Antibody response to *Babesia bigemina* and *Babesia bovis* by vaccinated and unvaccinated cattle in an endemic area of South Africa

by

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Submitted in partial fulfillment of the requirements for the

degree of

MASTER OF SCIENCE IN VETERINARY SCIENCE

in the

DEPARTMENT OF VETERINARY TROPICAL DISEASES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

submitted: July 2001

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to the following persons:

- Prof B L Penzhorn, my supervisor, for his guidance and help in identifying the ranches and assistance with specimen collection throughout the project.
- Dr N R Bryson, my co-supervisor, for his guidance and assistance during the project.
- Dr D T De Waal, (Onderstepoort Veterinary Institute) for advice on aspects involving serological tests.
- Dr H Hansen, (private veterinary practitioner) for help in identifying the two ranches.
- Mr F S H Du Preez, for using his ranch (Nooitgedacht ranch) to conduct the research and for providing all required information.
- Mr J Maritz, for using his ranch (Vlakplaas ranch) to conduct the research and for proving all required information.
- The managers of both ranches, for their assistance during sampling.
- The farm workers on both ranches for their help in restraining the animals.
- Mrs Rina Owen and Mr Sollie Millard, for help in data analysis.
- The Department of Veterinary Tropical Diseases, University of Pretoria, for their support.
- Above all, to my Lord Jesus Christ, for his unfolding love, protection and help during the project.

DEDICATION

This work is dedicated to my wife, Berhane Dugassa, my children, Telile Assefa, Olyad Assefa, Meti Assefa and Mati Assefa, for their support and, to my parents, Regassa Geleta and Ayantu Duressa, who believed in the importance of education.

DECLARATION

I declare that the dissertation, which I hereby submit for Master of Science in Veterinary Science at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at another University.

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SIGNATURE: _____

DATE: _____

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SUMMARY

Antibody response to B*abesia bigemina* and B*abesia bovis* by vaccinated and unvaccinated cattle in an endemic area of South Africa

by

ASSEFA REGASSA GELETA

Promoter: Prof B L Penzhorn

Co-promoter: Dr N R Bryson

The main objective of the study was to investigate whether there were significant differences in prevalence of antibodies to *Babesia bigemina* and *Babesia bovis* between vaccinated and unvaccinated cattle in a tick-borne disease endemic area of South Africa. The study was carried out between August 2000 and June 2001, in the Northern Province of South Africa at Nooitgedacht ranch (24° 33' S and 28° 36' E), where calves were vaccinated against *B. bigemina* and *B. bovis* infections, and at Vlakplaas ranch (24° 58' S and 28° 05' E), where calves had not been vaccinated against these parasites.

Sera were collected from cattle of different age groups at both ranches and the presence of antibodies against *B. bigemina* and *B. bovis* determined using the indirect fluorescent antibody (IFA) test.

It was found that *B. bovis* was absent from both ranches while *B. bigemina* antibody was more prevalent in cattle at Vlakplaas (unvaccinated) than at

Nooitgedacht (vaccinated). The difference in *B. bigemina* antibody response between the ranches may have been due to variations in tick populations. Vlakplaas, which had been operated for 14 years with relaxed tick control, probably had sufficient numbers of vector ticks for frequent transmission and maintenance of endemic stability to *B. bigemina*. At Nooitgedacht, however, livestock farming had been interrupted for three years before it was resumed in 1999 and it is postulated that the tick population had been substantially reduced due to lack of hosts to a level insufficient for the establishment and maintenance of endemic stability to *B. bigemina*. The vaccinated cattle and breeding cows might therefore have lost IFA reacting antibody titres due to low levels of superinfections.

The findings show that an endemically stable situation to *B. bigemina* could be achieved by adapting a tick control method that allows sufficient number of ticks on cattle rather than relying entirely on intensive tick control and vaccination. Therefore, it may not be necessary to vaccinate calves against *B. bigemina* on ranches located in *B. bigemina*-endemic areas and stocked with *Bos indicus* cattle or their crosses.

Key words: *Babesia bigemina, Babesia bovis,* bovine babesiosis, tick-borne diseases, endemic stability, immunization, antibody response, Brahman, Bonsmara, South Africa

SAMEVATTING

Teenliggaamreaksie teen *Babesia bigemina* en *Babesia bovis* in ingeënte en nie-ingeënte beeste in 'n endemiese streek van Suid-Afrika

deur

ASSEFA REGASSA GELETA

Promotor: Prof B L Penzhorn

Medepromotor: Dr N R Bryson

Die hoofdoel van die studie was om te bepaal of die voorkoms van teenliggame teen *Babesia bigemina* en *Babesia bovis* in beeste wat ingeënt is betekenisvol verskil van dié wat nie ingeënt is nie. Die studie is tussen Augustus 2000 en Junie 2001 in die Noordelike Provinsie van Suid-Afrika uitgevoer in 'n streek waar bosluisoorgedraagde siektes endemies is. Die betrokke plase was Nooitgedacht (24°33' S en 28°36'O), waar kalwers teen albei parasiete ingeënt is, en Vlakplaas (24°58'S en 28°05'), waar inenting nie plaasgevind het nie.

Serum is van beeste van verskillende ouderdomme versamel en die voorkoms van teenliggame teen *B. bigemina* en *B. bovis* is deur die indirekte fluoresserende teenligaamtoets (IFA) bepaal.

Babesia bovis was afwesig op albei plase, terwyl *B. bigemina* in 'n endemies stabiele toestand op Vlakplaas voorgekom het, maar onstabiel op Nooitgedacht was. Die verskil in teenliggaamreaksie tussen die twee plase mag aan verskille in

die bosluisbevolkings te wyte wees. Op Vlakplaas, waar minder streng beheer toegepas is, was daar waarskynlik voldoende vektorbosluise om *B. bigemina* dikwels oor te dra en endemiese stabiliteit in stand te hou. Op Nooitgedacht is beesboerdery egter vir drie jaar onderbreek, voordat dit in 1999 hervat is. Weens die gebrek aan gashere het die bosluisbevolking waarskynlik aansienlik gedaal, tot ‘n vlak wat te laag is om endemiese stabiliteit tot stand te bring en te onderhou.

Hierdie bevindinge toon dat ‘n endemies stabiele toestand bereik kan word deur ‘n bosluisbeheerstrategie toe te pas wat voldoende bosluise op beeste verseker, eerder as om op intensiewe bosluisbeheer en inenting staat te maak. Op plase met *Bos indicus*-beeste en hul kruisings in ‘n streek waar *B. bigemina* endemies is, is dit dus waarkynlik onnodig om kalwers teen *B. bigemina* in te ent.

Sleutelwoorde: *Babesia bigemina*, *Babesia bovis*, babesiose van beeste, bosluisoorgedraagde siektes, endemiese stabiliteit, inenting, serologiese status, Bramaan, Bonsmara, Suid-Afrika

ABBREVIATIONS

˚C	degrees Celsius
E	East
IgG	immunoglobulin G
mg/kg	milligram(s) per kilogram(s)
LA	Long Acting
m asl	meters above sea level
mℓ	milliliter(s)
mm	millimeter(s)
%	percent
PBS	Phosphate buffered saline
RBC	Red blood cells
rpm	revolution per minute
S	South
SAS	Statistical Analysis System
Spp	Species
v/v	volume/volume

CHAPTER ONE

1. INTRODUCTION

Bovine babesiosis or redwater is a tick-borne disease caused by the intra-erythrocytic protozoan parasites *Babesia bovis*, *Babesia bigemina, Babesia divergens* and *Babesia major* (McCosker, 1981). The genus *Babesia* belongs to the phylum Apicomplexa, class Sporozoasida, order Piroplasmorida, and family Babesiidae (Levine, 1985).

The parasite was first described by Babès in 1888 in the blood of cattle showing haemoglobinuria (Babès, 1888), and the name *Babesia* was adopted in his honor (Ristic and Levy, 1981). *Babesia bovis* and *B. divergens* are small type *Babesia*, whilst *B. bigemina* and *B. major* are the large type (Purnell, 1981).

Ticks were first recognized as vectors of babesiosis in 1893 when Smith and Kilbourne described *Boophilus annulatus* as the vector of *B. bigemina* (Smith and Kilbourne, 1893 cited by Ristic and Levy, 1981). It is now established worldwide that ticks are the main vectors of *Babesia* of domestic animals (Friedhoff and Smith, 1981).

Bovine babesiosis causes serious economic losses worldwide (Carson and Phillips, 1981) and in tropical and subtropical countries, redwater caused by *B. bovis* and *B. bigemina* is of great economic importance (McCosker, 1981). It has

been estimated that bovine babesiosis endangers half a billion cattle throughout the world (Ristic and Levy, 1981).

Redwater, caused by *B. bigemina*, was first recorded in 1870 in South Africa, but the organism responsible for the disease was only identified by Koch, in the Cape Province and Transvaal, and by Hutcheon in Natal and the Orange Free State in 1898 (Neitz, 1941). *Babesia bovis* was first reported in South Africa in 1941 (Neitz, 1941) and was probably introduced with the Asian blue tick (*Boophilus microplus*) during the later part of the 19th century (Henning, 1956).

Babesia bigemina was probably present in Africa before the arrival of European settlers and their exotic cattle breeds and it was most likely introduced to southern Africa from East Africa together with its vector along with the livestock of migrating indigenous tribes (Henning, 1956).

At present, bovine babesiosis is widespread in South Africa, and the distribution of both *B. bovis* and *B. bigemina* is determined by the distribution of their vectors (De Vos, 1979). *Babesia bovis* has a more limited distribution, as it is only transmitted by *B. microplus*, which occurs in the higher rainfall areas of the Eastern Cape, KwaZulu-Natal, and the eastern parts of Mpumalanga and Northern Province. *Babesia bigemina,* which is transmitted by both *Boophilus decoloratus* and *B. microplus,* exists throughout South Africa and is absent only from the drier parts of

the Western Cape, Northern Province and western Free State Province (De Vos, 1979).

Bovine babesiosis is one of the major causes of cattle mortality in South Africa (Bigalke *et al.*, 1976) and has been reported to cause annual losses of 8000 cattle in Kwazulu-Natal alone (Anon, 1972). Three hundred million cattle in tropical and subtropical regions of the world are at risk to infection with *B. bovis, B. bigemina* and *Anaplasma marginale* (Wright, 1990) and the economic losses inflicted by these diseases in South Africa alone are estimated to be between R70 and R200 million per annum (Bigalke, 1980).

Various tick-borne disease control methods including vector control, vaccination and chemoprophylaxis have been employed in South Africa (Bigalke *et al.*, 1976; De Vos, 1979; Purnell and Schröder, 1984). In most instances farmers decide on control measures without really considering the distribution of the vector ticks or the endemic stability situation of tick-borne diseases in the area or on the farm (Du Plessis *et al.*, 1994). Specific serological data on tick-borne diseases are lacking and are needed to successfully apply the principles of endemic stability to the control of tick-borne diseases. Commercial farmers apply a range of haphazard vector and parasite control measures. Some of the farmers vaccinate their calves against all three important tick-borne diseases (redwater, heartwater and anaplasmosis), whilst others vaccinate against two or one or none of them, regardless of the endemic stability status of the diseases in the area (Du Plessis

et al., 1994). The lack of clear a tick and tick-borne disease control strategy indicates widespread confusion in the practical field application of tick-borne disease control in cattle in South Africa.

The main **objectives** of the present project were to:

- Investigate possible serological status differences between vaccinated and unvaccinated cattle to bovine babesiosis in endemic areas.

- Determine whether *B. bovis* was present in the two study areas

- Determine factors that may affect the establishment of endemic stability to redwater (*B. bigemina/B. bovis)* in unvaccinated cattle.

- Generate data on tick-borne diseases, which could be used by the local livestock farming community and disease-control policy makers, as a basis to supplement existing tick and tick-borne disease control strategies, and if necessary to consider alternative measures.

