differences in the period of persistence of latent infection in Zebu-cross cattle as compared to European (*Bos taurus*) breeds (Johnston, Leatch & Jones 1978) may be responsible for differences in the duration of immunity.

Persistence of antibodies after vaccination for *B. bovis* in unexposed animals at LLC and at government farms in the southern region of Malawi was similar to that found by Mahoney *et al.* (1979). The finding by Johnston & Tammemagi (1969) that latent infections persisted in 10/10 unexposed calves until 13 months after vaccination, combined with the demonstration of a sharply declining proportion of seropositive animals within 6 months after chemosterilization of latent infection (Callow, McGregor, Parker & Dalgliesh 1974a), suggests that the high percentage of seropositives after 2 years is probably the result of a persistent latent infection. However, Callow *et al.* (1974a) found that disappearance of antibodies after elimination of latent infection was not accompanied by immediate loss of immunity. In the present study, however, there was a good correlation between the persistence of antibodies and the duration of immunity at herd level.

TABLE 1 Incidence of clinical babesiosis in weekly dipped, vaccinated animals following the breakdown in dipping at Likasi Livestock Centre in 1993

Health status	Interval between vaccination and exposure (in months)				Total
	4–15	16–27	28–39	40–51	
Sick	0 (0 %)	1 (2,7 %)	7 (21,9 %)	1 (33,3 %)	9 (5,9 %)
Well	81	36	25	2	144
Total	81	37	32	3	153

TABLE 2 Serological status of cattle at Likasi Livestock Centre related to time elapsed since vaccination against *B. bovis*, *B. bigemina* and *A. centrale*

Months since vaccination	No. of animals	% seropositive		
		B. bovis	*B. bigemina*	*A. centrale*
1–2	24	91,7	91,7	75,0
12–14	30	80,0	56,7	73,3
16–18	8	100,0	37,5	87,5
22–24	30	83,3	20,0	56,7
31–33	11	81,8	27,3	36,4
41–42	3	66,7	0,0	66,7

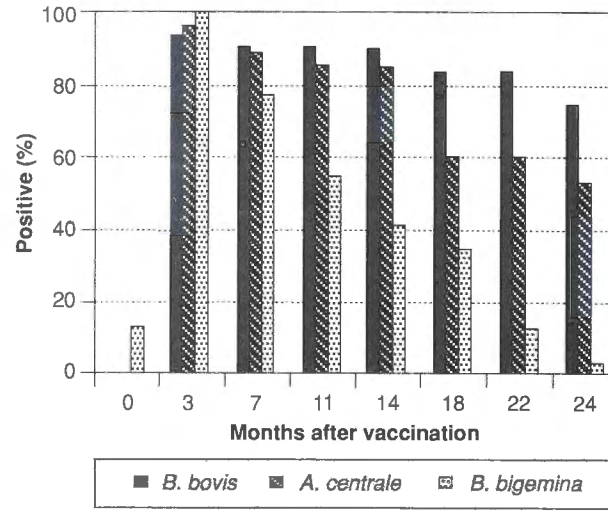

FIG. 1 Persistence of antibodies in 33 heifers after vaccination against *B. bovis*, *B. bigemina* and *A. centrale*

The rapid decline in the proportion of seropositive animals after vaccination with *B. bigemina* corresponds with the findings of Callow, McGregor, Parker & Dalgliesh (1974b). They found that, within 6 months of vaccination, the parasite had been eliminated from a high proportion of animals which subsequently became seronegative in the IFT, although they showed that immunity persisted for 7 months after chemosterilization. On the other hand, De Vos (1979) found that 60% of the animals vaccinated against *B. bigemina* were still seropositive after 21 months. It is possible that breed differences may affect the persistence of infection with this parasite as well.

If one considers the long-term latent infection that is believed to exist with *A. marginale* (Ristic 1960), the number of animals seropositive for *A. centrale* 2 years after vaccination was less than expected. This result could be an indication of more rapid loss of infection with *A. centrale* than with *A. marginale* in unexposed cattle, but it is not known how this would affect the duration of immunity.

Findings from the studies reported in this paper support the recommendations of Timms *et al.* (1981), that cattle should be revaccinated after 18 months if they are not being exposed to tick challenge. To combine vaccination against TBD with continued intensive dipping is, however, counterproductive and expensive. For *B. bovis* and other TBD, tick control should be relaxed after immunity has been established, in order to save acaricide, maintain immunity and avoid any need for revaccination.

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