THE IMPACT OF TWO DIPPING SYSTEMS ON ENDEMIC STABILITY OF BOVINE BABESIOSIS AND ANAPLASMOSIS IN CATTLE AT FOUR COMMUNAL GRAZING AREAS IN LIMPOPO PROVINCE, SOUTH AFRICA

BY

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A twelve-month study was conducted at four communal grazing areas namely, Oakley, Cunningmore, Mkhuhlu and Ronaldsy in the Bushbuckridge region, Limpopo Province, South Africa. The main objective of the study was to investigate the impact of reduced acaricide application on the endemic stability to bovine babesiosis (*Babesia bigemina* and *Babesia bovis*) and anaplasmosis in a sample of the local cattle population. The study should be of assistance to farmers who are attempting to move from intensive to strategic tick control strategies and reduce the frequency of dipping, whilst maintaining endemic stability.

Sixty cattle per communal grazing area were bled at the beginning and the end of the experimental period and the sera were assayed for *B. bovis, B. bigemina* and *Anaplasma* antibodies. Cattle in the intensively dipped group were dipped 26 times and maintained on a fourteen-day dipping interval throughout the study, whereas, cattle in the strategic group had their acaricide application frequency reduced and were only dipped 13 times. Three cattle per village were selected from which adult ticks were collected and immature ticks were also collected by dragging the veld. A questionnaire to assess the prevalence of clinical cases of tick-borne diseases, abscessation and mortalities was completed by an Animal Health Technician at each diptank during dipping. This was done to determine the number of clinical cases of bovine babesiosis, anaplasmosis as well as abscessation.

An increase in seroprevalence to *B. bovis* and *B. bigemina* and a decrease in seroprevalence to *Anaplasma* was detected in the strategically dipped group whilst in the intensively dipped group a decrease in seroprevalence to *B. bovis* and *B. bigemina* and an increase in seroprevalence to *Anaplasma* was detected. *Amblyomma hebraeum* was the most abundant tick species found on the cattle in this region, whilst *Rhipicephalus (Boophilus) microplus* and *Rhipicephalus (Boophilus) decoloratus* were also collected and *R. (B.) microplus* was the more abundant of the two species. Drag samples yielded more *A. hebraeum* immatures than *Rhipicephalus (Boophilus*) and a seasonal pattern was displayed. An increase in the number of clinical cases of tick-borne diseases and abscesses was recorded at the beginning of the survey in the strategically dipped group.

SAMEVATTING

‘n Twaalf-maande studie is by vier gemeenskaplike weidingsgebiede, naamlik Oakley, Cunningmore, Mkhuhlu en Ronaldsy in die Bosbokrand-gebied, Limpopo Provinsie, Suid-Afrika, gedoen. Die hoofdoel van die studie was om die inwerking van verminderde bosluisdoderaanwendings op die endemiese stabiliteit van babesiose (*Babesia bigemina* en *Babesia bovis*) en anaplasmose in ‘n proefgroep uit die plaaslike beesbevolking te bepaal. Die studie behoort van nut te wees vir boere wat poog om van intensiewe na strategiese bosluisbeheer te beweeg en die dipintervalle te verleng terwyl endemiese stabiliteit gehandhaaf word.

Sestig beeste per gemeenskaplike weidingsgebied is aan die begin en aan die einde van die proeftydperk gebloei en die sera is vir teenliggame teen *B. bovis*, *B. bigemina* en *Anaplasma* getoets. Beeste in die intensief-gedipte groep is 26 keer gedip en op ‘n veertien-dag dipinterval gehandhaaf vir die duur van die proef, terwyl die dipintervalle van die beeste in die strategies-gedipte groep verleng is en is hulle net 13 keer gedip. Drie beeste per stat is gekies om volwasse bosluise vanaf te versamel en onvolwasse bosluise is ook versamel deur middel van slepe in die veld. ‘n Vraelys om die voorkoms van kliniese gevalle van bosluisoorgedraagde siektes, absesvorming en vrektes te beraam, is deur ‘n dieregesondheidstegnikus by elke diptenk tydens elke dipgeleentheid voltooi. Hierdie is gedoen om die aantal kliniese gevalle van beesbabesiose en anaplasmose, asook absesvorming te bepaal.

In die strategies-gedipte groep is ‘n toename in die seroprevalensie van *B. bovis* en *B. bigemina* en ‘n afname in die seroprevalensie van *Anaplasma* waargeneem, terwyl in

die intensief-gedipte groep ‘n afname in die die seroprevalensie van *B. bovis* en *B. bigemina* en ‘n toename in die seroprevalensie van *Anaplasma* waargeneem is. *Amblyomma hebraeum* was die volopste bosluisspesie wat op beeste in hierdie gebied gevind is, terwyl *Rhipicephalus* (*Boophilus*) *microplus* en *Rhipicephalus* (*Boophilus*) *decoloratus* ook versamel is en *Rhipicephalus* (*Boophilus*) *microplus* die volopste van hierdie twee spesies was. Versamelings van veldslepe het meer *A. hebraeum* as *Rhipicephalus* (*Boophilus*) onvolwassenes opgelewer en het ‘n seisoenale patroon gevolg. ‘n Toename in die aantal kliniese gevalle van bosluisoorgedraagde siektes en absesse is aan die begin van die opname in die strategies-gedipte groep aangeteken.

Abbreviations used.

AHT	Animal Health Technician
BBR	Bushbuckridge
CGA	Communal grazing areas
CI	Competition Inhibition
Fem	Female
Imm	Immature
Mal	Male
OVI	Onderstepoort Veterinary Institute
TBD	Tick-borne Diseases

CHAPTER 1

INTRODUCTION

Tick infestations and the diseases transmitted by ticks occur widely in Africa and are a major problem for farmers in the tropical and subtropical areas of the world (Dipeolu, Mongi, Punyua, Latif, Amoo & Adhiambo 1992; Masika, Sonandi & Van Averbeke, 1997). Ticks and tick-borne diseases (TBD) cause important economic losses to cattle owners in communally grazed areas of southern Africa with high mortalities due to TBD, tick worry and tick abscessation (Norval 1981; Norval, Perry & Hargreaves 1992).