- Develop appropriate recommendations on the necessity of vaccination against bovine babesiosis (*B. bigemina/B. bovis)* in endemic areas.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Endemic stability to bovine babesiosis

The principle of endemic stability to tick-borne disease was first described in Australia, in a model for *B. bovis* and *B. microplus* in *Bos taurus* cattle (Mahoney and Ross, 1972). The principle is that, when the inoculation rate of *Babesia* by the ticks into cattle is sufficiently high to infect all calves whilst they are protected by the innate and colostral immunity, then clinical disease will be minimal and endemic stability will be achieved. Conversely, if the inoculation rate is not sufficiently high and young calves are not infected during the period of innate and colostral immunity, then endemic instability and clinical cases will result.

Mahoney and Ross (1972) also developed a model which relates the infection rate or the proportion of animals infected (I), measured by serological surveys on animals of known age in days (t), and inoculation rate (h) based on the model used by MacDonald (1950). It was given by the following formula:

$$I = 1 - e^{-ht} \qquad e=2.71828$$

Mahoney and Ross (1972) found out that, in an endemically stable situation in *Bos taurus* cattle, the inoculation rate ranged from 0.005 to 0.05. A minimum disease outbreak risk occurred when the inoculation rate was between 0.0005 and 0.005, and the chance of outbreaks diminished when the inoculation rate was less

than 0.0005, as the frequency of infection was extremely low (Mahoney and Ross, 1972).

The Food and Agriculture Organization of the United Nations (Anon, 1984) recommends that, in general terms, the model developed by Mahoney and Ross (1972) can be extended to other tick-borne diseases in similar studies.

Norval *et al*. (1983) defined five different epidemiological situations for bovine babesiosis based on the frequency of serologically positive animals and disease history:

1. Endemically stable situations (81 to 100% positive sera)
2. Approaching endemic stability (61 to 80 % positive sera)
3. Endemically unstable situation (21 to 60 % positive sera)
4. Minimal disease situation (1 to 20% positive sera)
5. Disease-free situation (0% positive sera)

Estimates of the minimum numbers of ticks needed to maintain endemic stability to bovine babesiosis without causing a reduction of weight gain in the host have been made using computer simulations (Smith, 1983). *Bos taurus* cattle were infested with *B. microplus* which had been infected with *B. bovis*, and it was concluded that eight or nine engorged ticks per animal per day was the cut-off point before economic effects were noticed (Smith, 1983). Below this range, the

risk of babesiosis outbreaks was significantly increased due to endemic instability. Whilst above the minimum tick count, the chance of a bovine babesiosis outbreak in native cattle was small, as all the cattle had been infected during the period of calfhood resistance. However, they were subjected to stresses due to tick feeding, which lead to reduced weight gains (Smith, 1983). Mahoney (1974) concluded that the critical level of tick infestation for the maintenance of *B. bovis* in *Bos taurus* cattle was one or two ticks per head per day.

In South Africa, *B. bovis* is transmitted by *B. microplus* (Potgieter and Els, 1976a), while *B. bigemina* is carried by both *B. decoloratus* (Potgieter and Els, 1976b) and *B. microplus* (Riek, 1964). Adult female ticks of both species engorging on infected hosts become infected during the last 24 hours of feeding and this is followed by the transovarial infection of a small proportion of the eggs (Callow, 1979; Friedhoff and Smith, 1981). *Babesia bovis* is transmitted only by the larvae of *B. microplus* (Mahoney and Mirre, 1979; Potgieter and Els, 1976a). The larvae lose the infection after transmission has occurred and the nymphs and adults that develop from these larvae are free of infection (Mahoney and Mirre, 1979). On the other hand, *B. bigemina* is transmitted by the nymph and adult stages of *B. decoloratus* (Potgieter, 1977; Buscher, 1988) and *B. microplus* (Riek, 1964; Callow and Hoyte, 1961) but not by the larvae (Callow and Hoyte, 1961; Potgieter, 1977). Ambient temperatures below 20ºC inhibit trans-ovarial transmission of *B. bovis* and *B. bigemina* by *B. microplus* (Riek, 1966).

It is well documented that there is a wide range of variation between *B. bovis* and *B. bigemina* with regard to tick infection and transmission rates. In Australia, Johnston (1967) reported that 4.2 % of ticks examined from Herefords and 5.9 % of ticks collected from Droughtmasters were infected with *B. bigemina,* whilst ticks infected with *B. bovis* had lower infection rates and only 0.3 % of ticks from Herefords and 0.2 % of ticks from Droughtmasters were infected.

Mahoney and Mirre (1971) demonstrated that, in endemic areas, the field infection rates of *B. microplus* with *Babesia* were generally low, but were much lower with *B. bovis* (0,04 %) when compared with *B. bigemina* (0,23 %). In South Africa, the prevalence and transmission rates of *B. bigemina* were found to be higher than those of *B. bovis* (De Vos, 1979). In serological surveys conducted on cattle from four communal grazing areas in the Northwest and Mpumalanga Provinces of South Africa it was found that the inoculation rate for *B. bigemina* was in the stable range, whilst that of *B. bovis* was unstable (Tice *et al.,* 1998).

In South Africa a number of studies were carried out to determine the effects of tick control methods on endemic stability to *B. bigemina* and *B. bovis* on individual commercial farms. Ardington (1982) reported that the maintenance of endemic stability to *B. bigemina* failed when strategic dipping allowed only light *B. decoloratus* infestations on the cattle. De Vos and Every (1981) demonstrated that endemic instability to bovine babesiosis caused by *B. bigemina* was related to the use of plunge dips, which resulted in low tick burdens, whilst the majority of the

farms which used spray races had endemic stability to the parasite. Bigalke (1980) showed that, in areas where both *B. bovis* and *B. bigemina* were present, dipping created a more unstable situation with *B. bovis* when compared with *B. bigemina*. De Vos and Potgieter (1983) concluded that with poor tick control *B. bovis* was in the endemically unstable situation in 30 % of the areas studied, whilst *B. bigemina* was endemically stable in all the study areas. They also reported that good tick control reduced *B. bovis* infection rates with minimal losses whilst with *B. bigemina* good tick control reduced infection rates but increased the risk of disease outbreaks (De Vos and Potgieter, 1983).

In South Africa, *B. decoloratus* has been collected from a number of wildlife species which include impala (*Aepyceros melampus*), hartebeest (*Alcelaphus buselaphus*), blue wildbeest (*Connochaetes taurinus*), blesbok (*Damaliscus dorcas philipsi*), waterbuck (*Kobus ellipsiprymnus*), reedbuck (*Redunca arundinum*), mountain reedbuck (*Redunca fulvorufula*), klipspringer (*Oreotragus oreotragus*), steenbok (*Raphicerus campestris*), grey duiker (S*ylvicapra grimmia*), eland (*Taurotragus oryx*), nyala (*Tragelaphus angasi*), kudu (*Tragelaphus strepsiceros*), gemsbok (*Oryx gazella*), warthog (*Phacochoerus aethiopicus*), bush pig (*Potamochoerus porcus*), black-backed jackal (*Canis mesomelas)* and porcupine (*Hystrix africae-australis*) (Boomker *et al.*, 1983; Horak *et al.*, 1983a; Horak *et al.*, 1983b; Horak *et al.*, 1988; Horak, 1995). Clinical disease due to *B. bovis* and *B. bigemina* infections is restricted to cattle and no important wildlife reservoir has been demonstrated (Friedhoff and Smith, 1981). However, the role

the wildlife play in the epidemiology of bovine babesiosis in South Africa has yet to be determined.

2.2 Immunity to bovine babesiosis

2.2.1 Breed resistance

A number of studies have been conducted to establish the susceptibility of different cattle breeds to babesiosis. European cattle breeds (*Bos taurus)* are more susceptible to *B. bovis* infections than the pure Zebu (*Bos indicus*) or their crosses (Mahoney *et al.*, 1981; Rogers, 1971). *Bos taurus* cattle can retain *B. bovis* infections for life (Neitz, 1969) and remain infective to ticks for up to four years (Mahoney, 1974), whilst pure-bred Zebu cattle as well as those with a significant amount of Zebu blood lose the infection within two years (Johnston *et al.,* 1978).

There are conflicting reports in the literature concerning the relative susceptibility of *Bos indicus* and *Bos taurus* breeds to *B. bigemina.* Callow (1984) and Hugh-Jones *et al.* (1988) reported that there are no strong indications for the existence of susceptibility differences between cattle breeds to babesiosis caused by *B. bigemina*. Infections with *B. bigemina* rarely persist for longer than a year, regardless of the breed, and infected cattle normally remain infective to ticks for only four to seven weeks (Johnston *et al.,* 1978; Mahoney, 1969). Johnston (1967) found no difference between Droughtmaster (Brahman x British beef cattle)

and Hereford (*Bos taurus*) calves for parasite rates of *B. bigemina* but Droughtmaster had significantly lower parasite rates for *B. bovis*. Daly and Hall (1955) also found no difference in susceptibility to *B. bigemina* between *Bos indicus* and *Bos taurus* breeds while *Bos indicus* cattle showed lower *B. bovis* infections when compared to *Bos taurus.* Mahoney *et al*. (1973b) showed that *Bos taurus* cattle maintained under tick-free conditions after natural infection at the age of 5-7 months, were carriers of *B. bovis* for the entire four-year study period but lost *B. bigemina* infections within two years; the cattle remained immune to both parasites after four years. On the other hand, Bock *et al.* (1999a) reported that *Bos indicus* and, to a lesser extent, crossbred cattle were much more resistant to *B. bigemina* than *Bos taurus* cattle. This result supports the observation that 10 times more outbreaks of *B. bigemina* are recorded in *Bos taurus* than in *Bos indicus* cattle in Australia (Bock *et al.,* 1999b). When *B. bigemina* challenge is mild, however, the difference between breeds is nowhere near as obvious (Bock *et al.,* 1997).

2.2.2 Age resistance

Age is an important factor in bovine babesiosis as the severity of clinical babesiosis increases with age (Trueman and Blight 1978). Calves less than two months of age, born to naive cows, were highly susceptible to both *B. bovis* (Hall, 1960; 1963) and *B. bigemina (*Hall *et al.,* 1968). Offspring of immune mothers were resistant to both parasites, because of the passive immunity they obtained through the colostrum (Hall, 1960; 1963; Hall *et al.,* 1968). After the age of two

months, calves were protected by a natural non-specific innate resistance that persisted for at least four to six months and did not depend on the immune status of the cow (Trueman and Blight 1978; Corrier and Guzman, 1977; Payne and Osorio, 1990). Six to nine months of age is therefore regarded as the practical limit within which calves must receive infection with *Babesia* in order to maintain an endemically stable situation Mahoney (1974).

2.2.3 Mechanisms of immunity

Both humoral and cellular immune systems are reported to be mobilized in bovine babesiosis (Callow, 1977). Evidence that the humoral immune system is involved in protecting against bovine babesiosis has been demonstrated in a number of studies. In early studies it was shown that immunity was passively transferred from immune dams to calves through colostrum (Hall, 1960; 1963). Mahoney (1967a) demonstrated the passive transfer of immunity by taking serum from *B. bovis* carrier cattle and passing it on to highly susceptible splenectomized calves. Although the mode of action of antibodies in acquired immunity to babesiosis has not been fully elucidated, Carson and Phillips (1981) proposed that a specific antibody would be directed against babesial antigens on free parasites, as they are briefly exposed in the plasma prior to invasion of the erythrocyte or against parasite antigens deposited on or inserted into surface membranes of infected RBC.

The role of the cellular immune mechanism in immunity to bovine babesiosis has not yet been fully substantiated (Aragon, 1976). However, the involvement of T-cells in a helper capacity for antibody synthesis and in the activation of macrophage (Carson and Phillips, 1981) and phagocytic elements (Aragon, 1976; Ristic and Levy, 1981) has been reported. It has also been suggested that the role of phagocytosis could be the removal of infected erythrocytes from the circulation, after the reaction of specific antibodies with antigens located on the surfaces of infected erythrocytes has occurred (Aragon, 1976; Ristic and Levy, 1981).

