Serological response to early vaccination against *Babesia bovis* and *Babesia bigemina* in dairy calves

by

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DECLARATION

I declare that this is my own original work and that it has not been presented for any other degree to this or another university.

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ABSTRACT

Calves infected with *Babesia bovis* or *Babesia bigemina* between 3 and 9 months of age can develop immunity without showing overt clinical signs. Thistransient immunity is not dependent on maternal immunity. After 9 months of age, they are fully susceptible to challenge. Dairy calves between 2 and 3 months of age were vaccinated with *B. bigemina* and *B. bovis* live frozen vaccines (Onderstepoort Biological Products®). Two months after vaccination, 90% of calves were serologically positive on IFA test to *B. bigemina,* and 70% were serologically positive to *B. bovis*. At this age, only 17% of the control group had seroconverted to *B. bigemina* and none of the calves had seroconverted to *B. bovis.* All experimental calves maintained positive serological status to both *B. bovis* and *B. bigemina* for at least 5 months after vaccination. It is sound practice to vaccinate dairy calves against babesiosis at 2–3 months of age. Endemic stability is achieved before the period of natural resistance wanes.

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CHAPTER 1

REVIEW OF THE LITERATURE

Introduction

Internationally babesiosis, caused by *Babesia bigemina* and *Babesia bovis,* is the most economically important arthropod-borne disease of cattle (Bock, Jackson, De Vos & Jorgensen, 2004). In 1980, it was estimated that the combined effect of *B. bovis, B. bigemina* and *Anaplasma marginale* cost South Africa up to R200 million per annum (Bigalke, 1980). The disease is well-researched and effective vaccines are available. Despite this, preventable mortalities continue to unnecessarily erode profit. This project highlights the importance of timing vaccination in such a way that protective immunity develops early enough to avoid mortalities.

A commercial dairy farmer in the Lydenburg district, Mpumalanga, experienced periodic losses due to confirmed cases of babesiosis on his farm. In addition to the confirmed cases, farm records provide evidence of unconfirmed babesiosis cases in adult cattle and in calves under 1 year of age. It was also noted that some of the calves, although not clinically ill, were doing poorly. The farmer decided to rule out babesiosis as a contributing factor to these problems. Vaccinating replacement heifers at 2–3 months of age was suggested as a possible intervention. Since this was an early age to vaccinate, serological follow-up was recommended to establish whether seroconversion had taken place. A control group of bull calves was available to compare the serological response in non-vaccinated calves kept under the same conditions. This on-farm study investigated whether immunity to babesiosis can be achieved earlier by vaccinating calves at 2–3 months of age.

Management of babesiosis in dairy cattle

Two different approaches can be used to control babesiosis in cattle (De Vos, De Waal & Jacobson, 2004). One way is to keep cattle completely tick free to prevent exposure to infection. The disadvantage of this approach is that cattle remain immunologically naïve, rendering them fully susceptible to challenge. The preferred approach, which strives to attain endemic stability in the herd, is achieved by a combination of vaccination and judicious tick control, or by simply allowing calves to develop resistance following field challenge (De Vos, 1979). Achieving endemic stability at an early age significantly reduces the risk of calves and older cattle developing babesiosis.

The importance of innate age-related immunity

A period of innate immunity, independent of maternal immunity, in 3–9-month-old calves, is reported in the literature (Pound, 1897; Legg, 1933; Hall, 1960; Mahoney, 1969; Lohr, 1969; Mahoney & Ross, 1972; Trueman & Blight, 1978; Latif, Said & Ali,1977; Bock, De Vos, Kingston, McLellan, 1997). The literature in which this period of innate resistance is mentioned is summarised in Table 1. Most field work has been done on Australian beef cattle and in some cases it was not clear which *Babesia* species was under investigation. There is a paucity of field work done on dairy cattle.

Despite the fact that calves elicit an early protective immune response to *B. bovis* infection, protection is not always enough to prevent mortality. Trueman and Blight (1978) is the reference most commonly cited to substantiate innate immunity in calves. These authors infected 12 5–6-month-old beef calves with an Australian strain of *B. bovis*. Four

Table 1: Literature review – Innate immunity: A synopsis of literature published in which the period of innate resistance was noted or tested under field conditions.