Two economically important TBD of cattle occur in the study area, namely bovine babesiosis caused by *Babesia bovis* and *Babesia bigemina* and bovine anaplasmosis which is predominantly caused by *Anaplasma marginale*. *B. bovis* is transmitted by *Rhipicephalus (Boophilus) microplus* which is its only known vector in southern Africa (Riek 1966; Potgieter 1977). Single *B. bovis* organisms are round, oval or irregular in shape whilst paired forms are piriform or club-shaped and easily visible on a blood smear (De Vos & Potgieter 1983). *B. bigemina* is transmitted by *Rhipicephalus (Boophilus) decoloratus* and *R. (B.) microplus* (Potgieter 1977; Norval, Fivaz, Lawrence & Brown 1983) and to a much lesser extent by *R. evertsi evertsi* (Buscher 1988). *B. bigemina,* the large *Babesia*, has single forms which are elongated or amoeboid in shape, whilst paired forms are piriform with an acute angle between the merozoites (De Vos & Potgieter 1983). The main clinical signs of bovine babesiosis are fever, inappetence, weakness, reluctance to move, anaemia, icterus, nervous signs (*B. bovis*) and haemoglobinuria.

A. marginale is mainly transmitted by *R. (B.) decoloratus* and *R.(B.) microplus*, and to a lesser extent by *Hyalomma marginatum rufipes, Rhipicephalus evertsi evertsi* and *Rhipicephalus simus*. Mechanical transmission by biting flies also occurs. On a Giemsa stained blood smear *A. marginale* organisms are commonly seen to be on the margins of the erythrocytes (De Vos & Potgieter 1983). Clinical signs of acute and chronic bovine anaplasmosis are fever, anaemia, icterus, rumen stasis, constipation and nervous signs.

Livestock production in southern Africa is heavily dependent on improved animal health and this entails good tick and TBD control systems (Van Rensburg 1981). Many commercial and some rural subsistence farmers use regular short-interval dipping to keep their cattle tick-free. The intensive dipping policies were first instituted during the East Coast fever era and this has lead to endemic instability to many of the TBD (Norval *et al*. 1992). More recently there has been a shift towards strategic and threshold tick control with less frequent application of acaricides during periods of low tick abundance and more frequent applications during the critical times of the year to avoid the damaging effects of the adult ticks (Fivaz & De Waal 1993; Norval 1983; Tatchell, Chimwari, Chirchir, Ongari, Mwangi, Rinkanya, & Whittington 1986).

The primary objective of the present study was to compare intensive dipping and strategic tick control in this region and to implement alternative ways of maintaining endemic stability to TBD without getting a significant increase in the incidence of tick damage and TBD.

CHAPTER 2

LITERATURE REVIEW

2.1 **Historical Preamble**

TBD and the damage associated with tick bites are often major constraints to livestock production in Africa, especially in communal farming areas (Norval, Barret, Perry & Mukhebi 1992). Ticks also cause direct tick worry which results in significant losses in livestock production (McCosker 1979; Norval *et al.* 1992). Intensive tick control has been an integral part of the disease control strategy of the Department of Veterinary Services in Limpopo Province for almost a century. Intensive tick control policies have recently become more difficult to implement because of the rising costs of acaricides as well as the maintenance of the personnel and the dipping infrastructure (Norval *et al.* 1992).

Despite the large financial investment in tick and TBD control strategies the problem has not been overcome. This is due mainly to a variety of factors, such as the introduction of susceptible taurine breeds and the development of acaricide resistance in tick populations, which has necessitated the use of progressively more expensive acaricides (Norval *et al.* 1992). If previous trends in tick and TBD control strategies are continued, then it is likely that dipping as is currently practiced would become less cost-effective and eventually unsustainable (Norval *et al.* 1992).

2.2 **Re-appraisal of present tick control strategies**

There is a need to re-appraise veterinary strategies with respect to the control of ticks and TBD not only because of the development of acaricide resistance in ticks, but also because of the financial constraints on government-funded control programmes as the farmers frequently bear the cost themselves (Norval *et al.* 1992).

2.2.1 **Development of acaricide resistance in ticks**

Tick resistance is defined as the development of an ability in a strain of ticks to tolerate doses of toxicants which would normally prove lethal to the majority of individuals in a normal population of the same species (World Health Organization 1957). Ticks have pre-existing resistance genes which are present at low levels in their populations, hence, this ability can also be inherited through the selective effect of the acaricides (Brown 1976; Sutherst & Comins 1979; Nolan 1990). Tick resistance has a genetic basis and mutation and amplification of the dominant genes result in quick development of resistance (Tellier, Steffan & Buhlmann 1991). Acaricides do not kill all the ticks on the host and those which survive may develop resistance and this may be enhanced by complete elimination of the susceptible ticks by excessive use of acaricides (Sutherst & Comins 1979).

Numerous studies have confirmed the development of acaricide resistance (Thompson & Bryson 1972; Baker 1982; Solomon 1983). Tick resistance to all the main groups of ectoparasiticides was firstly recorded against arsenic in South Africa (Du Toit, Graf & Bekker 1941), and subsequently to chlorinated hydrocarbons (Whitnall, Thornburn, Mchardy, Whitehead & Meerholz 1952; Whitehead 1956), cyclodienes (Fiedler 1952), organophosphates (Whitnall *et al.* 1952), amidines (Taylor & Oberem 1995)

and the synthetic pyrethroids (Coetzee, Stanford & Davies 1987). It has been shown that resistance to one member of a group of acaricides commonly implies resistance to others (Baker, Jordaan & Robertson 1979,1981). Roulston (1980) confirmed that ticks resistant to gamma benzene hexachloride (gamma BHC), were also resistant to toxaphene, dieldrin and aldrin.