2.2.4 Antigenic variation

The existence of different strains and antigenic variation has been reported in both *B. bovis* (Curnow, 1973a) and *B. bigemina* (Callow, 1964; Callow, 1967). Babesial infections persist in cattle through the phenomenon of antigenic variation (Doyle, 1977) and by superinfection with antigenically different parasite populations (Ross and Mahoney, 1974). Each change in antigenic type is believed to give the parasite a temporary respite from attack by the host immune system and prolongs the infection period (Aragon, 1976). The number of antigenically distinct relapses that could occur in a herd as a result of any babesial infection could be more than 100 (Ross and Mahoney, 1974). Curnow (1973a) has shown that *B. bovis* parasites collected at each relapse from one infected animal were antigenically different from one another and when these parasites were transmitted through the vector tick, *B. microplus*, they reverted to a common antigenic type.

Strain differences and antigenic variation do not appear to be of major importance either as a cause of disease or in the preparation of vaccines, since cross-immunity tests between strains usually give adequate clinical protection against each other (Callow, 1977; McElwain *et al.*, 1987; Wright, 1990). Vaccination with a specific vaccine strain is more effective if carried out in an area where only a limited number of similar basic antigens circulate (Zwart and Brocklesby, 1979). However, there is strong evidence for the existence of immunological similarity between *B. bovis* and *B. bigemina* from different countries (Dalgliesh *et al*., 1990). Australian *B. bovis* and *B. bigemina* vaccines were found to be safe and provided adequate immunity to local field strains of the parasites in Paraguay (Brizuela *et al.*, 1998). Vaccine strains of *B. bovis* from Australia have been shown to protect cattle against pathogenic strains in South Africa (De Vos *et al.*, 1982a). Based on a comparison of strains from Australia and Mozambique, Callow *et al.* (1981) concluded that Australian *B. bovis* vaccine should protect cattle in southern Africa. Likewise an Australian vaccine strain of *B. bigemina* was found to immunize cattle against a pathogenic field strain of the same species in South Africa (De Vos *et al.,* 1982b).

Studies on the mechanism of cross-immunity showed that the protective antigens of a strain prime the host immune system, so that a secondary response against a heterologous strain occurs soon after challenge (Mahoney *et al.,* 1979a). Furthermore, cross-immunity between different *Babesia* species has also been

demonstrated and Wright *et al.* (1987) reported that *B. bigemina*-immune cattle were cross-protected against challenge with virulent *B. bovis*.

2.2.5 Premunity

Premunity has long been considered to be a prerequisite for protective immunity to babesiosis. Neitz (1969) reported that immunity to *B. bigemina* was related to the maintenance of the parasite in the animal through continuous re-infection from the infected vector and speculated that the same phenomenon occurred in *B. bovis*.

It is now known that persistence of infection is not necessary to ensure immunity. Cattle that had been drug sterilized of *B. bovis* (Callow *et al.,* 1974a) and *B. bigemina* (Callow *et al.,* 1974b) infections retained immunity after sterilization. Likewise, cattle which naturally eliminated *B. bovis* (Johnston *et al.,* 1978) and *B. bigemina* (Callow, 1967; Callow *et al.* 1974b; Johnston *et al.,* 1978) infections had strong immunity. It was also shown that cattle vaccinated with killed *B. bovis* and *B. bigemina* parasites had a high degree of sterile immunity (Todorovic *et al.,* 1973).

2.2.6 Immunization

Vaccination against bovine babesiosis was initially based on the principles of premunition, where susceptible animals were inoculated with blood from naturally infected animals at the acute stage of infection or recovered carriers, followed by treatment of severely reacting animals with antibabesial drugs (Dalgliesh, 1968; Todorovic, 1975a; Aragon, 1976; Callow, 1977; 1984). However, variability in the infectivity of the blood of carrier animals and severity of the infections produced by reaction blood were the obstacles to effective field vaccinations (Callow and Tammemagi, 1967). To overcome these problems, the method of preparation of babesiosis vaccine in splenectomized calves was introduced (Callow and Mellors, 1966). This was followed by the production of the currently used, standardized, relatively safe and quality controlled vaccines of babesiosis attenuated in splenectomized calves (Callow, 1977; Callow *et al.,* 1979; Dalgliesh *et al.,* 1981; Dalgliesh *et al.,* 1990). Passaging of *Babesia* in splenectomized calves reduces the virulence of the parasite and abolishes its infectivity for the vector tick (Callow and Mellors, 1966; O'Sullivan and Callow, 1966; Callow, 1976).

Many other babesiosis vaccines have been tried over the years. These include killed parasites from infected erythrocytes (Mahoney, 1967b; Todorovic *et al.* 1973; Mahoney and Wright, 1976), plasma from infected animals (Mahoney and Goodger 1972; Todorovic *et al.* 1973), culture-derived immunogens (Smith *et al.,* 1979; Smith and Ristic, 1981; Timms *et. al.,* 1983), irradiated intraerythrocytic forms of *B. bigemina* and *B. bovis* (Mahoney *et al.,* 1973a; Bishop and Adam,

1974), attenuation by *in vitro* cultivation (Yunker *et al.,* 1987), recombinant vaccine (Wright, 1991), and antigens produced by recombinant DNA technology (Gale *et al.,* 1992). However, none of these immunization methods have been used for large-scale vaccination against babesiosis.

The strains of *B. bovis* and *B. bigemina* currently used in the vaccine are live organisms of reduced virulence and non-transmissible by the vector ticks, as a result of passage in splenectomized calves (Callow and Mellors, 1966; Callow, 1977; Mason *et al.,* 1986). The vaccine is not entirely safe and as a consequence, its use should be limited to calves in which nonspecific resistance will minimize the risk of any vaccine reaction (De Vos & Potgieter, 1994).

Following inoculation with the vaccine, protective immunity develops in three to four weeks and in the case of *B. bovis* it lasts for several years (De Vos, 1979; Anon, 1996), but in the absence of natural challenge, the immunity to *B. bigemina* may break down (Neitz, 1969). However, it is generally advised that animals be vaccinated only once against both *B. bovis* and *B. bigemina* infections (Callow, 1977; De Vos, 1978).

In South Africa and Zimbabwe, vaccination against redwater has been practised since 1911, when the *B. bigemina* vaccine was introduced (Lawrence and Norval, 1979). *Babesia bovis* has been included in the vaccine since 1953. These early vaccines used a carrier-donor system whereby recovered cattle, some of them

splenectomized, were used as donors of infective blood and this blood was then used as a vaccine (De Vos & Potgieter, 1994). Due to the considerable variation in the levels of infectivity of the vaccine, the production procedure at the Onderstepoort Veterinary Institute (OVI) was changed in 1973 to follow the same procedure used in Australia (De Vos, 1978). Here, the blood of splenectomized animals in the acute stage of infection is used to produce a standardized vaccine presented in chilled or frozen form (Callow, 1977; De Vos, 1978).

Studies conducted in Australia (Mahoney *et al.,* 1973b), Colombia (Corrier & Guzman, 1977), Zimbabwe (Norval *et al.,* 1983) and Paraguay (Payne & Osorio, 1990) revealed that vaccination of native calves in babesiosis endemic areas was unnecessary and the vaccine should only be used on imported susceptible cattle or when cattle are moved from disease-free areas to endemic areas.

Detailed studies have not been conducted on the advantages of vaccinating against bovine babesiosis in South Africa, although a questionnaire survey carried out in tick-borne disease endemic areas of South Africa (Du Plessis *et al.,* 1994) indicated that the mean mortality rate in calves vaccinated against bovine babesiosis was higher than that in unvaccinated ones. These finding obviously question the credibility of vaccinating against bovine babesiosis in endemic areas. One of the main objectives of the present survey was to do a detailed comparison to see if there were any advantages to the farmers in vaccinating against bovine babesiosis in endemic areas.

2.3 Serological techniques for bovine babesiosis

In bovine babesiosis caused by *B. bigemina* and *B. bovis,* the parasites are readily detectable in stained blood films only during the first few weeks of infection and as a consequence, serological diagnosis is the most reliable method of detecting infection in carrier animals (Mahoney, 1964; Ross and Löhr, 1968). Various serological diagnostic techniques have been used to demonstrate the presence of antibodies against *B. bigemina* and *B. bovis* infections in cattle, with varying levels of accuracy.

The indirect fluorescent antibody (IFA) test (Ross and Löhr, 1968; Joyner *et al*., 1972; Johnston *et al*., 1973) is the most popular method for the diagnosis of bovine babesiosis and has been widely used in South Africa (De Vos *et al.*, 1982b), Zimbabwe (Norval *et al.*, 1983) and Mozambique (Callow *et al.,* 1981). Leeflang and Perie (1972) reported that the four *Babesia* species of cattle (*B. bigemina, B. bovis, Babesia divergens* and *Babesia major*) could successfully be differentiated with the IFA test. The IFA test is highly specific at species level for all *Babesia* species (Anon, 1984). Joyner *et al.* (1972) also found that the IFA test was species specific and could be used to differentiate between *Babesia divergens* and *Babesia major*. Johnston *et al.* (1973) also reported that the IFA test was very specific for *B. bovis*.

Todorovic (1975b) demonstrated that IFA-reacting antibody titres of *B. bigemina*-infected cattle peaked 21 days post infection. The titres decreased gradually thereafter but were still detectable after six months.

Callow *et al.* (1974b) reported that in *B. bigemina*-infected, self-cured cattle IFA reactivity declined sharply during the six months after infection with the parasite, coinciding with the time *B. bigemina* was eliminated from a high proportion of the cattle. Callow *et al.* (1974a) found that in *B. bovis*-infected, drug-sterilized cattle the IFA reactivity dropped sharply six months after the animals were sterilized of the infection with drug. De Vos (1977, unpublished data, cited by De Vos, 1979) also observed a decline in the IFA reactivity in cattle vaccinated with attenuated live vaccines against *B. bigemina* and *B. bovis*.

The complement fixation (CF) test has also been used for the detection of *B. bigemina* and *B. bovis* infection in cattle (Mahoney, 1962; 1964). The test was useful for the diagnosis of babesiosis only at an early stage of infection, and negative results cannot be reliably interpreted as proof of the absence of infection (Mahoney, 1962; 1964).

The rapid latex (slide) agglutination test was found to be effective for the diagnosis of *B. bovis* in natural and experimental infections (Lopez and Todorovic, 1978). It could also be used to classify the herd according to *B. bovis* infection which could then be used as a guide for future babesiosis control programmes (Goodger and

Mahoney, 1974a). The latex agglutination test for *B. bovis* infections in cattle also showed a high degree of specificity and sensitivity when compared with the IFA test (Montenegro *et al.*, 1981).

Todorovic and Kuttler (1974) developed a babesiosis capillary agglutination (CA) test for the detection of specific antibodies in cattle infected with *B. bigemina*. They reported that it showed 100 % agreement with the CF test and because of its simplicity and apparent specificity, the babesiosis CA test could be used as a field test for *B. bigemina* infections.

Curnow (1973b) used the slide agglutination test and found it to be useful in detecting subclinical *B. bigemina* infections in recently infected herds where a build-up of infection has occurred after the introduction of a single infection and where a homologous antigen can be used. Curnow and Curnow (1967) described the indirect haemagglutination test for the diagnosis of *B. bovis* infections in cattle, and reported that the test had the same sensitivity and specificity as the CF test. Goodger and Mahoney (1974b) evaluated the passive haemagglutination test for *B. bovis* infections in cattle, and reported that the test was 99.3 % specific in natural infections and 100 % sensitive in experimental infections.

Currently an internationally validated enzyme-linked immunosorbent assay (ELISA) kit is available for the diagnosis of *B. bovis* infections (Anon, 1996), whilst an ELISA of adequate sensitivity, which can discriminate between *B. bigemina*

and *B. bovis*, has not yet been achieved for *B. bigemina* (El-Ghaysh *et al*., 1996). Other forms of ELISA such as dot-ELISA for *B. bigemina* (Mishra *et al.,* 1998) slide ELISA for *B. bovis* (Kung'u and Goodger, 1990), indirect ELISA for both *B. bovis* and *B. bigemina* (Waltisbuhl *et al.*, 1987; El-Ghaysh *et al*., 1996) and microplate enzyme immunoassay for *B. bovis* (Barry *et al.*, 1982) are also all available.