References	**Age <2 Mo**	**Age 3–9 Mo**	**Age >1 Yr**	**%Morbidity/ %Mortality**	**Type**	**Country**
Pound,1897			X	0% Mortality	Beef/dual	Australia
Pound, 1897			2-5y	36% Mortality	Beef/Dual	Australia
Legg, 1933				Observations	N.A.	Australia
Mahoney, 1969				Observations	N.A.	Australia
Hall,1960	X				Not Specified	Australia
Hall,1963	X				Not Specified	Australia
Lohr, 1969			X	48% Morbidity	Beef	Kenya
Mahoney & Ross,1972	X		X		Beef	Australia
Latif, Said & Ali,1977		X	X		Dairy	?
Trueman and Blight, 1978		X		33% Morbidity 8% Mortality	Beef	Australia
Guglielmone, 1995	X	X		Observations	Dairy	South America
Bock, Kingston & de Vos, 1999			X	0% 30% 8% 0%	Beef	Australia

of these calves required treatment (33% morbidity) and one died (mortality rate 8%). This trial illustrated that a protective immunity existed, but was not absolute.

Innate immunity to babesiosis in young calves was already reported in the 19th century (Smith & Kilborne, 1893, cited in Callow, 1984). One of the earliest studies mentioned was an observation made by Pound (1897), who found that naive Australian Shorthorn cattle under 2 years of age were resistant to babesiosis. Pound inoculated 35 Shorthorn bulls with *Babesia*-infected blood and noted no clinical reactions requiring treatment. When the same procedure was performed on cattle over 2 years of age, 36% of the bulls died of babesiosis. Legg (1933) did not perform experimental work, but noted that younger animals suffered a slight malaise, while aged bulls were more susceptible. Legg observed that over a 20-year period, negligible losses had occurred after inoculating against *B. bigemina*.

Calves under two months of age are protected from *B. argentina* (= *B. bovis*) infection by maternal immunity (Callow, 1979). Hereafter, a non-specific immunity which protected the calf for at least an additional 5 months was observed.

Latif *et al*. (1977) observed that challenging calves with *B. bigemina* usually did not result in clinical disease. Four 6-month-old Holstein calves were challenged with *B. bigemina* and there were few, if any, clinical manifestations of disease. When four 1-year-old calves were challenged, however, they developed severe clinical signs.

Observations by Guglielmone (1995) are that calves under 7 months of age usually do not exhibit clinical signs following *Babesia* spp. infection and that lasting immunity is not necessarily dependent on re-infection. However, he also reports that calves under 4 months of age, infected with *Babesia* spp, have a reduced appetite and lose weight.

A clear difference in susceptibility to babesiosis between *Bos indicus* and *Bos taurus* breeds has been demonstrated by various authors. Bock, Kingston and De Vos (1999)

investigated age-related resistance in different breeds of 15–18-month-old *Bos indicus*, *Bos taurus* and *Bos indicus* x *Bos taurus* steers. Findings were that all breeds were equally resistant to the Australian *B. bigemina*, but *Bos taurus* breeds were more susceptible than other breeds of cattle to the Australian strain of *B. bovis*. It is also possible that a difference in susceptibility could occur within the *Bos taurus*-type animals and that *Bos taurus* dairy cattle may well be more susceptible to infection than *Bos taurus* beef cattle.

A molecular basis for this period of innate immunity to *B. bovis* has been demonstrated and elucidated in young calves (Levy, Clabaugh and Ristic, 1982; Goff, Johnson, Parish, Barrington, Tuo & Valdez, 2001; Goff, Johnson, Tuo, Valdez, Parish, Barrington & Davis, 2002; Goff, Johnson, Horn, Barrington & Knowles, 2003; Brown, Norimine, Knowles & Goff, 2006). Compared to adult cattle, calves elicit an immune response which results in a decreased parasitaemia, more rapid cytokine response and a well-regulated reduction of inflammatory by-products. During this period of innate immunity, exposure to field challenge or vaccination is well-tolerated by calves. This can be used as a management tool, ensuring that calves become immunocompetent prior to field challenge when the period of innate immunity ends.