2.2.2 **Constraints to government veterinary programmes**

In the study area (BBR), farmers had previously been dependent on the Department of Veterinary Services in the Limpopo Province to provide free acaricide, maintain diptanks and to supply labour and diptank personnel. In other countries such as in South America, funding for compulsory dipping was given as part of a national tick eradication campaign (Nari 1995; Vaughan 1998). In Australia, government funding was used to maintain a strategic barrier to the southward expansion of a tick infested area (Norval *et al*. 1992; Norton, Sutherst & Maywald 1983).

2.2.3 **Financial burden to the farmers**

In extensive beef production systems, especially on the commercial ranches in southern Africa, gathering cattle for dipping has been labour-intensive and costly (Norval *et al.* 1992). Many commercial beef farmers would have liked to have moved away from intensive dipping but are not convinced that the alternative control strategies are cost-effective and do not carry a high risk of TBD outbreaks (Onen-Okello, Mukhebi,Tukahirwa, Musisi, Bode, Heinonen, Perry & Opuda-Asibo 1998). These beef farmers also suffer production losses due to stress, abortions, drowning and physical injuries incurred during dipping and in addition, they also lose a day's animal traction and labour when dipping occurs (De Castro, James, Minjauw, Di Giulio, Permin, Pegram, Chizyuka & Sinyangwe 1997).

2.3 **Alternative control strategies**

There are several important reasons to re-appraise the present day tick control strategies in Africa, most of which emphasize the maintenance of endemic stability, the use of vaccines against TBD and the use of tick resistant cattle.

2.3.1 **Endemic stability to TBD**

Where the vector density is high, host infection with TBD is common and usually occurs early in the host's life resulting in reduced host morbidity and mortality to TBD (Norval *et al.* 1992; Perry & Young 1995). A sustainable ecological relationship between the host, the vector and the environment and an endemically stable disease situation also exists between the TBD, the vector challenge and the agro-economical sustainability of the TBD (Perry & Young 1995). Endemic stability to heartwater, anaplasmosis and bovine babesiosis is common in endemic areas in Africa (De Vos 1979; De Vos & Every 1981; Howell, De Vos, Bezuidenhout, Potgieter & Barrowman 1981; Norval, Fivaz, Lawrence & Brown 1983; Bezuidenhout & Bigalke 1987). Infestation with *R. (B.) decoloratus* usually indicates endemic stability to *Babesia bigemina* (De Vos & Every 1981; De Vos & Potgieter 1983; Norval *et al.* 1983) which is a desirable situation as it reduces the risk of losses to the parasite (De Vos & Every 1981). Intensive dipping interferes with the development of endemic stability to TBD (Cook 1991). A study conducted in South Africa showed that different cattle populations could pass from endemic instability and low prevalence of seropositivity to endemic stability and high prevalence of seropositivity without clinical disease outbreaks (Tice, Bryson, Stewart, Du Plessis & De Waal 1998). There are now many proponents of the view that it is better to aim for endemic stability to TBD in

communal areas in Africa because it is the more sustainable option (Norval 1981; Norval *et al*.1983; Norval *et al.* 1992).

2.3.2 **Availability of vaccines against TBD and ticks**

Vaccines against TBD

Blood-based vaccines for bovine babesiosis and anaplasmosis using attenuated or less pathogenic organisms have been available for many years (Callow & Dagliesh 1979). Blood from cattle which had recovered from babesiosis was used as a vaccine in the early years of vaccine development. This is no longer used due to its high failure rate (De Vos & Potgieter 1983; Bok & De Vos 2001) and was superceded by a chilled (unfrozen) babesiosis vaccine made from blood obtained from splenectomized cattle infected with *B. bovis* and *B. bigemina.* An isolate of *A. centrale* is used to produce a chilled live-blood anaplasmosis vaccine (De Vos & Potgieter 1983). The chilled babesiosis and anaplasmosis vaccines are relatively easy to transport, however they have a short shelf life and therefore need to be used soon after being produced (De Vos & Potgieter 1983).

Frozen vaccines first used in Australia and Israel, have been introduced into Africa. Although the same vaccine strains are used in the vaccines, they are cheaper to produce and have the advantage of a long shelf life (Bok & de Vos 2001). However, they have to be stored in liquid nitrogen. Both chilled and frozen vaccines are mainly used in calves because vaccine reactions in this age group are minimal although severe vaccine reactions may occur in older animals. A single vaccination should generally provide long-lasting protection

(Bok & de Vos 2001; Rogers & Shiels 1979), however, field usage in Africa has so far shown variable results (Bok & de Vos 2001). Research today is focused on developing a safe, inactivated bovine babesiosis vaccine (Bok & De Vos 2001).

Vaccines against ticks

A recombinant DNA vaccine against the one host tick *Rhipicephalus (Boophilus) microplus* has been developed (Dayton 1991). It targets the gut cells of the tick by destroying the digestive tract of the tick. The vaccine gives a 90% reduction in the weight and the egg production capacity of the tick. Vaccination with recombinant *R. (B.) microplus* gut antigens have been shown to control tick infestations in South America but more field work needs to be conducted before commercial implementation is possible (Fuente, Rodriguez & Garcia-Garcia 2000).

2.3.3. **Use of tick resistant cattle**

Indigenous African cattle (*Bos indicus*) are known to be more resistant to ticks than European breeds (Spickett, De Klerk, Enslin & Scholtz, 1989; Rechav & Kostrzewski 1991; Miller, Diall, Craig & Wagner 1984). This is attributed to both innate resistance (Bourne, Sutherst, Sutherland, Maywald & Stegeman 1998; Spickett *et al*. 1989; Fivaz, De Waal & Lander 1992; Bok, Kingston & De Vos 1999) and genetic resistance (Bok *et al*. 1999) which *Bos indicus* breeds develop to ticks and TBD. Tick resistant cattle are able to attain a state of endemic stability to most TBD without any tick control (Norval *et al.* 1983). Endemic stability is maintained by continuous exposure of the cattle to infected ticks, and calves become infected and they take

advantage of an age-related resistance or colostral immunity which minimizes the effects of the TBD (Norval *et al*. 1992).