In a survey comparing the ELISA, IFA and rapid conglutination tests for *B. bovis* infections in cattle, Araújo *et al.* (1998) found the performances of the three tests to be similar.

Other tests, such as Polymerase Chain Reaction (PCR) for the diagnosis of *B. bovis* infections (Fahrimal *et al.,* 1992), and solid-phase radioimmunoassay, for the diagnosis and determination of antibody titres against *B. bovis* (Kahl *et al*., 1982), have also been used.

CHAPTER THREE

3. MATERIALS AND METHODS

The study was carried out on two ranches known as Nooitgedacht and Vlakplaas (Fig. 1).

3.1 Nooitgedacht ranch

3.1.1 The area

Nooitgedacht, a ranch of 2780 ha, is located at 24º 33' S and 28º 36' E, in the Potgietersrus district of the Northern Province of South Africa. The ranch was established in 1872. Ranching activities were interrupted in 1996 when the ranch was sold to the present owner, Mr F.S.H. du Preez, and resumed in 1999. The main objective of the ranch as a Brahman stud to produce steers, breeding heifers and stud bulls. The property is undulating with an average altitude of 1380 m asl. The vegetation is classified as Sour Bushveld (Acocks, 1988). The annual rainfall for the years 2000 and 2001 were 1000 mm and 670 mm, respectively. In 2000, the main rainfall period was from mid-January to May but in 2001 this was delayed by a month. The area is very dry in the winter months. The minimum (3 ºC) and maximum (33 ºC) temperatures usually occur in June and in December, respectively.

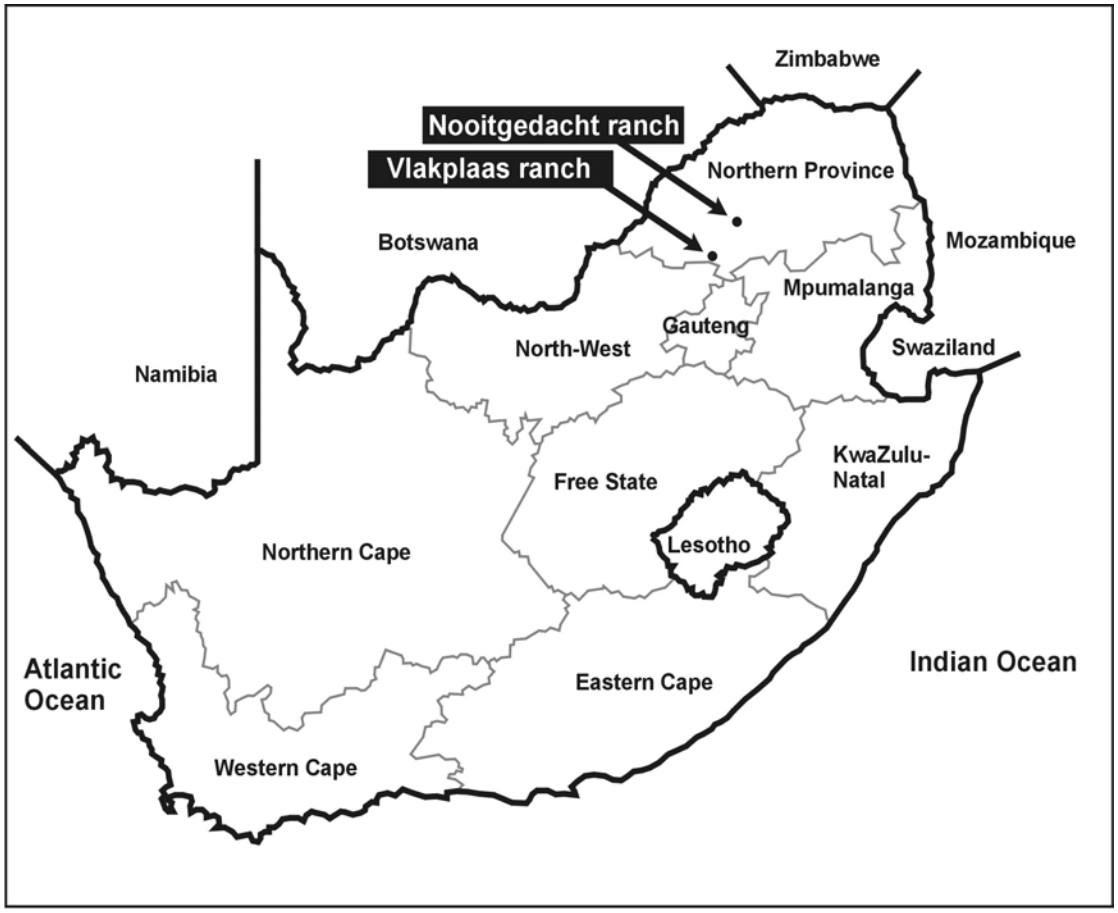

Fig. 1: Map of South Africa showing the locality of the two study sites.

3.1.2 The cattle

All cattle at the Nooitgedacht were Brahman, a breed which was developed in the southern United States of America in the early 1900s from humped cattle of India (*Bos indicus*), often referred to as Zebu (Thomas, 1986; Anon, 1995). The name “Brahman" was given by the American Breeders Association, which was established in 1924 (Thomas, 1986). In South Africa, the Brahman Breed Society was founded in 1958 and the cattle population is still growing (Anon, 1995). Brahman cattle are known to be more resistant to tick infestations and can withstand tropical and subtropical conditions better than European (*Bos taurus*) breeds of cattle (Thomas, 1986).

The founding stock at Nooitgedacht was obtained in 1999 from Kareefontein ranch, 100 km south of Nooitgedacht, in the Warmbaths district of the Northern Province. They included 65 stud and 50 commercial breeding cows and two stud and two commercial breeding bulls. During the study period, the cattle at Nooitgedacht comprised 115 breeding cows (30 to 140 months old), 4 breeding bulls, 50 stud bulls and heifers, born in October 1999, and 58 stud and commercial calves, born in October 2000. They were predominantly white-gray in colour with the characteristic long, drooping ears, large dewlap and a prominent hump.

The breeding season was from January to March, when bulls were allowed to run with the cows in a 1:35 ratio. Calves born in October every year and were weaned

at seven months. Stud bulls and heifers were sold at 24 months of age, whilst commercial bull calves were sold at about seven months. Cattle were in excellent condition and largely maintained on natural grazing, supplemented with winter and summer licks as required throughout the year.

Other domestic animals kept on the ranch included 60 crossbred Dorper sheep. A number of different wildlife species were also maintained on the ranch and included 26 eland, 21 gemsbok, 30 hartebeest, 38 blue wildebeest, 180 blesbok, 200 impala, 60 kudu, 3 nyala, 30 waterbuck, 28 reedbuck, 20 mountain reedbuck, 40 grey duiker, 10 steenbok and 10 klipspringer.

3.1.3 Tick-control and occurrence of bovine babesiosis

Ticks were controlled by hand-spraying with Bayticol (2 % flumethrin, Bayer), at irregular intervals, whenever the farmer believed that the tick burden was excessive. The nucleus of the breeding stock came from an area where tick-borne diseases were endemic and vaccination or blanket treatments for babesiosis and ticks were not given to the animals before they moved to Nooitgedacht. No clinical cases of bovine babesiosis were reported at Nooitgedacht during the study period (August 2000 to June 2001).

3.1.4 Vaccination

The first calf crop on Nooitgedacht since the resumption of ranching activities was born during October 1999. The calves were vaccinated against bovine babesiosis (*B. bovis* and *B. bigemina*) at four months by a private practitioner (Dr H. Hansen). Thirty calves (22 stud, 8 commercial herd) of the subsequent crop, born during October 2000, were vaccinated against *B. bovis* and *B. bigemina* at seven months by the researchers. The remainder (all commercial herd) were not vaccinated.

The vaccines used were deep-frozen, live *B. bigemina* and *B. bovis* blood vaccines, attenuated by passage through splenectomized calves (Onderstepoort Biological Products, South Africa). The vaccine was taken to the ranch in a frozen state on dry ice (-70 °c), thawed in lukewarm water and 1 mℓ was administered intramuscularly to each calf.

3.2 Vlakplaas ranch

3.2.1 The area

Vlakplaas, a ranch of 820 ha, is located at 24º 58' S and 28º 05' E, in Warmbaths district of the Northern Province of South Africa. The present owner, Mr J. Maritz, has been producing Bonsmara stud bulls since 1987. The ranch is flat with an average altitude of 1090 m asl. The vegetation is Sourish Mixed Bushveld (Acocks, 1988). The rainfall during 2000 and 2001 was 1010 mm and 320 mm, respectively. In 2000 the main rainfall occurred from mid-January to May and in

2001 was a month late. The area is very dry during winter months. The minimum temperature of 0 ºC usually occurs in June and the maximum of about 35 ºC in January.

3.2.2 The cattle

All the cattle on Vlakplaas were Bonsmara, a beef breed produced by cross-breeding the indigenous South African *Bos indicus* breed, the Afrikander, with two European *Bos taurus* breeds, the Hereford and the Shorthorn (Bonsma, 1980). Bonsmara cattle contain 5/8 Afrikander, 3/16 Hereford and 3/16 Shorthorn genes (Anon, 1995). The breed was developed with the objective of resolving problems associated with the lack of adaptability of the more productive exotic breeds (*Bos taurus*) to subtropical, semi-arid areas of South Africa and the poor economic performance of *Bos indicus* (Bonsma, 1980). Bonsmara cattle were also selected for their adaptability to a tropical and subtropical climate, resistance to tick-borne diseases and their docile temperament (Anon, 1995). The name Bonsmara was given as a tribute to Jan Bonsma, the producer of the breed and Mara Research Station in Transvaal, South Africa, the site where the crossbreeding research was carried out (Bonsma, 1980).

The Bonsmara cattle at Vlakplaas comprised 120 breeding cows (30 to 120 months old), 4 breeding bulls, 115 stud bulls and heifers, born in October 1999 and 116 calves, born in October 2000. They were all red-brown in colour and in excellent body condition. The breeding season was January - February, when the

bulls were allowed to run with the cows at a 1:30 ratio. Calves were born in October every year and were weaned at seven months. Stud bulls and heifers were sold at 24 months of age. Cattle were maintained almost entirely on natural grazing, supplemented with winter lick (Voermol) during the winter months.

Other domestic animals kept on the ranch were 250 Mutton-Merino sheep maintained for wool and meat production. The wildlife species on the ranch included: 5 impala, 5 kudu, 15 steenbok, 5 grey duiker, 20 warthog, 5 bush pig, 30 porcupine and 10 black-backed jackal.

3.2.3 Tick-control and occurrence of bovine babesiosis

Ticks were controlled by the application of Amipor (Amitraz 1 % and Cypermethrin 1 %, Logos Agvet) pour-on, at irregular intervals, and by dipping with Triatix (12.5 % Amitraz, Hoechst), every month and every two months during summer and winter seasons, respectively. Vaccination against bovine babesiosis was not carried out at Vlakplaas ranch.

During 2000, the farmer reported 10 suspected cases of redwater which were treated with imidocarb dipropionate (Forray 65, Schering-Plough), 1.2 mg/kg, and oxytetracycline (Terramycin LA, Pfizer), 20 mg/kg and recovered. The researchers did not encounter any cases of babesiosis on any of the visits to Vlakplaas.

3.3 Experimental procedures

At both ranches, the study involved calves born during October 1999 and October 2000, as well as the breeding cows. When sampling commenced in August 2000, calves born during October 1999 were 10 months old. At Nooitgedacht, they were sampled at this age (n = 49) and re-sampled at the age of 17 months (n = 39) and 20 months (n = 29). Likewise, calves at Vlakplaas were sampled at the age of 10 months (n = 49), 17 months (n = 52) and 20 months (n = 35). Breeding cows were sampled only once at Nooitgedacht (n = 50) and at Vlakplaas (n = 49).