This period of innate resistance wanes by approximately 9 months of age (De Waal & Combrink, 2006; De Vos *et al*., 2004). If calves have not been exposed to *Babesia* spp by this time, allowing them to build up a baseline immunity to the disease, they will be fully susceptible at this age. Dairy calves are particularly at risk, as they are often intensively dipped and housed until 7–9 months of age. Releasing them onto tick-infested pasture at a time when innate immunity is waning, without any acquired immunity, puts these calves at enormous risk of succumbing to babesiosis. A management goal on any dairy or beef farm should be to ensure that more than 75%

of calves are seropositive to *B. bigemina* and *B. bovis* by 9 months of age (Regassa, Penzhorn & Bryson, 2003).

It is generally accepted that cattle between 3 and 9 months of age are less susceptible to *B. bovis* and *B. bigemina* infection than older cattle. That this immunity is infallible, is false; that this applies to all breeds of cattle in all areas, is questionable, and the exact length of this period has not been experimentally determined.

Innate vs acquired immunity

A fundamental distinction must be made between age-related innate immunity and acquired immunity: Innate immunity is independent of previous exposure and also independent of maternal immunity. It is effected by activation of splenic macrophages which release inflammatory products, notably nitric oxide, leading to destruction of the parasite (Brown, 2001). The acquired response is, by definition, dependent on previous exposure to an antigen. There is a humoral component to this response, but the cell-mediated response is of greater importance in eliminating the parasite (Brown *et al*., 2006). Measuring a cell-mediated response is possible, but is not in routine laboratory use (J.E. Crafford, personal communication, 2010). Since there is a humoral component to this reaction, this is used as an indirect measure of immune competence. If no response is serologically measurable, however, it does not necessarily mean that the animal is not immunocompetent. This is of importance when interpreting serological data, especially from animals vaccinated against, or exposed to *Babesia* spp. some time previously. An animal can exhibit no titre to either *B. bovis* or *B. bigemina*, yet be protected from field challenge (Callow, McGregor, Parker & Dalgliesh, 1974).

The role of vaccination

One of the most important considerations when vaccinating calves early is whether maternal immunity interferes with the development of protective immunity. This was assessed by measuring titres to both *B. bovis* and *B. bigemina*, at the time of vaccination.

Live vaccines are available against both *B. bovis* and *B. bigemina*. Being frozen, however, these vaccines have specific handling and administration requirements and potentially cause clinical disease if administered to adult cattle. Farmers are therefore reluctant to use the vaccines on adult cattle. However, very few calves vaccinated between 3 and 9 months of age show ill effects (De Waal *et al*., 2006.)

The bovine babesiosis vaccines produced by Onderstepoort Biological Products are registered for use on calves from 3–9 months of age. Conventionally, the vaccine is rarely administered as early as 3 months of age. As dairy calves turned out to pasture are frequent victims of babesiosis, it is of cardinal importance that calves seroconvert effectively before the period of innate immunity wanes.

The indirect fluorescent antibody (IFA) test

A semi-quantitative serological test was required to measure the response of calves to vaccination. The various tests available for the diagnosis of *Babesia* spp were reviewed by Bose, Jorgensen, Dalgliesh, Friedhoff & De Vos (1995). A complement fixation test (CFT) exists, and enzyme-linked immunosorbent assays are being developed, but currently, the IFA test is the most widely used test, and is offered routinely at the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI).

The sensitivity of the IFA test, especially for detecting early infections, is good. Kutler and Todorovic (1977) compared the sensitivity of the CF test to the IFA test for the

detection of antibodies to both *B. bovis* and *B. bigemina*. They found that the IFA test was superior to the CFT, with a sensitivity of 100% during the first 84 days of infection, and 95% from 98–175 days. False positive reactions to *B. bovis* have been reported, but these are usually low titre responses and are associated with a *B. bigemina* titre of over 1:160 (Bessenger & Schoeman, 1983). For this reason, titres are only regarded as positive if they are 1:80 or more.

CHAPTER 2

MATERIALS AND METHODS

Study site

Holstein-Friesian dairy calves were used for this field experiment. Calves were raised in a closed herd in Mpumalanga, South Africa: Welgedacht Dairy (*025°deg25'S 030°16'E alt 1250m)*. The farm is pasture-based: Irrigated 20 ha Kikuyu, 20 ha annual ryegrass (Enhancer, Italian type).