2.4 **Future options for tick control**

In southern Africa compulsory dipping was seen as a way to eradicate ticks but today it is clear that it is virtually impossible to achieve this by intensive dipping alone (Howell *et al.* 1981; Dipeolu *et al.* 1992; Norval 1983). In future, communally grazed indigenous cattle will probably require minimal tick control, ranging from total absence of control during the dry season to strategic and threshold control during the wet seasons (Matthewson1984). Strategic tick control means the application of acaricide only at critical times of the year in order to minimize the damaging effects of adult ticks (Norval, Sutherst, Kurki, Gibson & Kerr 1988). Threshold tick control is the application of acaricides only when the number of ticks (tick challenge) per individual host exceeds a predetermined economic threshold (Cook 1991), and is used mainly in cross-bred cattle (Nari 1995).

Tick parasitoids could also play an important role in the control of ticks. A study in Kenya showed how tick parasitoids such as tick predators (oxpeckers) and tick parasites (nematodes) could be strategically used to manage ticks on subsistence farms (Mwangi, Hassan, Kaaya & Essuman 1997). The tick parasitoid *Ixodiphagus hookeri* parasitizes several *Ixodid* tick species such as *Ixodes scupularis, Ixodes dammini* and *Dermacentor variabilis* in the United States of America (Hu, Hyland & Mather 1993). The Kenyan strain of *I. hookeri* seems to parasitize only *A. variegatum* even in the presence of other tick species such as *Rhipicephalus appendiculatus* and *Amblyomma cohaerens* (Mwangi, Hassan, Kaaya & Essuman 1997; Mwangi, Newson

& Kaaya 1993). The parasitoid is attracted by the chemical extracts from engorged nymphs or adult ticks (Takasu, Takano, Sasaki, Yagi & Nakamura 2002). Their potential for tick management in Africa has not been extensively evaluated even though it was reported as early as the beginning of the 20th century (Wood 1911). More research on the capability of these parasitoids is needed to evaluate their sustainability (Mwangi, Hassan, Kaaya & Essuman 1997).

2.5 **Seasonal activity of the various ticks**

Most economically important tick species display a clear seasonal pattern of activity which would support the idea of strategic control. (Horak 1982; Baker, Ducasse, Sutherst & Maywald 1989; Horak 1999). Baker *et al.* (1989) also found that the seasonal peaks of immature R. (*B.) microplus*, *A. hebraeum, R. (B.) decoloratus* and *R. appendiculatus* were in spring and autumn and the adults in summer. Baker *et al.* (1989) indicated that *A. hebraeum* may complete its life cycle in one year and this was supported by other field studies in the Eastern Cape (Rechav 1982). Spickett & Fivaz (1992) found that *R. appendiculatus* R. (*B.) decoloratus* and *Hyalomma* ticks showed distinct seasonal abundance, with immatures peaking in spring and adults in summer. The above studies strongly suggest that dipping during peak tick activity should control the prevalent tick species in this region (Dreyer, Fourie & Kok 1998).

2.6 **Serological tests for TBD**

There are a number of serological diagnostic screening tests which are commonly used in South Africa to detect TBD infection, including the Indirect Fluorescent Antibody Test (IFAT) which was first used to distinguish *B. bovis* and *B. bigemina* (Joyner, Donnelly, Payne & Brocklesby 1972). The IFAT is the standard test used to

detect antibodies to *Babesia* in the sera of cattle in South Africa and it has good sensitivity and specificity and can reliably detect seropositive cattle (Morzaria, Brocklesby, Harradine 1977; Burridge & Kimber 1972; Goncalves, Passos & Ribeirio 1999). Sera were also screened for antibodies to *Anaplasma* using the Competition Inhibition (CI) ELISA test. This test uses a recombinant *E. coli* which expresses an immunogenic major surface protein 5 (MSP5) of *Anaplasma marginale* as an antigen, and has a specificity of 94% and sensitivity of 99%. (Visser, McGuire, Palmer, Davis, Skhap, Pipano & Knowles 1992; Ndungu, Aguirre, Rurangirwa, McElwain, Mcguire, Knowles & Palmer (1995)).