At Nooitgedacht, some calves (n = 30) born during October 2000 were vaccinated against *B. bigemina* and *B. bovis* while the others (n = 28) were left unvaccinated. All the vaccinated calves (n = 30) and some unvaccinated calves (n = 17) were sampled on vaccination day at seven months of age. Twenty-eight days post vaccination the vaccinated (n = 27) and unvaccinated calves (n = 20) were sampled (at the age of eight months). At Vlakplaas, where vaccination against *B. bigemina* and *B. bovis* was not carried out, calves born during October 2000 were also sampled at the ages of seven (n = 37) and eight months (n = 33).

At each collection, cattle were selected by simple random sampling technique (Thrusfield, 1995). Animals belonging to age groups in which horizontal sampling was applied, could have been sampled repeatedly on successive dates.

3.4 Serum samples

Serum samples were collected between August 2000 and June 2001. The animals were restrained in a crush with a neck-clamp. Blood was collected aseptically from the caudal vein into 10 mℓ plain vacutainer tubes (Sherwood Medical) using 20-gauge needles (Becton Dickinson). At the laboratory, the tubes were centrifuged and the serum decanted. The sera were frozen and stored at the Department of Veterinary Tropical Diseases until they were transferred to the Onderstepoort Veterinary Institute, where serological testing was performed.

3.5 Indirect fluorescent antibody test

All serum samples were tested for the detection of antibodies against *B. bigemina* and *B. bovis* using the IFA test. The IFA test is the most widely used method for the diagnosis of *B. bovis* and *B. bigemina* infections in South Africa (De Vos *et al.*, 1982b).

3.5.1 Antigen Preparation

Fifty mℓ of blood was collected into 250 mℓ PBS from either *B. bovis* or *B. bigemina* infected splenectomized calves when the parasitaemia was about 3 %. The blood was washed, by centrifuging twice in PBS at 2000 rpm, transferred to a 10 mℓ tube and again centrifuged three more times, whilst removing the white blood cells with each wash. After the fifth wash, one part of the RBC was reconstituted with two parts 4 % bovine albumin fraction V in PBS (1:2 v/v),

poured into a dish and carried to prepared wells on glass slides (24 wells on each slide). The slides were air dried, wrapped in soft paper, marked with the name of the parasite antigen and date and stored at –20 ºC, for use in the IFA test.

3.5.2 Test procedure

Antigen slides and test and control sera were taken from storage at –20 ºc and incubated at 37 ºC for 10 minutes. Test and control sera were diluted to 1/80 and 1/160 in PBS and the antigen slides fixed in cold acetone (-20 ºC) for 1 minute. A drop of the diluted positive and negative control sera were placed into the first and second wells of the antigen slides, respectively, followed by a drop of each of the 1/80 and 1/160 dilutions of each test serum into the rest of the wells. The slides were then incubated in a humid chamber at 37 ºC for 1 hour. After incubation, the sera were rinsed from the slides by dipping into a container with a 200 mℓ PBS. This was followed by washing in 1 ℓ PBS and then in 1 ℓ distilled water for 10 and five minutes, respectively, on a magnetic stirrer set at very low revolutions. Conjugate (rabbit anti-bovine IgG conjugated to fluorescein isothiocyanate, Sigma), was diluted to 1/80 in Evans blue. Excessive distilled water was dispensed and each slide was covered with conjugate by placing a drop into each well. The slides were then incubated in a humid chamber at 37 ºC for 1 hour. After incubation, the slides were rinsed in PBS and washed in PBS for 10 minutes on a magnetic stirrer and then left to air dry. A drop of 50 % glycerin in PBS was placed on each slide, covered with 24 x 50 mm cover slip and examined under a

fluorescent microscope using a 50x water objective. Serum samples that showed fluorescence at the dilution rate of 1/80 were regarded as positive.

3.6 Data analysis

All data generated from the work were recorded and analyzed in collaboration with Mrs. Rina Owen and Mr Sollie Millard, statisticians from the Department of Statistics, University of Pretoria. The SAS statistical package, Version 8.1 was used for the analyses. Comparative analyses were carried out using the chi-square test.

CHAPTER FOUR

4. RESULTS

4.1 Nooitgedacht ranch

The antibody response to *B. bigemina* and *B. bovis* of the seven and eight-month-old vaccinated and unvaccinated calves (calves born during October 2000) is summarized in Table 1. Thirteen percent of the seven-month-old calves sampled immediately before inoculation on vaccination day, and 18 % of calves of the same age group, which were sampled on the same day but left unvaccinated, were positive to *B. bigemina*. All the calves in both groups were negative to *B. bovis.* Twenty-eight days later (at the age of eight months), 44 % of the vaccinated and 70 % of the unvaccinated calves were positive to *B. bigemina* while 11 % of the vaccinated and none of the unvaccinated calves were positive to *B. bovis*.

Within 28 days, both vaccinated and unvaccinated calves showed a significant (P=0.0091 and P=0.0015, respectively) seroconversion to *B. bigemina* whilst the seroconversion of the vaccinated calves to *B. bovis* was not significant (P=0.0607). There was no significant difference (P=0.0814) in antibody response to *B. bigemina,* between the eight-month-old vaccinated and unvaccinated calves, even though more of the unvaccinated calves were sero-positive. The eight-month-old vaccinated and unvaccinated calves also exhibited no significant difference (P=0.1234) in antibody response to *B. bovis*.

Table 1. Percent positive to *Babesia bigemina* and *Babesia bovis* in vaccinated and unvaccinated groups of Brahman calves at Nooitgedacht ranch on day-zero (seven-month-old) and 28 days post vaccination (eight-month-old), as determined by the IFA test

Days post vaccination	Vaccinated group		Unvaccinated group	
	B. bigemina	*B. bovis*	*B. bigemina*	*B. bovis*
Day zero	13	0	18	0
28 days	44	11	70	0

Day zero refers to the day the vaccinated group was inoculated against *Babesia bigemina* and *Babesia bovis*. Both vaccinated and unvaccinated groups of calves were sampled on that day (at the age of seven months) and 28 days later (at the age of eight months).

The prevalence of antibodies to *B. bigemina* and *B. bovis* in vaccinated and unvaccinated cattle of various age groups is shown in Table 2. Fifty-nine percent and 39 % of the 10-month-old calves were positive to *B. bigemina* and *B. bovis*, respectively, six months post vaccination day. When these animals were re-sampled at the age of 17 months, 49 % and 15 % were positive to *B. bigemina* and *B. bovis*, respectively. The same group of animals was sampled when they were 20 months old. This time 31 % and 21 % were found to be positive to *B. bigemina* and *B. bovis*, respectively.

The prevalence of antibodies to *B. bigemina* in 10 and 17-month-old cattle did not differ significantly (P=0.3273), while the 10-month-old calves had significantly (P=0.0156) higher antibody prevalence to *B. bovis* than the 17-month-old cattle. The prevalence of antibodies to *B. bigemina* and *B. bovis* in 17 and 20-month-old cattle did not differ significantly (P=0.1428 and P=0.5704, respectively).

Seventy-two percent of the breeding cows (30 to 140 months old) were positive to *B. bigemina* two years after being transferred to Nooitgedacht ranch. Prevalence of antibodies to *B. bigemina* was significantly higher (P=0.0004) in breeding cows than in the 20-month-old cattle. The breeding cows were all negative to *B. bovis.*

In general, prevalence of antibodies to *B. bigemina* in cattle at Nooitgedacht was relatively high between eight and 17 months of age but had started to decline at the age of 20 months (Table 2). Prevalence was the highest in the breeding cows

Table 2. Prevalence of antibodies against *Babesia bigemina* and *Babesia bovis* in vaccinated (eight, 10, 17 and 20-month-old) and unvaccinated (seven and 30-140 month-old) Brahman cattle at Nooitgedacht ranch as determined by IFA test

Antibody Prevalence		Age (months)					
		7	8	10	17	20	30-140
Percent positive	*Babesia bigemina*	13	44	59	49	31	72
	Babesia bovis	0	11	10	15	21	0

(30 to 140 months old). The *B. bovis* antibody prevalence of vaccinated animals followed similar trends as *B. bigemina* (Table 2). However, vaccinated cattle of all age groups showed higher seropositivity to *B. bigemina* than to *B. bovis* (Fig. 2).

4.2 Vlakplaas ranch

The seroprevalence of antibodies to *B. bigemina* of the seven, eight, 10, 17 and 20-month-old cattle and the breeding cows on Vlakplaas is shown in Table 3. Forty-six percent of the seven month-old and 70 % of the eight-month-old calves were positive to *B. bigemina*. The difference is significant (P=0.045).

Ninety, 92, 54 and 82 percent of the 10, 17 and 20-month-old cattle and breeding cows, respectively, were positive to *B. bigemina* (Table 3). No significant (P=0.9211) difference was observed between the 10 and 17-month-old cattle while seroprevalence among the latter was significantly higher (P=0.001) than among the 20-month-old cattle. Seroprevalence among the breeding cows was also significantly higher (P=0.0069) than that of 20-month-old cattle. All cattle at Vlakplaas ranch were seronegative to *B. bovis*.

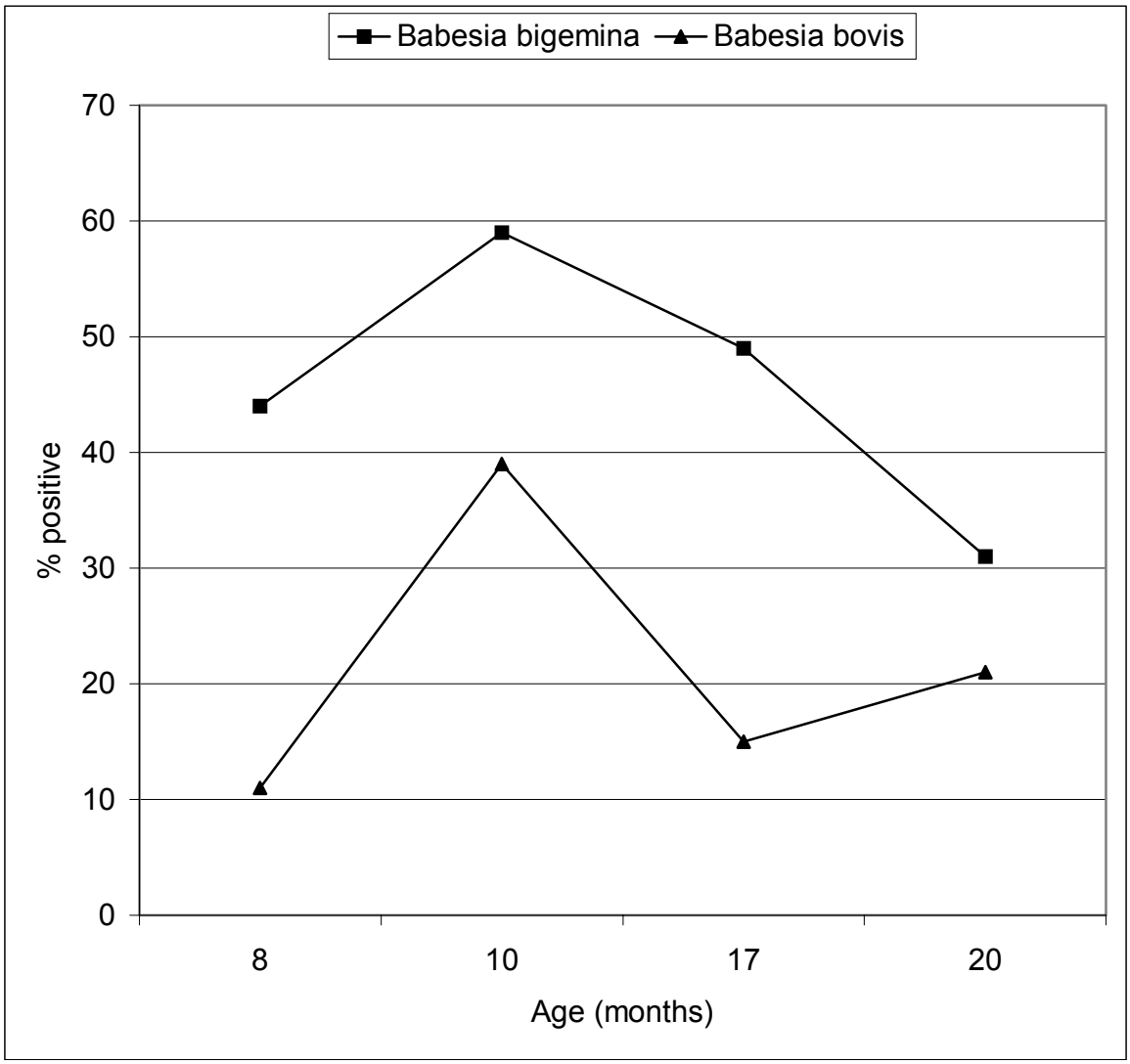

Fig. 2. Prevalence of antibodies against *Babesia bigemina* and *Babesia bovis* in vaccinated cattle at Nooitgedacht ranch