Study population

Ten Holstein-Friesian heifer calves raised on the farm were assigned to the experiment and nine Holstein-Friesian bull calves were used as unvaccinated controls. Calves are raised on concrete with an exercise pen until at least12 months of age and then go out to pasture. The farm makes use of regular veterinary herd visits and applies a vaccination and deworming program recommended by the attending herd veterinarian.

Tick control

Tick control is stringent. Cattle are checked every two weeks, and if ticks are found, they are dipped. Tick burdens in winter are low.

Vaccines

The Onderstepoort frozen Asiatic redwater vaccine® and African redwater vaccine® were purchased from Onderstepoort Biological Products. The vaccine vials were delivered in the standard manner in dry ice. The vaccine vials were then transferred to a managed liquid nitrogen flask at the Sterkspruit Veterinary Practice, Lydenburg,

Mpumalanga. On the day of vaccination, both vaccines (*B. bovis* and *B. bigemina*) were removed from the liquid nitrogen, transferred onto crushed ice and transported to the farm.

Procedure

One millilitre of the *B. bigemina* vaccine and 1 ml of the *B. bovis* vaccine were inoculated into the calves using a deep intramuscular injection with an 18G needle. This was done within 4 hours of removing the vaccine from the liquid nitrogen flask. At the same time, blood was collected either from the caudal vein or the jugular vein into a 6 ml sterile serum tube. Blood was collected from calves at approximately 2-monthly intervals. Calves were vaccinated 2–3 months after birth (See Table 2). The number of days from vaccination to blood-sampling was calculated for each calf. Calves were bled at 2–3, 4–7, 9–11 and 12–16 months after vaccination. Since visits could not be timed exactly, there are several instances where a result is absent from a specific group, and this is indicated on the results tables by NC (Not collected). Blood collected from calves was kept on ice and then taken to Ampath laboratories, Lydenburg, for centrifugation. Serum was then collected and stored in a refrigerator, maintained between 3ºC and 10ºC, managed twice daily with a minimum/maximum thermometer. Serum samples were sent on ice to the ARC-OVI where an indirect fluorescent antibody (IFA) test was performed on the samples. At the time of vaccination, blood smears were also made using peripheral blood collected from the tip of the tail. These smears were stained with Diff-Quick® (Kyron Laboratories) to check for the presence of *Anaplasma marginale* and any other parasites.

Table 2: Birth dates of experimental and control calves.

CALF NR	DATE OF BIRTH	VACCINATION DATE
EXPERIMENTAL GROUP		
9001	7-Jan-09	11-Mar-09
9003	16-Jan-09	11-Mar-09
9004	21-Jan-09	11-Mar-09
9008	22-Mar-09	20-Jun-09
9009	24-Mar-09	20-Jun-09
9010	2-May-09	21-Aug-09
9011	3-May-09	21-Aug-09
9012	18-May-09	21-Aug-09
9013	20-May-09	21-Aug-09
9014	16-Jul-09	8-Oct-09
CONTROL GROUP		
B912	16-May-09	UNVACCINATED
B913	23-May-09	UNVACCINATED
B914	30-May-09	UNVACCINATED
B915	9-Jun-09	UNVACCINATED
B916	24-Jun-09	UNVACCINATED
B917	5-Aug-09	UNVACCINATED
B918	29-Aug-09	UNVACCINATED
B919	10-Sep-09	UNVACCINATED
B920	23-Oct-09	UNVACCINATED

Serology

Antibody titres were determined using the IFA test method. The method conforms to OIE specifications (De Vos & Jorgensen, 1991). Blood from donor cows, housed at the ARC-OVI, infected with either *B. bovis* or *B. bigemina*, was used to prepare slides. Test sera were diluted serially with phosphate-buffered saline (PBS), 1:40, 1:80 and 1:160, and added to test wells on the slides. Prepared positive and negative controls were also included. Hereafter, rabbit-anti-bovine IgG conjugated to fluorescein isothiocyanate was added. The plates were incubated in a humid chamber for 20 minutes at 37ºC. After removal from the chamber, the slides were rinsed with PBS and then washed twice with PBS for 10 minutes each, followed by a wash with water. The samples were then examined using fluorescence microscopy. The test was regarded as positive if clear fluorescence of the majority of cells on the slide occurred. Samples were interpreted as positive if fluorescense occurred at titres of 1:80 or 1:160. The samples were not diluted beyond 1:160.