CHAPTER 3

MATERIALS AND METHODS

3.1 **Study Area**

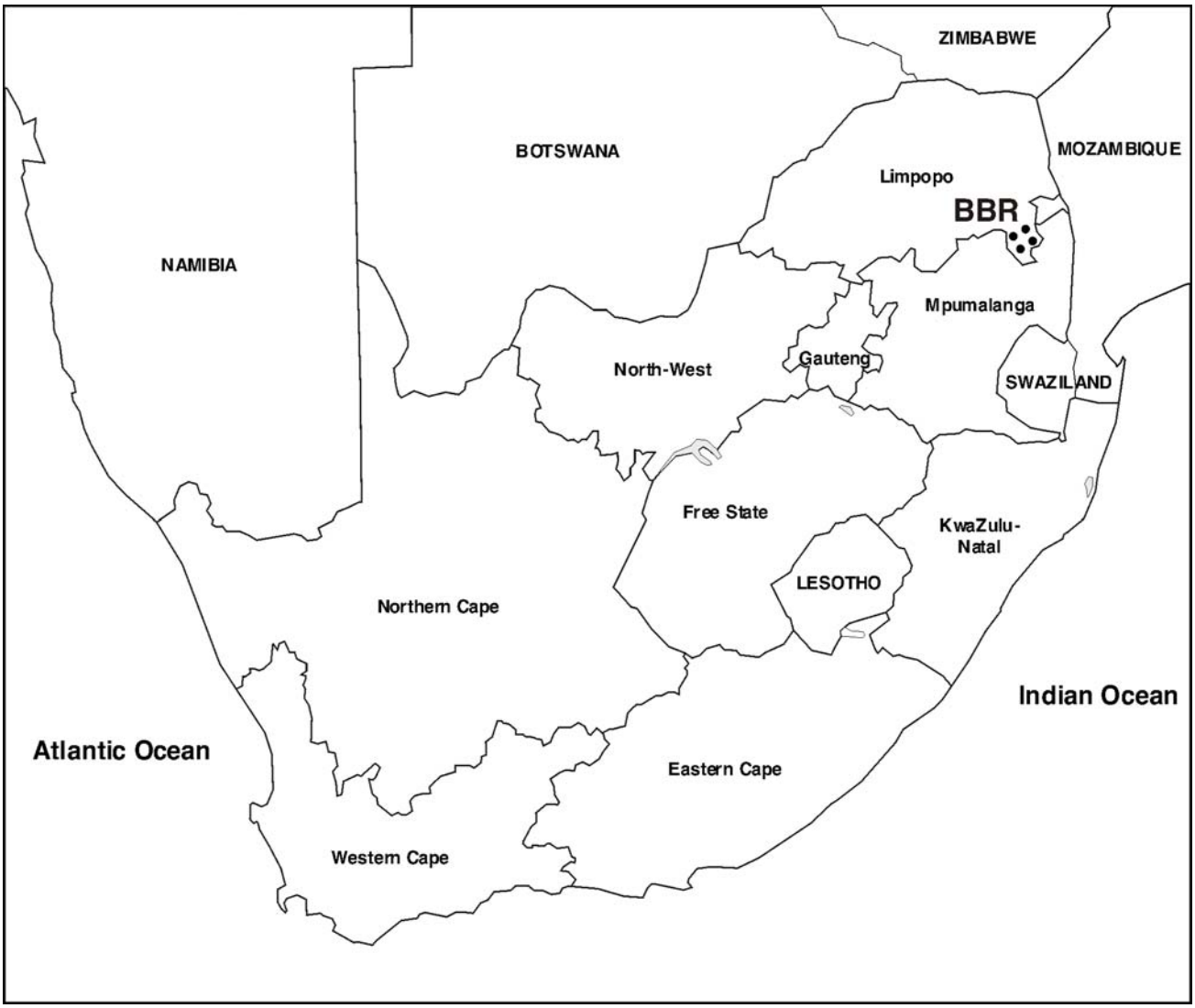


Fig1: Map of South Africa illustrating the location of the four study sites **BBR** ● ● ● ●

The study was conducted at BBR, Limpopo Province, South Africa at four CGA namely: Oakley (31°15′s to 24° 58′ E), Cunningmore (31°16′S to 24°56′ E), Mkhuhlu (31°16′S to 25°00′E) and Ronaldsy (31°18′ S to 24°55′E) (Fig.1). Fences separated

the farms but all four communal grazing areas (CGA) were located in one ward with similar vegetation and climatological and ecological conditions. This is a summer rainfall area with a high tick challenge during the summer months (November to February) and grazing is on natural sourveld (Acocks 1988).

3.2 **Experimental animals**

Two hundred and forty (n=240) predominantly Nguni cattle, aged between six months to fully grown adults were selected from the four CGA. One hundred and sixty of the cattle were adults (n=160) older than two years and eighty were calves (n=80) less than one year old. All the cattle/calves in the study area had been on a 14 day dipping interval prior to the start of the experiment.

3.3 **Tick counts**

All cattle/calves were examined for ticks and tick collection from three animals (1 calf and 2 adult cattle) occurred once a month prior to dipping.

3.4 **Serological testing**

Two hundred and forty cattle/calves (n=240) were bled from the caudal vein at the beginning of the study and two hundred and forty (n=240) at the end of the study period and the sera were assayed for *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* antibodies using the IFAT as described by Joyner *et al.* (1972); Morzaria *et al.* (1977); Burridge & Kimber (1972) for *Babesia* and the CI- ELISA as described by Visser *et al.* (1992); Ndungu *et al.* (1995) for *Anaplasma*.

Cattle/calves were selected for sampling by a random sampling technique (Thrusfield 1995). The four diptanks in the study region were the primary sampling units and the individual cattle/calves were the secondary sampling units (Thrusfield 1995). The prevalence of bovine babesiosis and anaplasmosis in the study area was not known, hence an estimation of 25% prevalence with a 95% confidence level was made. The sample size was determined by assuming that the estimated prevalence was within 5% of the true level and the formula n=4PQ ÷ by L squared, where Q= 1-P and L is the allowable error or required precision was used (n=4∗0,25∗0,75÷0,0036=208. 208÷4= 52 cattle) (Thrusfield 1995).

3.5 **Experimental procedures**

The two groups of the sample population of cattle/calves were run in separate but similar grazing camps from April 2002 to March 2003.

3.5.1 Serum collection: 240 blood samples (80 samples from calves and 160 from adult cattle) were collected in April 2002 and 240 samples (80 samples from calves and 160 samples from adult cattle) were collected in March 2003 and the sera were assayed for *B. bovis, B. bigemina* and *Anaplasma*.

3.5.2 Tick collection: Three cattle (1 calf and 2 adult cattle) per village, per month were chosen for tick collection (Fivaz, De Wet & Lander 1992; Thrusfield 1995). Cattle/calves were restrained in a crushpen and adult ticks from one half of the cow/calf were collected for identification and counting. The ticks were all identified by comparing with reference specimens obtained from the OVI (Spickett, personal communication). The number of ticks collected from each host was then doubled to give an estimate of the total tick burden on each animal (Fivaz *et al.* 1992). Immature ticks were collected by

the larval dragging technique described by Rechav (1982) and 3 larval drags per month per village were done.

3.5.3 Dipping:

Intensive group: Dipping in the intensive group was continued as had been done previously, 26 dippings during the entire study period from April 2002 to March 2003 using Triatix (Amitraz, Intervet) at 14 day intervals.

Strategic group: These were also dipped in Triatix (Amitraz, Intervet), but only 13 times during the same period, which was half the dipping frequency of the intensively dipped group. (Once during the period April to May 2002, once between June and July 2002, once between August and October 2002, and twice per month from November 2002 to March 2003). The strategic group was allowed to acquire moderate to heavy tick burdens between acaricide treatments especially during peak adult tick activity

3.6 **Estimation of the tick damage and the effects of TBD in the cattle/calves**

A questionnaire was given to the relevant Animal Health Technician working at each diptank for completion during dipping. The questionnaire attempted to estimate the damage caused by the adult ticks, which included abscessation, clinical disease and any mortality in the cattle.