Table 3. Prevalence of antibodies against *Babesia bigemina* in different age groups of Bonsmara cattle at Vlakplaas ranch as determined by the IFA test

Antibody prevalence	Age (months)					
	7	8	10	17	20	30-140
Percent positive to *Babesia bigemina*	46	70	90	92	54	82

4.3 Comparison of the two ranches

The seroprevalences of antibodies to *B. bigemina* among cattle of similar age groups at Nooitgedacht (vaccinated) and Vlakplaas (unvaccinated) are compared in Fig. 3. Seroprevalence of antibodies to *B. bigemina* in the seven-month-old calves was significantly higher (P=0.0042) at Vlakplaas than at Nooitgedacht. By eight months of age, seroprevalence of antibodies to *B. bigemina* in the unvaccinated, eight-month-old calves at Vlakplaas was significantly higher (P=0.048) than that of the vaccinated calves of the same age group at Nooitgedacht. Pardoxically, the eight-month-old unvaccinated calves at Nooitgedacht showed the same seroprevalence of antibodies to *B. bigemina* (P=0.9814) than calves of the same age at Vlakplaas.

Seroprevalence of *B. bigemina* among the 10-month-old calves and 17-month-old cattle at Vlakplaas was significantly higher (P=0.0005 and P=0.0001, respectively) than that of cattle of the same age at Nooitgedacht. However, seroprevalence of antibodies to *B. bigemina* in the 20-month-old and 30 to 140-month-old (breeding cows) cattle at both ranches did not differ significantly (P=0.0620 and P=0.2565, respectively). *Babesia bovis* was absent from both ranches.

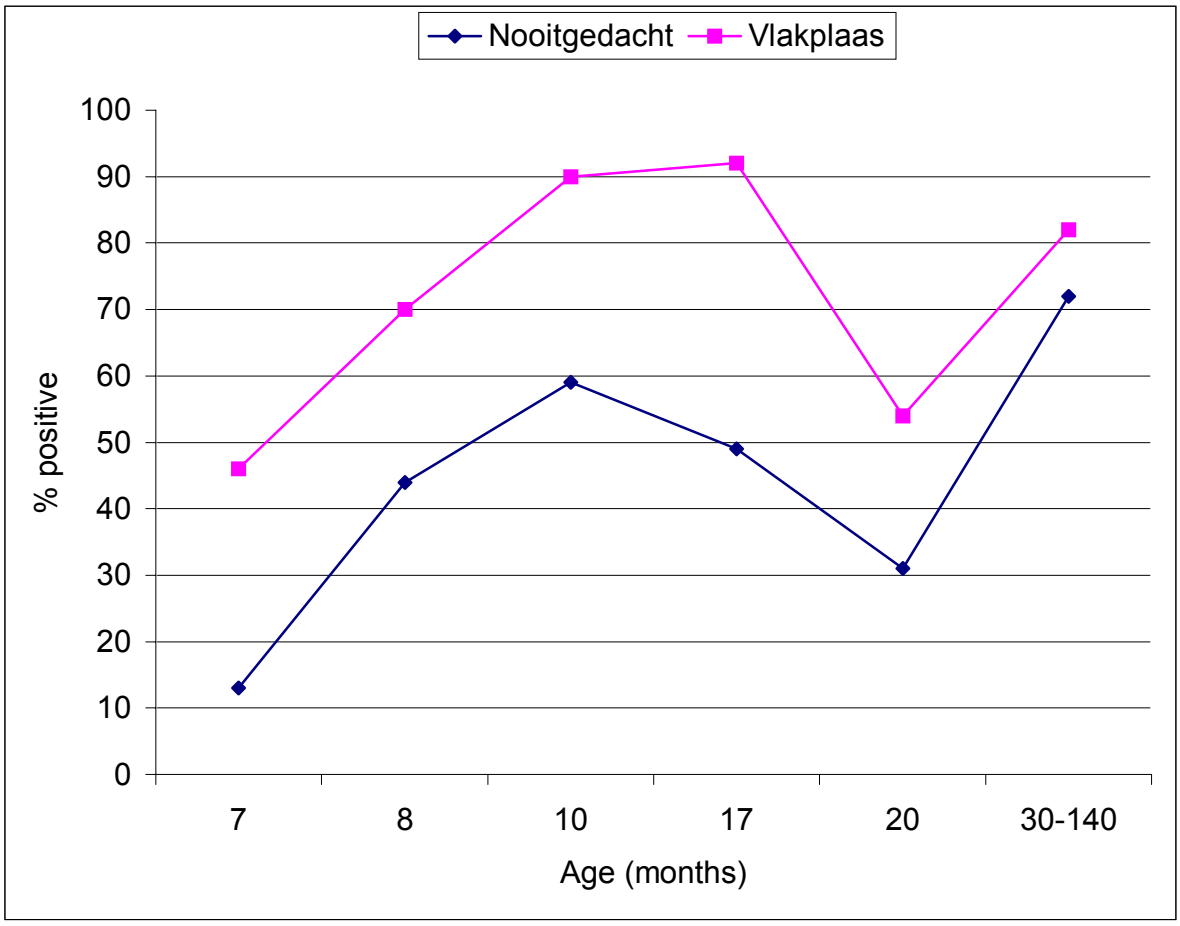

Fig. 3. Prevalence of antibodies against *Babesia bigemina* in cattle of different age groups at Nooitgedacht and Vlakplaas ranch, as determined by IFA test

(Vlakplaas: cattle not vaccinated; Nooitgedacht: 8-, 10-, 17- and 20-month-old cattle had been vaccinated)

CHAPTER FIVE

5. DISCUSSION

5.1 General

The two ranches were situated in a tick-borne disease endemic area (Du Plessis *et al.,* 1994) in the same province and their vegetation was fairly similar (Acocks, 1988). Similar livestock management and tick-control methods were employed.

The cattle breeds involved in this study were also closely related genetically. Brahman is pure Zebu while Bonsmara is 62.5 % Zebu. Like Zebu cattle, Bonsmara was bred for resistance to tick-borne disease and adaptability to the subtropical and tropical environment.

The age groups of cattle involved in the study were largely determined by availability. The 10-month-old calves were sampled to represent the serological response of older calves to vaccination and natural infections with *Babesia* parasites. They were then re-sampled at the age of 17 and 20 months to observe any changes in serological status of vaccinated and unvaccinated young adult cattle to *B. bigemina* and *B. bovis*.

Breeding cows were included in order to follow the serological status of older adult cattle and to get some idea of the levels of colostral antibody protection lin the new-born calves.

Calves born during October 2000 were sampled at the age of seven months on the day vaccination was done at Nooitgedacht ranch and were re-sampled 28 days later. The calves were re-sampled to determine whether seroconversion had occurred in response to vaccination in the vaccinated group and to natural infections in the unvaccinated group. Similar data could not be obtained from calves born during October 1999, as they were first sampled at the age of 10 months, which is six months post vaccination, and had probably lost IFA-reacting antibody titres (Callow *et al.,* 1974a; 1974b).

Detailed immunological studies were not the objective of the present study. However, positive serological reactions to *B. bigemina* and *B. bovis* would be used as an indicator of the existence of immunity to the parasites, as the humoral immune system has long been demonstrated to be involved in immunity of cattle against bovine babesiosis (Hall, 1960; 1963; Mahoney 1967a). On the other hand, negative results would not conclusively prove the absence of immunity to *B. bigemina* and *B. bovis* (Callow *et al.,* 1974a, 1974b).

5.2 Nooitgedacht ranch

5.2.1 *Babesia bigemina*

5.2.1.1 Ten, 17 and 20-month-old cattle

The percentage of animals that showed positive IFA-test reactions to *B. bigemina* decreased with increasing age. This may have been due to loss of IFA-reacting antibody titres as a result of lack of superinfections due to low tick populations.

At Nooitgedacht livestock ranching had been interrupted for about three years until it was resumed in 1999. It was likely, therefore, that the field population of *B. decoloratus*, the vector tick of *B. bigemina,* may have been reduced due to starvation as a result of lack of hosts. It has been shown that free-living *B. decoloratus* larvae cannot survive for more than six months without feeding (Bigalke *et al.,* 1976). Payne and Osorio (1990) also reported that the build-up of tick populations on a farm in Paraguay was slowed down by the low stocking rates of cattle.

In a study to investigate the effect on the immunity of cattle to *B. bigemina* following drug-cure or self-cure, Callow *et al.* (1974b) found that IFA reactivity (positivity) declined sharply during the six-month period post inoculation. The parasite in the self-cure group was eliminated from a high proportion of the cattle. In some of the cattle which had been drug-sterilized four weeks after infection, the IFA positivity soon declined, but at a slower rate than in the self-cured cattle (Callow *et al.,* 1974b). On the other hand, no change in IFA reactivity was

observed in cattle that remained infected until the end of the study (32 weeks). However, all groups had an appreciable degree of immunity on subsequent challenge with pathogenic *B. bigemina*. They concluded that low titres or absence of IFA test reactivity could not be taken as an indication of loss of immunity in naturally infected or vaccinated animals (Callow *et al.,* 1974b).

Todorovic (1975b) reported that cattle challenged with *B. bigemina*-infected blood reached peak level of IFA-reacting antibody titres 21 days post infection and the titres decreased gradually thereafter but were still above minimum levels after six months. He indicated the existence of a continued downward trend with minimum positive IFA response being detectable 18 to 24 months after a single experimental infection.

In South Africa, De Vos (1977, unpublished data cited by De Vos, 1979) using the IFA test found that a herd in which 93 % of the animals were positive to *B. bigemina* two months after vaccination were only 60 % positive 21 months post vaccination. He observed that, in the absence of adequate natural challenges, IFA-test-reacting antibody titres of vaccinated cattle decreased and more animals became IFA test negative as time progressed.

Tice *et al.* (1998) reported a situation in South Africa where the serological status of *B. bigemina* shifted from endemic stability to instability and then back to stability over a three-year period without being accompanied by disease outbreaks. This

condition was probably created as a result of fluctuations in the vector population due to climatic changes or vector control activities.

In studies done in different areas of South Africa (Boomker *et al.*, 1983; Horak *et al.*, 1983a; Horak *et al.*, 1983b; Horak *et al.*, 1988; Horak, 1995), *B. decoloratus* was collected from the same wildlife species as those on Nooitgedacht. However, after the resumption of cattle farming activities at Nooitgedacht the stocking rate of wildlife on the ranch was probably not high enough to have made a significant contribution to the build-up of tick populations large enough to establish and maintain endemic stability to *B. bigemina*. Furthermore, the susceptibility of wildlife to tick infestations has yet to be determined. Friedhoff and Smith (1981) have reported, however, that clinical infections with *B. bigemina* and *B. bovis* are restricted to cattle and no important wildlife reservoir has been demonstrated.