CHAPTER 3

RESULTS

Results of blood smears made on the day of vaccination are given in Table 3.

Table 3: Blood smear results – Taken at time of vaccination (Experimental group).

Calf nr	***Babesia* spp.**	***Anaplasma marginale***
9001	NEG	NEG
9003	NEG	NEG
9004	NEG	NEG
9008	NEG	NEG
9009	NEG	NEG
9010	NEG	NEG
9011	NEG	NEG
9012	NEG	<1/HPF
9013	NEG	NEG
9014	NEG	<1/HPF

Table 4 and Table 6 depict serological changes following vaccination of heifer calves at 2–3 months of age. Two to three months after vaccination, 70% of these calves had seroconverted to *B. bovis* and 90% of the calves had seroconverted to *B. bigemina.* Four to seven months after vaccination, 100% of calves had seroconverted to *B. bovis*

and 71% had seroconverted to *B. bigemina*. At 9–11 months following vaccination, seroconversion rate was 88% to both *B. bovis* and *B. bigemina*. In contrast, only 50% of unvaccinated control calves had seroconverted to *B. bigemina* by 7–8 months of age, and none of the control calves had yet seroconverted to *B. bovis.*

All calves tested were still seropositive to both *B. bovis* and *B. bigemina* between 12 and 16 months after vaccination. Since control calves were also serologically positive from 11 months of age, it is not possible to determine whether this persistence of immunity was due to the vaccine or due to reinforcement of immunity by field challenge (Table 5 & Table 7).

Neither *B. bigemina* nor *B. bovis* were detected on blood smears taken from experimental calves on the day of vaccination, but calves 9012 and 9014 did have low parasitaemias of *Anaplasma marginale* (see Table 3).

Babesia bovis

Table 4: EXPERIMENTAL GROUP: IFA titres of heifer calves (n = 10) after vaccination with Onderstepoort *Babesia bovis* vaccine at 2–3 months of age.

	AT	**MONTHS AFTER VACCINATION**			
CALF NR	**VACCINATION**	**2–3**	**4–7**	**9–11**	**12–16**
9001	0	160	160	160	*NC
9003	0	160	160	160	160
9004	0	160	160	160	160
9008	NC	160	160	NC	160
9009	NC	160	160	NC	NC
9010	20	0	80	160	NC
9011	80	160	160	0	NC
9012	0	0	NC	160	NC
9013	NC	160	NC	160	NC
9014	40	0	NC	160	NC
Positive (number)	**1**	**7**	**7**	**7**	**3**
Total	**7**	**10**	**7**	**8**	**3**
% Positive	**14**	**70**	**100**	**88**	**100**

*NC=Not collected

Table 5: CONTROL GROUP: IFA titres to *B.bovis* of unvaccinated bull calves (n = 9).

	AGE IN MONTHS			
CALF NR	**2–3**	**4–6**	**7–8**	**11–16**
B912	*NC	0	0	160
B913	NC	0	0	160
B914	NC	NC	0	160
B915	NC	20	NC	NC
B916	NC	0	0	NC
B917	0	0	NC	160
B918	0	0	NC	NC
B919	NC	NC	NC	160
B920	NC	NC	NC	160
Positive (number)	**0**	**0**	**0**	**6**
Total	**2**	**6**	**4**	**6**
% Positive	**0**	**0**	**0**	**100**

*NC = Not collected

Babesia bigemina

Table 6: EXPERIMENTAL GROUP: IFA titres of heifer calves (n=10) after vaccination with Onderstepoort *B. bigemina* vaccine at 3-4 months of age.