3.6.1 Questionnaire

A questionnaire was structured to determine if there was an increase or a decrease in the number of abscesses during 2003 as compared with the previous year (2002). It

was also used to get an indication of the number of mortalities in each ward and whether there were any changes in the tick burdens in either group during the study. It was also used to determine whether there had been an increase or a decrease in the number of clinical cases of bovine babesiosis and anaplasmosis during the study period.

3.7 **Statistical analyses**

The statistical analyses were performed at the Department of Statistics of the University of Pretoria using comparative analyses with the Chi-square test. The Chi-square test was used to test for association between the two variables. If the test gave a p-value of less than 0.05 it was concluded that there was a significant association between the two variables at a 95% confidence level. A low p-value indicated that the difference was not due to random error/chance. The tests were then performed using 2-way frequency tables. Table 1 highlights the serological data from the cattle and calves compared with the dipping system used during 2002 and 2003. The statistical analyses were performed using the SAS v 8.2 programme.

CHAPTER 4

RESULTS

The results of the study are summarized in Tables 1, 2, 3 and 4 and Figs 2, 3, 4 and 5.

4.1 **Serological results**

The results of the serological tests for *B. bovis, B. bigemina* and *Anaplasma* for both the strategic and intensive groups of cattle are shown in Table 1 and Fig 2. The percentage positive sera for *B. bovis* in the strategic group showed a significant increase in both the adult cattle and the calves ($p<0.05$) in 2003 when compared to 2002. The *B. bigemina* seroprevalence also showed a significant increase in the adult cattle ($p<0.05$) in 2003 but the increase in the calves was not significant. The seroprevalence of both *B. bovis* and *B. bigemina* in the intensive group did not change significantly from 2002 to 2003 ($p>0.05$).

4.1.1 **Serological results (Table 1)**

Table 1: **Serological results:a+c calves ,b+d adult cattle, a+b in 2002, c+d in 2003**

a: Serological results of calves (2002)

	Farm	***B.bovis***		***B. bigemina***		***Anaplasma***	
		pos	**neg**	**pos**	**neg**	**pos**	**neg**
Intensive group	Cunningmore n=20	12	8	6	14	17	3
	Mkhuhlu n=20	8	12	3	17	13	7
Strategic group	Oakley n=20	6	14	5	15	17	3
	Ronaldsy n=20	8	12	4	16	15	5

b: Serological results of adult cattle (2002)

	Farm	***B.bovis***		***B.bigemina***		***Anaplasma***	
		pos	neg	pos	neg	pos	neg
Intensive group	Cunningmore n=39	22	17	13	26	33	6
	Mkhuhlu n=40	15	25	6	34	24	16
Strategic group	Oakley n=29	10	19	6	23	24	5
	Ronaldsy n=27	10	17	7	20	21	6

c: Serological results of calves (2003)

	Farm	***B.bovis***		***B.bigemina***		***Anaplasma***	
		pos	neg	pos	neg	pos	neg
Intensive group	Cunningmore n=15	8	7	2	13	11	4
	Mkhuhlu n=15	2	13	1	14	7	8
Strategic group	Oakley n=20	13	7	8	12	11	9
	Ronaldsy n=20	14	6	7	13	15	5

d: Serological results of adult cattle (2003)

	Farm	***B. bovis***		***B. bigemina***		***Anaplasma***	
		pos	neg	pos	neg	pos	neg
Intensive group	Cunningmore n=21	12	9	4	17	16	5
	Mkhuhlu n=18	2	16	1	17	8	10
Strategic group	Oakley n=30	21	9	15	15	17	13
	Ronaldsy n=38	25	13	15	23	26	12