The cattle kept at Nooitgedacht were Brahman, a *Bos indicus* breed known for its tick resistance (Frisch and Neill, 1998; Payne and Osorio, 1990). This characteristic alone may have played an important role in limiting any increase in the tick population on the ranch and may have affected the establishment and maintenance of endemic stability to *B. bigemina*.

Although the percentage positivity to both parasites at Nooitgedacht was low, there were more *B. bigemina*-positive cattle than *B. bovis*-positive ones. This may have been due to the existence of low levels of natural challenges with *B.*

bigemina as compared with *B. bovis*, which did not exist on the ranch. However, the inoculation rate of *B. bigemina* was not high enough to keep up with the rate of loss of IFA-reacting antibody titres in the herd.

Calves born during October 1999 were weaned at seven months, after which they no longer shared the same grazing land with the breeding cows, which were considered to be the main source of *B. bigemina* infections on the ranch. It appears therefore that, as a result of low tick populations many calves were not infected before seven months and could not maintain high enough levels of *B. bigemina* to maintain endemic stability. The low tick populations coupled with low numbers of reservoir hosts, may also have contributed to the reduced parasite transmission rate and hence low IFA test reactivity in the first batch of calves born on the ranch.

The progressively lower IFA-reacting antibody titres in vaccinated cattle at Nooitgedacht could therefore be due to self-cure of the vaccinated animals from the infections established from the live vaccines and the lack of natural challenges due to low tick populations on the ranch.

5.2.1.2 Breeding cows

The breeding cows at Nooitgedacht were obtained in 1999 from Kareefontein ranch in the Warmbaths district of the Northern Province, *ca.* 100 km south of Nooitgedacht. As *Babesia bigemina* infection was endemic at Kareefontein, the

cows were not blanket-treated with antibabesial drugs before they were moved and the majority of the cows were believed to be latently infected with *B. bigemina*. The cows were also not treated with acaricides before the move, and consequently maintained their existing tick populations.

The seropositivity status of the breeding cows at Nooitgedacht with72 % IFA-positive to *B. bigemina* was close to endemic stability (Norval *et al*., 1983). The cows were tested two years after being transferred to Nooitgedacht, where the tick population was much lower and would not have effected the transmission of *B. bigemina*. The prevalence of antibodies to *B. bigemina* would therefore have declined. It is believed that some of the cows may have lost *B. bigemina* infections while still being immune to the parasite (Callow, 1967; Callow *et al.*, 1974b; Johnston *et al.,* 1978) as a result of lack of superinfections.

In an endemically stable situation, the age incidence of *B. bigemina* parasitaemia is generally lower in older animals than in younger ones (Mahoney 1969). Although the *B. bigemina* antibody prevalence of the breeding cows at the time of transfer to Nooitgedacht was unknown, they still appeared to have retained a higher seroprevalence than their calves. This higher seroprevalence was maintained despite the tick population on the ranch being low, so the breeding cows were probably the main source of *B. bigemina* infections for the younger cattle. The older cattle were also maintained together and would have had a better chance of being superinfected. The calves were separated from their dams after

weaning at seven months and as a consequence most of the calves had not been infected. Johnston *et al.* (1978) and Mahoney (1969) found that *B. bigemina* infections rarely persist for longer than a year in all breeds of cattle, and infected cattle normally only remain infective to ticks for four to seven weeks. Any loss of infection would further contribute to a reduced parasite transmission rate, as fewer animals would serve as sources of infection to ticks. *Babesia bigemina* may persist longer in an infected herd through vertical transmission in the tick from one generation to the next without the need for the tick to feed on an infected animal (Gray and Potgieter 1981). This feature may have played a role in allowing *B. bigemina* to persist at high levels in the herd despite low tick numbers.

5.2.1.3 Seven and eight-month-old calves

The seroconversion in response to vaccination 28 days post inoculation was very low. Seropositivity of calves increased from 13 % on vaccination day to only 44 % 28 days post vaccination. On the other hand, seropositivity of unvaccinated calves increased from 18 % to 70 % during the same period. It therefore appears that the vaccinated calves did not respond to the vaccination. This has made the objective to study the seroconversion differences between vaccinated and unvaccinated calves impossible. As the vaccines had been carefully administered in accordance with the instructions of the manufacturer, there is a need to conduct an infectivity test of the vaccine batch used.

Although not statistically significant, the vaccinated calves had lower IFA-test reactivity than the unvaccinated group. This may have been due to differences in tick control measures applied to stud and commercial cattle at Nooitgedacht. The majority (74 %) of the vaccinated calves were stud animals, while the rest of the vaccinated and all unvaccinated calves were from the commercial herd. The commercial calves were destined for sale immediately following weaning at seven months of age. The commercial and stud cattle were kept separately on different grazing lands. Although the tick control measures at the ranch were erratic, the owner may have considered the stud cattle more valuable, and consequently applied relatively more aggressive tick control measures to these cattle in comparison to the commercial herd. This may have caused reduced parasite transmission and have led to a slow seroconversion rate in the stud calves.

In general, both the vaccinated and unvaccinated groups showed a sharp increase in serological status eight months of age. A similar trend was observed in calves of the same age group at Vlakplaas. Vaccination took place in May. The substantial increase in seroconversions form May to June imply a high level of tick activity in late autumn / early winter on both ranches. A similar pattern was found in Free State Province where the *Boophilus decoloratus* burden on cattle peaked in June (Dreyer, Fourie and Kok, 1998). Mahoney (1969) found that the age incidence of *B. bigemina* parasitaemia rose from zero at birth, attained a maximum between six months and two years of age and then declined sharply in the older animals. As the onset of parasitaemia precedes the production of

antibodies, the rise in the number of serologically positive animals at the age of eight months is in agreement with this report.

At Nooitgedacht there had been a build-up of the tick population over two years, which was probably due to the tick control measures applied at the ranch. Erratic tick control measures had been in operation since the resumption of cattle ranching to enhance the increase in the tick population to a level where endemic stability to *B. bigemina* could be achieved and maintained. It was likely, therefore, that the commercial calves would attain endemic stability to *B. bigemina* before the non-specific innate resistance waned at nine months, by which time they would have been sold.

It is not easy to predict whether the stud calves would also attain endemic stability to *B. bigemina* before nine months of age. Severe losses may not necessarily occur, as *B. bigemina* usually causes mild disease (Rogers, 1971; Mahoney *et al.*, 1973b; James *et al.*, 1985; Jongejan *et al.,* 1988).

De Vos (1979) attributed the unstable situations in bovine babesiosis in South Africa to unfavourable climatic conditions and intensive tick control. The interruption of livestock farming activities on a ranch for an extended period, as had occurred at Nooitgedacht, could also be a cause of instability due to the reduction of the vector ticks.

5.2.2 *Babesia bovis*

5.2.2.1 Ten, 17 and 20-month-old

The percentage of cattle seropositive to *B. bovis* at 10, 17 and 20 months decreased with increasing age. This was most likely be due to loss of IFA-reacting antibody titres as a result of the absence of natural challenge. In South Africa, De Vos (1977, unpublished data cited by De Vos, 1979) using the IFA test determined that antibody titres in a vaccinated herd dropped progressively in the absence of adequate natural challenges. A herd that was 97 % positive to *B. bovis* two months post vaccination, was only 60 % sero-positive 21 months after vaccination.

The loss of IFA-reacting antibody titres may not be paralleled with loss of immunity. Callow *et al.* (1974a) found that *B. bovis* IFA reactivity dropped sharply six months after drug sterilization, while immunity to subsequent challenge with the parasite was maintained. Mahoney *et al*. (1973b) demonstrated that vaccinated cattle were immune to *B. bovis* four years after vaccination. Mahoney *et al.* (1973b), Johnston *et al.* (1978) and Mahoney *et al.* (1979b) reported that cattle which naturally eliminated *B. bovis*, after vaccination or natural infection, maintained strong and lasting immunity to heterologous and homologous strains of the parasite, regardless of the fall in IFA titres. It was thus concluded that cattle could lose IFA reacting antibody titres with time following vaccination or natural infections whilst remaining immune to the parasite.

The loss of IFA reactivity (positivity) was more obvious with *B. bovis* than *B. bigemina.* This may be due to the absence of superinfections with *B. bovis,* as the parasite did not occur on the ranch. Previous reports indicated that Nooitgedacht is located in a *B. bigemina*-endemic but *B. microplus* and *B. bovis*-free area (De Vos 1979). The present study indicated that *B. bovis* had not yet spread to the Nooitgedacht area.

Mahoney and Ross (1972) demonstrated that the IFA positivity after the age of four months could only be due to natural infections or vaccination, as colostral antibody could not last beyond four months. The *B. bovis* positive results on Nooitgedacht only involved the vaccinated animals and were therefore due to vaccination. Therefore, vaccination against *B. bovis* was done unnecessarily without studying the distribution of the parasite and its vectors and assessing the potential risk of the disease occurring.

In South Africa, *B. microplus* is less widespread than *B. decoloratus* and, as a result, *B. bovis* has a more limited distribution when compared with *B. bigemina* (De Vos, 1979). In Zimbabwe, Norval *et al.* (1983) found that *B. bigemina* occurred all over the country together with *B. decoloratus* and was endemically stable in most of these areas. The distribution of *B. bovis* and its vector *B. microplus* was limited to the eastern part of the country and it was endemically unstable in most of the areas. Jongejan *et al.* (1988) reported that *B. bigemina*

occurred throughout Zambia whereas *B. bovis* closely followed the distribution of its vector tick and was limited to the northeastern part of the country.

5.2.2.2 Breeding cows and seven and eight-month-old calves

The calves did not respond well to vaccination against *B. bovis*; only 11 % of the vaccinated calves were positive to *B. bovis,* twenty-eight days after vaccination.All the breeding cows and the seven-month-old calves were negative to *B. bovis* and It was therefore concluded that *B. bovis* was absent from the ranch.

5.2.3 Absence of clinical babesiosis

The serological status of the calves born during October 2000 indicated that there was an increase in the transmission rate of *B. bigemina*, two years after livestock ranching had resumed at Nooitgedacht. There was no record of clinical babesiosis in any of the cattle at Nooitgedacht during the project, however,. This was probably due to the good immunity in the cattle. The inherent resistance of Brahman cattle to babesiosis (Bock *et al.,* 1999a; 1999b) should also be taken into account. The breeding cows were obtained from an area where *B. bigemina* was endemic. They may have been superinfected repeatedly and had become immune to the parasite. In addition, Callow *et al.* (1974a) found that the duration of prior exposure to the parasite was an important factor in immunity to bovine babesiosis. Furthermore, the serological status of the breeding cows to *B. bigemina* was close to that required for endemic stability (Norval *et al.*, 1983) and consequently few clinical cases would have been seen.

Calves born during October 1999 were protected by vaccination. Although the percentage of positive cattle at 10, 17 and 20 months of age was low, probably as a result of loss of IFA-reacting antibody titres, they were probably immune to *B. bigemina.* Strong and lasting sterile immunity to heterologous and homologous strains of *B. bigemina* persists in cattle which have been drug-sterilized or have naturally eliminated the parasite, regardless of loss of IFA-reacting antibodies titres (Callow, 1967; Callow *et al.,* 1974b; Mahoney *et al.,* 1973b; Johnston *et al.,* 1978).

Calves born during October 2000 were probably protected by natural non-specific resistance, a well-documented phenomenon (Hall, 1960, 1963; Hall *et al.*, 1968; Trueman and Blight 1978; Corrier and Guzman 1977; Payne and Osorio 1990). The calves also got good immunity as a result of a high natural tick challenge. In Colombia, Corrier and Guzman (1977) reported the occurrence of 100 % positive reactions to the CF test in calves born to cows of which only 57 % reacted positively to the same test. It was very likely, therefore, that calves born to cows of which 72 % were IFA positive to *B. bigemina,* would be protected by colostral antibodies until innate resistance took over.