	AT	**MONTHS AFTER VACCINATION**			
CALF NR	**VACCINATION**	**2–3**	**4–7**	**9–11**	**12–16**
9001	0	160	40	160	*NC
9003	0	160	160	160	160
9004	0	0	0	80	160
9008	NC	160	160	NC	160
9009	NC	160	160	NC	NC
9010	0	80	160	160	NC
9011	40	160	160	0	NC
9012	0	80	NC	160	NC
9013	NC	80	NC	160	NC
9014	0	160	NC	160	NC
Positive (number)	**0**	**9**	**5**	**7**	**3**
Total	**7**	**10**	**7**	**8**	**3**
% Positive	**0**	**90**	**71**	**88**	**100**

*NC = Not collected

Table 7: CONTROL GROUP: IFA titres to *B. bigemina* of unvaccinated bull calves (n = 9).

	AGE IN MONTHS			
CALF NR	**2–3**	**4–6**	**7–8**	**11–16**
B912	NC	0	0	160
B913	NC	0	160	160
B914	NC	NC	80	160
B915	NC	0	NC	NC
B916	NC	0	0	NC
B917	0	0	NC	160
B918	0	160	NC	NC
B919	NC	NC	NC	160
B920	NC	NC	NC	160
Positive (number)	**0**	**1**	**2**	**6**
Total	**2**	**6**	**4**	**6**
% Positive	**0**	**17**	**50**	**100**

*NC = Not collected

CHAPTER 4

DISCUSSION

The experimental model used for this study is that of an on-farm clinical trial, and is therefore subject to the inherent design limitations of such a model. The essential research question posed was whether 2–3-month-old calves would seroconvert when vaccinated with redwater vaccine. Several variables could potentially influence this outcome. Vaccine efficacy, vaccine storage, handling of the vaccine, and vaccination procedure all influence the presentation of vaccine to the experimental group. Besides vaccine efficacy, these factors were under the author's direct supervision. The manufacturer's recommendations were followed and the vaccine was administered intramuscularly by the author.

Variables applicable to the calves in the experimental group include general condition of the calves, presence or absence of ticks and level of maternal immunity.

All calves were raised on a set feeding regimen and were all in similar condition. As ticks are present and redwater cases are known to occur on this farm, field challenge cannot be ruled out. A policy of stringent tick control i followed on the farm, however, especially of the young calves. Calves are not released onto pasture until over 1 year of age. They are housed on concrete in stalls until approximately 2–3 months of age, and then moved to camps without pasture until approximately 10 months of age. The risk of field challenge in calves under 3 months of age is therefore very low, and supportive evidence for this is the seronegative status of control calves at this age. The

experiment therefore demonstrates an effective seroconversion rate amongst experimental calves at a time in their lives where field challenge was very unlikely.

Blood smear results

No *Babesia* piroplasms were seen on blood smears made on the day of vaccination. Two calves had low parasitaemias of *Anaplasma marginale.*

Response of calves to vaccination

A positive serological response is not necessarily synonymous with protective immunity. In the case of age-related immunity, the immune mechanism responsible for the elimination of *Babesia* spp is chiefly cell-mediated, involving a soluble babesiacidal factor (Goff *et a*l, 2001). As mentioned in the introduction, however, the humoral component is easier to measure, but the cell-mediated component is responsible for destruction of the parasite.

At the time of vaccination, one out of seven calves (14%) had a positive titre to *B. bovis*. Field challenge usually results in a detectable parasitaemia on blood smear. No *Babesia* parasites were seen on a blood smear taken on the day of vaccination (See Table 3). The positive titre recorded in this calf is therefore more likely to be due to maternal immunity than field challenge. A higher positive titre was recorded after vaccination, so the presence of a positive titre at vaccination did not interfere with the serological response induced by the vaccine. None of the calves in the experimental group had positive titres to *B. bigemina* at the time of vaccination, and none of the calves in the control group demonstrated titres to either *B. bovis* or B. *bigemina* at this age.

Within 2–3 months of vaccination, 90% of calves had seroconverted to *B. bigemina* (n = 10) and 70% had seroconverted to *B. bovis* (n = 10). At least 70% of experimental calves maintained seropositivity to both *B. bovis* and *B. bigemina* at subsequent blood collections: 4–7, 9–11 and 12–16 months after vaccination (See Table 4 & Table 6). Twelve to 16 months after vaccination, three calves sampled had positive IFA titres to both *B. bigemina* and *B. bovis*. However, it is not possible to determine whether the immunity seen in calves older than 7 months of age is maintained by natural challenge or by the vaccine, since 50% of the control calves had positive titres to *B. bigemina* by 7–8 months of age.