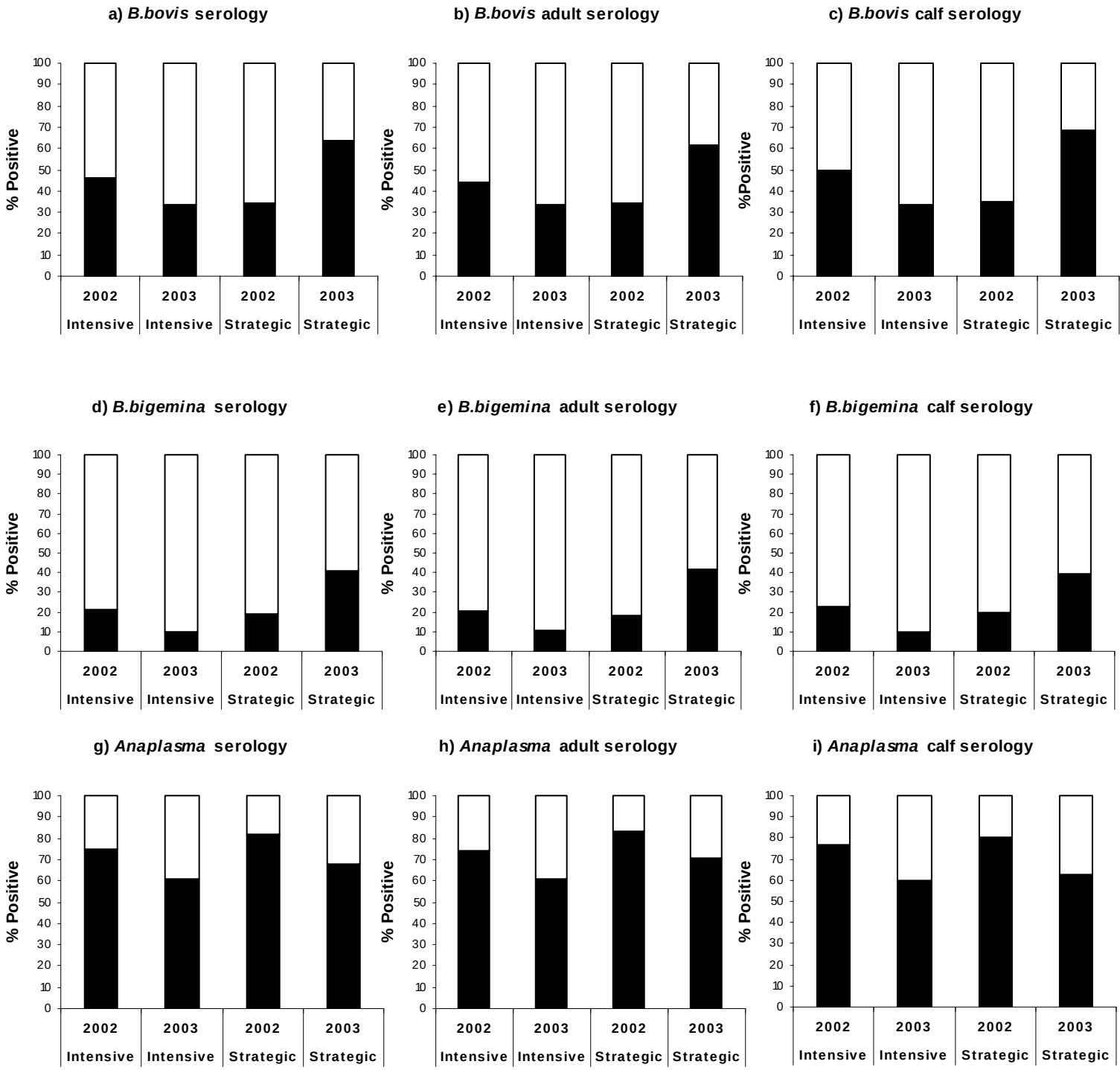


Fig 2: Comparison of the seroprevalence of *B. bovis*, *B. bigemina* and *Anaplasma* of adult cattle and calves dipped intensively and strategically between April 2002 and March 2003

a) Total *B. bovis* serology
b) *B. bovis* adult serology
c) *B. bovis* calf serology
d) Total *B. bigemina* serology
e) *B. bigemina* adult serology
f) *B. bigemina* calf serology
g) Total *Anaplasma* serology
h) *Anaplasma* adult serology
i) *Anaplasma* calf serology

4.2 Total tick counts from individual adult cattle, calves and the veld collected during the study period (April 2002 to March 2003, Table 2)

The adult and immature tick counts from individual cattle, calves and the veld in both the intensive and strategic groups were pooled and the results are presented in Tables 2 and 3 and Figures 3, 4 and 5. *A. hebraeum* was the most common tick species collected with *R. (B.) decoloratus, R. (B.) microplus, R. appendiculatus* and *Hyalomma marginatum* also collected. Adult ticks peaked in spring and summer whilst immature ticks peaked in autumn and spring (p-value of less than 0.05).

Table 2: **Total adult and immature ticks collected off the intensive (a+c) and strategic (b+d) groups of adult cattle (a+b) and calves (c+d) during 2002/2003**

a: **Intensive group adult cattle (2002 and 2003)**

Month		*Boophilus*			*Amblyomma*			*Rhipicephalus*			*Hyalomma*		
		mal	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**
Apr	2002	2	4	41	3	3	108	2	3	28	1	3	37
May	2002	2	5	71	4	3	153	1	2	53	3	1	32
Jun	2002	3	4	52	2	4	110	2	4	40	2	5	15
Jul	2002	2	2	29	3	3	98	2	3	19	2	4	17
Aug	2002	4	3	69	2	6	99	3	3	47	1	6	52
Sept	2002	2	6	125	5	1	68	4	5	115	4	3	98
Oct	2002	5	4	111	6	5	122	3	4	37	5	4	142
Nov	2002	3	2	79	4	4	141	4	3	36	3	5	65
Dec	2002	5	3	66	3	5	100	5	4	43	5	4	29
Jan	2003	4	7	82	4	6	75	5	8	98	6	5	70
Feb	2003	8	2	56	5	7	77	6	7	64	7	2	59
Mar	2003	5	6	70	4	3	92	6	5	59	6	3	62

b: **Strategic group adult cattle (2002 and 2003)**

Month		*Boophilus*			*Amblyomma*			*Rhipicephalus*			*Hyalomma*		
		mal	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**
Apr	2002	3	5	52	7	8	122	3	3	62	1	6	82
May	2002	3	4	62	6	10	109	4	2	51	3	4	56
Jun	2002	2	3	31	4	9	98	2	2	48	4	6	66
Jul	2002	3	6	43	5	8	111	1	5	23	2	3	31
Aug	2002	5	5	16	8	8	73	4	3	20	3	3	18
Sept	2002	4	3	28	11	3	88	2	6	39	4	4	34
Oct	2002	5	7	57	8	12	65	9	14	34	1	4	52
Nov	2002	4	5	121	13	9	133	4	6	94	4	6	88
Dec	2002	3	6	119	17	12	159	7	3	135	5	6	99
Jan	2003	2	6	162	15	17	188	5	6	110	7	5	107
Feb	2003	5	8	149	11	11	162	11	8	154	3	6	121
Mar	2003	3	9	113	10	13	322	13	6	123	6	9	145

c**: Intensive group calves (2002 and 2003)**

Month		*Boophilus*			*Amblyomma*			*Rhipicephalus*			*Hyalomma*		
		mal	**fem**		**mal**	**fem**		**mal**	**fem**		**mal**	**fem**	
Apr	2002	1	2		2	1		3	2		4	6	
May	2002	2	2		3	1		4	2		3	5	
Jun	2002	3	3		2	2		2	2		1	4	
Jul	2202	3	1		4	4		4	3		2	2	
Aug	2002	5	2		3	2		4	4		3	6	
Sept	2002	2	3		2	5		7	6		8	5	
Oct	2002	4	3		3	6		5	3		5	7	
Nov	2002	3	5		5	4		5	6		3	3	
Dec	2002	4	4		4	6		3	4		4	3	
Jan	2003	5	5		7	3		5	5		2	16	
Feb	2003	5	4		6	5		8	7		7	11	
Mar	2003	6	5		5	6		7	11		6	4	

d: **Strategic group calves (2002 and 2003)**

Month		*Boophilus*		*Amblyomma*		*Rhipicephalus*		*Hyalomma*	
		mal	**fem**	**mal**	**fem**	**mal**	**fem**	**mal**	**fem**
Apr	2002	1	3	4	7	6	2	1	1
May	2002	2	2	6	8	7	8	1	5
Jun	2002	2	4	7	8	7	8	1	5
Jul	2002	2	3	4	5	3	3	3	2
Aug	2002	1	3	8	4	1	5	5	3
Sept	2002	5	9	9	9	3	4	1	4
Oct	2002	7	6	7	14	6	7	4	5
Nov	2002	4	5	10	12	6	3	3	7
Dec	2002	6	7	11	9	6	6	2	2
Jan	2003	10	9	16	14	5	9	7	6
Feb	2003	8	13	11	16	10	4	8	6
Mar	2003	3	4	9	17	3	5	4	5