The strain of *B. bigemina* involved may have been of low pathogenicity as reported by several worklers Rogers (1971) and Mahoney *et al.* (1973b) in Australia, James *et al.* (1985) in Venezuela and Jongejan *et al.* (1988) in Zambia reported the absence of clinical babesiosis in areas where *B. bigemina* occurs and

suggested that the strain involved may be of low pathogenicity and was not causing clinical disease. On the other hand, Norval (1979) found that outbreaks of babesiosis caused by *B. bigemina* occurred in the second rainy season following the collapse of dipping in Zimbabwe, probably because the build-up of ticks and infection rates in the ticks takes about two years to reach levels high enough to cause transmission and outbreaks of disease.

5.3 Vlakplaas ranch

5.3.1 *Babesia bigemina*

According to the definition of endemic stability by Mahoney and Ross (1972) and Norval *et al.* (1983), *B. bigemina* at Vlakplaas was endemically stable in the 10, 17, and 20-month-old cattle and breeding cows. The seven and eight-month-old calves were still in the endemically unstable range, however. As there have been no major climatic changes in recent years in the area and the tick-control methods have remained unchanged since the inception of ranching in 1987, it is assumed that the seven and eight-month-old calves will achieve endemic stability to *B. bigemina* by nine months of age. This will hopefully occur before the non-specific innate resistance wanes, in line with the calves born during October 1999.

The tick-control method at Vlakplaas was probably the main reason for the establishment and maintenance of endemic stability to *B. bigemina* on this ranch. One-host ticks such as *B. decoloratus* could only be effectively controlled when

cattle are dipped every 21 days (Sutherst and Tatchell, 1980). Monthly or bi-monthly dipping of the cattle, as was done at Vlakplaas, did not result in a severe reduction in the tick population. Treatment of animals with pour-ons also did not prevent the transmission of babesiosis in an endemic area (Aguirre *et al*., 1993). The tick control at Vlakplaas was not aggressive enough to reduce the vector tick population to a level which would disrupt the establishment and maintenance of endemic stability to *B. bigemina*. Similar scenarios have been recorded in South Africa where De Vos and Potgieter (1983) reported that with poor tick control *B. bigemina* was in an endemically stable situation. Ardington (1982) found that the maintenance of endemic stability to *B. bigemina* failed when strategic dipping allowed only light *B. decoloratus* infestations on the cattle.

The percentage of cows seropositive to *B. bigemina* was lower than that in the 10 and 17-month-old cattle but higher than in the 20-month-old ones. Mahoney (1969) reported that in an endemically stable herd in Australia, the age incidence of *B. bigemina* parasitaemia rose from zero at birth, attained a maximum between six months and two years and then declined sharply in the older animals. The overall trend of the IFA reactivity of cattle at Vlakplaas appears to be similar to Mahoney;s findings. However, re-sampling after 20 months of age may be necessary to establish the trend in the serological status of cattle at this age.

Mahoney (1962, cited by Curnow, 1973a) reported that the incidence of *Babesia* parasitaemias was higher in calves when compared to cattle of two to three years

old. He suggested that the decline in the incidence of parasitaemia with age was probably due to a continued superinfection leading to a build-up in immunity. The higher IFA positive percentage in the younger animals in this project may be linked to the fact that by two years of age the cattle had been exposed to almost all strains of the parasite on the farm through superinfections and antigenic variation (Doyle, 1977; Ross and Mahoney, 1974) allowing little chance for the establishment of a parasitaemia thereafter.

5.3.2 *Babesia bovis*

All animals studied at Vlakplaas tested negative to *B. bovis*. As the parasite and its vector, *B. microplus*, had not previously been identified in the area, it was concluded that *B. bovis* was absent from the ranch.

5.3.3 Clinical babesiosis

The owner reported 10 cases of clinical babesiosis during the period from December 1999 to February 2000 and these mostly occurred during the high tick season. In Zimbabwe, Norval *et al.* (1983) found that babesiosis was not an important cattle disease in areas where more than 80 % of the animals were serologically positive. In endemically stable situations the inoculation rate ranges from 0.005 to 0.05 (Mahoney and Ross, 1972), which corresponds to 75 % to 100 % infection rate, respectively (Friedhoff and Smith, 1981). At an inoculation rate of about 0.005, approximately 75 % of the herd became infected by nine months of

age and 85 % by one year, bus as the inoculation rate approached 0.05, so all the animals became infected by the age of one year (Friedhoff and Smith, 1981).

When the inoculation rate is in the endemic stability range, then only a small proportion of the herd become infected at an age when clinically severe babesiosis could occur and outbreaks would be unlikely (Friedhoff and Smith, 1981). However, in areas where the disease is in an endemically stable situation, sporadic cases of babesiosis can still occur when animals escape infection before nine months of age and become infected at an older age (Friedhoff and Smith, 1981).

In general, clinical babesioses due to *B. bigemina* would not be expected to be common at Vlakplaas, where the prevalence of antibodies to the parasite in the older cattle was high. A few cases may occur, however.

There was no evidence to support the acquisition of new strains of parasites either through the introduction of new cattle or by contact with animals from neighboring properties. Strain differences were also unlikely to cause clinical disease within a closed herd, and one infection transmitted by ticks during calfhood should confer protection from babesiosis for several years on the property of origin, as well as against some of the strains introduced from other localities (Mahoney and Ross, 1972). Furthermore, Rogers (1971) and Mahoney *et al.* (1973b) in Australia,

James *et al.* (1985) in Venezuela and Jongejan *et al.* (1988) in Zambia reported that *B. bigemina* usually caused only mild disease.

The most likely reason for the occurrence of clinical babesiosis on the ranch was therefore the possibility that animals which had escaped infection before nine months became infected later. The possibility of misdiagnosis of some clinical cases by the farmer should also not be ruled out.

5.4 Comparison of the ranches

In general, the cattle at Vlakplaas had a higher prevalence of antibodies to *B. bigemina* when compared with those at Nooitgedacht. The difference was probably be due to variations in the tick populations. Vlakplaas ranch had been operating uninterruptedly for 14 years and has adopted a tick-control scheme which permitted sufficient ticks on the ranch for the establishment and maintenance of endemic stability to *B. bigemina*.

On the other hand, Nooitgedacht ranch had been established for only two years on land where livestock ranching had been interrupted for three years. Hence, tick numbers may already have been dramatically reduced over the previous years due to a lack of suitable hosts. Since the resumption of ranching activities, the stocking rate on the ranch was low. The tick-resistance quality of the Brahman cattle on the ranch would also have contributed to limiting the rate at which tick populations would build up on the ranch (Frisch and Neill, 1998; Payne and

Osorio, 1990). Therefore, even though tick control at Nooitgedacht was erratic, it may take some time before tick populations build up to the level where they would effect frequent transmission of *B. bigemina* and hence endemic stability.

The lower prevalence of antibodies to *B. bigemina* at Nooitgedacht was probably due to the loss of IFA titres brought on by vaccination and natural infections, in the absence of superinfections due to the low vector tick populations on the ranch. Similar results have been recorded in previous studies (Callow, 1967; Callow *et al.*, 1974b; Mahoney *et al.*, 1973b; Johnston *et al.*, 1978; De Vos, 1979).

The difference in *B. bigemina* seroprevalence in the cattle on both ranches, however, was not necessarily paralleled by immunological differences, as immune animals could lose IFA-reacting antibody titres in the absence of superinfections (Callow, 1967; Callow *et al.*, 1974b; Mahoney *et al.*, 1973b; Johnston *et al.*, 1978; De Vos, 1979). This was partly manifested by the absence of clinical bovine babesiosis at Nooitgedacht and also made it difficult to make conclusive statements on the immunological differences between cattle from both ranches, on the basis of IFA reactivity alone.

Only those animals vaccinated against *B. bovis* at Nooitgedacht were positive to *B. bovis* and all animals at Vlakplaas were negative. It was concluded, therefore, that *B. bovis* was absent from both ranches.

CHAPTER SIX

6. CONCLUSION

It was concluded that *Babesia bovis* was absent from both ranches while *Babesia bigemina* was more prevalent in cattle at Vlakplaas than at Nooitgedacht. The difference was probably due to the variation in the number of ticks on the two ranches. Vlakplaas probably had sufficient ticks to effect frequent transmission of the parasite and, as a consequence, the percentage of IFA positive animals was higher. The tick population at Nooitgedacht was probably not large enough to maintain superinfections and many animals were not IFA positive due to the loss of IFA titres. However, the IFA-based serological status differences on the ranches could not be taken as immunological differences, as immune animals may also lose IFA-reacting antibody titres. It was therefore very difficult to deduce the immunological status differences of cattle on both ranches, solely on the basis of IFA reactivity.

At Nooitgedacht, vaccination against *B. bovis* was done without first studying the distribution of the parasite in the area and its potential risk. As Nooitgedacht is located in a *B. bigemina*-endemic and *B. bovis*-free area, vaccinating against *B. bovis* was unnecessary.

When ranching was resumed at Nooitgedacht, the tick population was probably quite low due to the lack of hosts over the previous years. One of the consequences of this was that the first calf crop (born during October 1999) would

have escaped infection with *B. bigemina* during the resistance period. The decision to vaccinate this calf crop against *B. bigemina* was therefore an appropriate one.

Our results indicate that an endemically stable situation with *B. bigemina* could be achieved by adopting a tick-control method that allows sufficient ticks on cattle for adequate transmission of the parasite, rather than relying on intensive tick-control and vaccination. It may not therefore be necessary to vaccinate calves against *B. bigemina* on ranches located in *B. bigemina*-endemic areas stocked with *Bos indicus* cattle or their crosses, provded that relaxed tick control is applied.

Managing *Boophilus* spp numbers for the maintenance of endemic stability to bovine babesiosis is not an easy task. In tropical and subtropical Africa, where the study area was situated, ticks other than *Boophilus* spp are potentially very important. Attempts to maintain endemic stability for babesiosis may be negated by the need to control other ticks such as *Amblyomma* spp.

Overall, two options are open to farmers for the management of ticks and tick-borne diseases in the study area: the establishment of either a disease-free situation or an endemically stable disease situation. The disease-free sicenario is a risky and costly operation. Tick eradication results in populations of cattle fully susceptible to tick-borne diseases. It is not recommended for where an extensive

farming system, especially those located in areas where tick-borne diseases are endemic.

An endemically stable disease situation implies living with ticks and tick-borne diseases by adopting a tick-control method that allows the existence of sufficient ticks for frequent transmission of tick-borne pathogens and the maintenance of endemically stable situations to tick-borne diseases.

From the present study, it appears that the principles of endemic stability for the control of tick-borne diseases are gaining popularity among farmers in South Africa. The key issue in the establishment and maintenance of endemic stability to tick-borne diseases, however, is the selection of the tick-control methods to be used.

Estimates of the minimum numbers of *Boophilus* ticks needed to maintain endemic stability to tick-borne diseases, without causing reduction of weight gains in the host, have been made using computer simulations in *Bos taurus* cattle infested with *B. microplus* infected with *B. bovis*. Such estimates are not available for *Bos indicus* cattle and their crosses infested with a range of one-, two- and three-host tick species infected with various tick-borne pathogens. The information would be important to assist in practical field control of ticks and tick-borne diseases in *Bos indicus* cattle and their crosses.

It is therefore suggested that a study be undertaken to accumulate data on the economic threshold of tick infestations in *Bos indicus* cattle and their crosses in tick-borne disease endemic areas of southern Africa. This would be done in order to recommend a tick-control strategy to assist in maintaining endemic stability to tick-borne diseases without causing reduced productivity in the cattle.

Until such information is available it is recommended that an erratic tick control method, which favours the existence of a reasonable number of ticks sufficient for frequent transmission of *B. bigemina* and causes the establishment and maintenance of endemic stability to the parasite be instituted. This policy would be preferable to relying on intensive tick-control and vaccination, especially on ranches located in tick-borne disease endemic areas of Africa which are stocked with *Bos indicus* cattle or their crosses.

At both ranches, wildlife shared grazing land with cattle. The interaction between wildlife and cattle on ranches in southern Africa is very common. it is therefore imperative that the role of wildlife in the epidemiology of bovine babesiosis should be researched.

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