Calf 9004 had a negative titre to *B. bigemina*, but a positive titre to *B. bovis* 1–3 months after vaccination (Table 4 & Table 6). False positive IFA reactions are known to occur in the case of *B. bovis* (Bessenger & Schoeman, 1983). In their study, a proportion of cattle infected with *B. bigemina* became seropositive to *B. bovis*, despite the fact that they were kept free of *B. bovis* infection. Their results indicated that in the presence of a positive titre to *B. bigemina* (160 or more), low *B. bovis* titres were also recorded (mostly 40). In the case of calf 9004 , the *B. bigemina* titre was not elevated, so this *B. bovis* titre cannot be the result of a false positive reaction.

Two calves died during the trial: 9007 (experimental group) and B915 (control group). The cause of 9007's death could not be definitively determined, but an *E. coli* septicaemia was suspected. Only one sample had been collected from 9007. One of the control animals (B915) also died. Blood smear was negative for *Babesia* spp. and *Anaplasma marginale.* One sample was collected from this calf at 4 months of age. Unvaccinated control animals seroconverted later than calves in the experimental group. Control group calves between 7 and 8 months of age were still fully susceptible to *B. bovis* (0% seroconversion), whereas half of the control calves had titres to *B.*

bigemina at this age (Table 7). This clearly demonstrates the risk faced by these unvaccinated animals. Their period of innate immunity is waning, but they have not yet achieved endemic immunity. By 11–16 months of age, however, all control group calves sampled had seroconverted to both *B. bovis* and *B. bigemina*. Field challenge is therefore present on this farm. In this case, these unvaccinated calves survived the field challenge.

Tick vectors on the farm

Accurate field studies to determine the distribution of the blue ticks *Rhipicephalus (Boophilus) microplus* and *R. (B.) decoloratus* have not yet been done in the Badfontein area, in which this farm is situated. A distribution map in Howell, Walker and Nevill (1978) indicates that ticks are present in Mpumalanga, but specific distribution data are not given. Tick burdens were generally low throughout the year, but no counts were done. Four tick species were collected from 1–2-year-old heifers in October 2011 (Table 8.)

Table 8: Cattle ticks collected from heifers in October 2011 (Identified by I.G. Horak)

Species	**Males**	**Females**
Haemaphysalis aciculifer	0	1
Rhipicephalus appendiculatus	0	1
Rhipicephalus evertsi	8	3
Rhipicephalus (Boophilus) microplus	0	18

Rhipicephalus (Boophilus) microplus is the only known vector of *B. bovis.* This tick is also a vector of *B. bigemina. Rhipicephalus evertsi* can transmit *B. bigemina. Rhipicephalus appendiculatus* and *Haemaphysalis aciculifer* have not been proven to be vectors of either *B. bovis* or *B. bigemina* (De Vos *et al*., 2004). Our clinical

experience is that bovine babesiosis is common in the Badfontein area. Both *B. bovis* and *B. bigemina* have been diagnosed on blood smear and post mortem examinations done on cattle from this farm.

Dipping practices and their effect on endemic stability

The farmer applies a very strict dipping regimen and cattle are generally kept tick-free. Cattle are observed carefully and dipped every two weeks if necessary. Cattle are also dipped before moving camps. The low tick burden and strict dipping strategy would certainly impede the development of endemic stability in this herd. This is most likely to be a situation common to most dairy farms where tick numbers have decreased due to years of intensive dipping. Unfortunately, in mid to late summer, when tick numbers are at their highest, exposure of these immunologically naïve cattle to *B. bovis* or *B.bigemina* frequently manifests in clinical disease. This is a pattern seen on this farm.

Mahoney and Ross (1972) developed a model illustrating the importance of achieving endemic stability early. After making their calculations, they focused on how best to achieve this end. The ideal situation would be to artificially infect calves at weaning to induce immunity for periods similar to those induce by natural infection. If 75–100% of calves can be infected before 9 months of age, endemic stability would have been achieved (De Vos & Potgieter, 1983). Clinical babesiosis rarely develops in herds in which 80% of cattle are serologically positive to *Babesia* spp. (Norval, Fivaz, Lawrence & Dailecourt, 1983).

Based on these figures, the results of this study indicate that calves vaccinated early achieve effective immunity, before the age of 9 months. Early vaccination therefore plays a practical and vital role to ensure acquisition of endemic stability prior to field challenge.

Persistence of immunity after vaccination.

This study demonstrated that early vaccination induced a significant serological response, and protection of calves for at least 5 months after vaccination. Determining the duration of this immunity was not within the scope of this project, but various authors have noted differences between the duration of immunity to *B. bovis* and *B. bigemina*.

Brown (2001) stated that cattle which recover from *B. bovis* infection become persistently infected and immune to clinical disease following re-exposure to homologous organisms. Immunity to *B. bovis* lasts at least 2 years (Neitz, 1969), even lifelong (De Vos, 1979, 2005; Brown, 2001; De Vos *et al*.). This does not appear to the case with *B. bigemina* (Neitz, 1969; Pipano, Shkap, Kriegel, Leibovitz, Savitsky & Fish, 2002; Fish, Leibovich, Krigel, Mcelwain & Shkap, 2008)

Persistent infection appears to create a reliable, sustained immunity in the case of *B. bovis* (Pipano *et al.*, 2002; Brown, 2001). Importantly, though, it has been demonstrated that persistent infection is not a prerequisite for immunity. Immunity can be present despite the absence of infection: Callow *et al*. (1974) demonstrated that cattle in which *B. bovis* infection had been sterilised, still mounted an immune response to reinfection up to 6 months later. Further evidence for persistence of immunity despite loss of infection was provided by Pipano *et al*. (2002), who found that cattle can spontaneously rid themselves of *Babesia* infection. Blood was passaged from older cattle to naïve, splenectomised calves to determine whether any parasites were present in the older cattle. The cattle whose recipients did not react to the blood transfusion were considered free of infection. These findings challenged the concept of premunity and the term fell into disuse (De Vos *et al*., 2004).

Despite the demonstration of this sterile immunity by Callow *et al*. (1974) and Pipano *et al.,* 2002), Brown *et al*. (2006) noted that persistent infection is a feature of *B. bovis*

infection and that these cattle are resistent to reinfection by related strains of *B. bovis*. This remains controversial: Fish, Leibovich, Krigel, McElwain & Shkap (2008) stated that the importance of parasite persistence for long-term protective immunity is not clear. Nevertheless, historical evidence indicates that a feature of *B. bigemina* infection is loss of infection and short-lived immunity, whereas *B. bovis* is characterised by persistent infection and long-term immunity to related strains.

Understanding the mechanisms of immunity to babesiosis is important to understanding how to protect cattle throughout their lives. This project covered only the first year of life, but loss of immunity, especially in the case of *B. bigemina*, is a concern. To maintain protective immunity, judicious tick control is necessary to ensure constant re-exposure of cattle to both *B. bovis and B. bigemina*, but especially to *B. bigemina.*

Conclusion

Young calves between 2 and 9 months of age demonstrate capacity to resist *Babesia* infection and this has been elucidated at molecular level (Brown *et al*., 2006). Given the lack of experimental evidence for an absolute period of innate resistance to both *B. bigemina* and *B. bovis* in dairy calves, however, achieving early endemic stability is a highly desirable management goal. It is essential that dairy calves, which are usually turned out to pasture at 7 months of age, are fully protected against both *B. bovis* and *B. bigemina* by this age. Even if they do not succumb to clinical disease, diminished weight gain and morbidity have been reported.

There are several major advantages to vaccinating early. Potentially, maternal antibodies could interfere with seroconversion when calves are vaccinated at 2–3 months of age, but in this study, this was not the case. Firstly, since the period of innate immunity has not been experimentally proven to be 100% protective, nor the impact of morbidity been established, acquired protection during this time is desirable.

A second advantage is that endemic stability is achieved before innate immunity wanes. In the case of *B. bovis*, a single vaccination may induce lifelong immunity, effectively reducing the risk of mortality associated with this parasite. Early vaccination therefore constitutes an extremely useful management tool which can prevent morbidity and mortality in calves under 1 year of age due to both *B. bigemina* and *B. bovis*.

CHAPTER 5

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