Table 3: **Summary of the tick data (accumulated tick data) collected off adult cattle (a+b) and calves (c+d) (2002/2003)**

a) **Intensive group (adult cattle)**

R. (B.) microplus/ R. (B.) decoloratus			***A. hebraeum***			***R. appendiculatus***			***H. marginatum***		
mal	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**
45	46	851	45	50	1243	43	51	639	45	46	678

b) **Strategic group (adult cattle)**

R. (B.) microplus/ R. (B.) decoloratus			***A. hebraeum***			***R. appendiculatus***			***H. marginatum***		
mal	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**	**mal**	**fem**	**imm**
43	67	953	115	120	1630	65	66	893	43	62	899

c) **Intensive group (calves)**

R. (B.) microplus/ R. (B.) decoloratus			***A. hebraeum***			***R. appendiculatus***			***H. marginatum***		
mal	**fem**		**mal**	**fem**		**mal**	**fem**		**mal**	**fem**	
43	39		46	45		57	55		48	72	

d) **Strategic group (calves)**

R. (B.) microplus/ R. (B.) decoloratus			***A. hebraeum***			***R. appendiculatus***			***H. marginatum***		
mal	**fem**		**mal**	**fem**		**mal**	**fem**		**mal**	**fem**	
51	68		102	123		63	64		40	51	

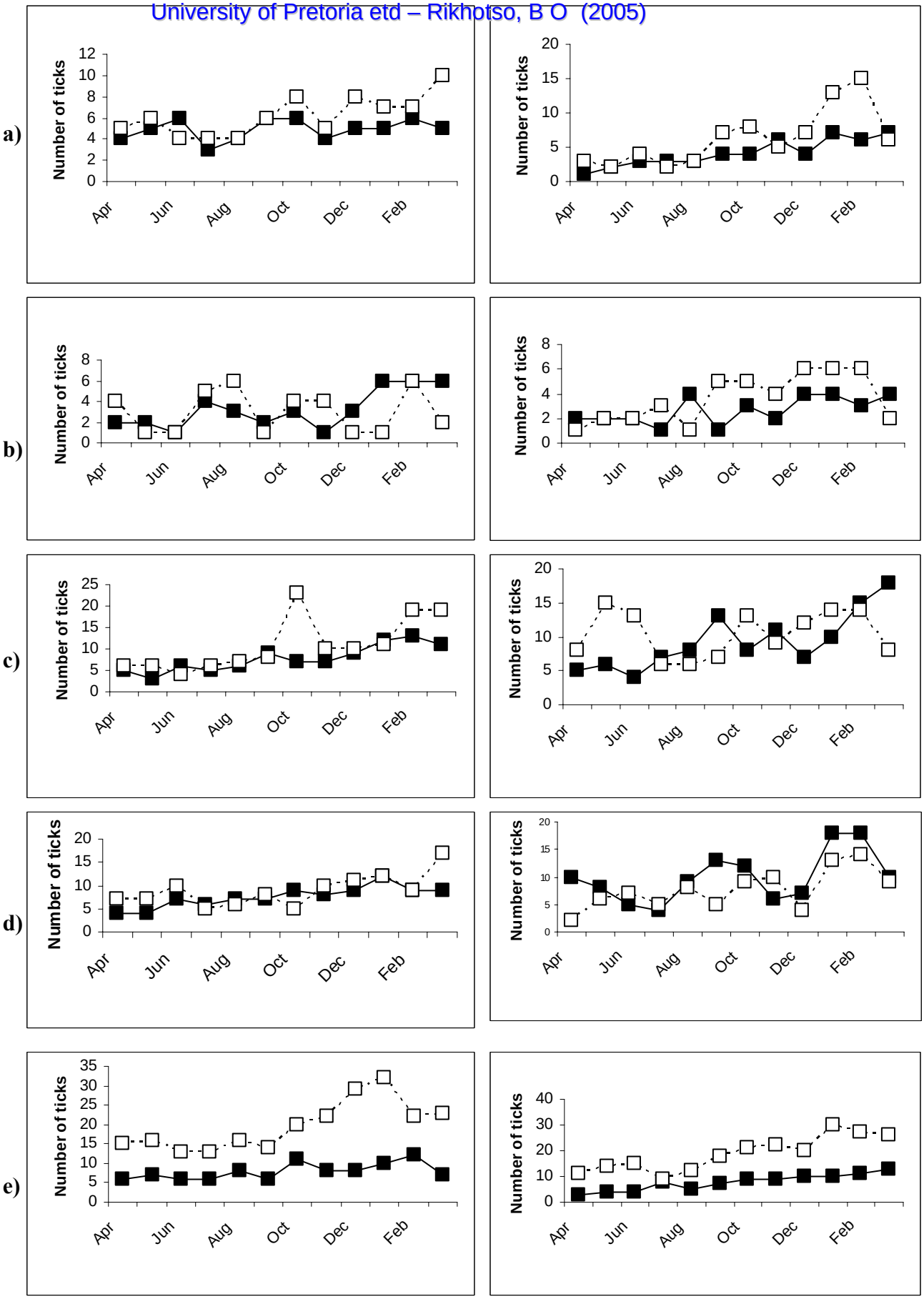
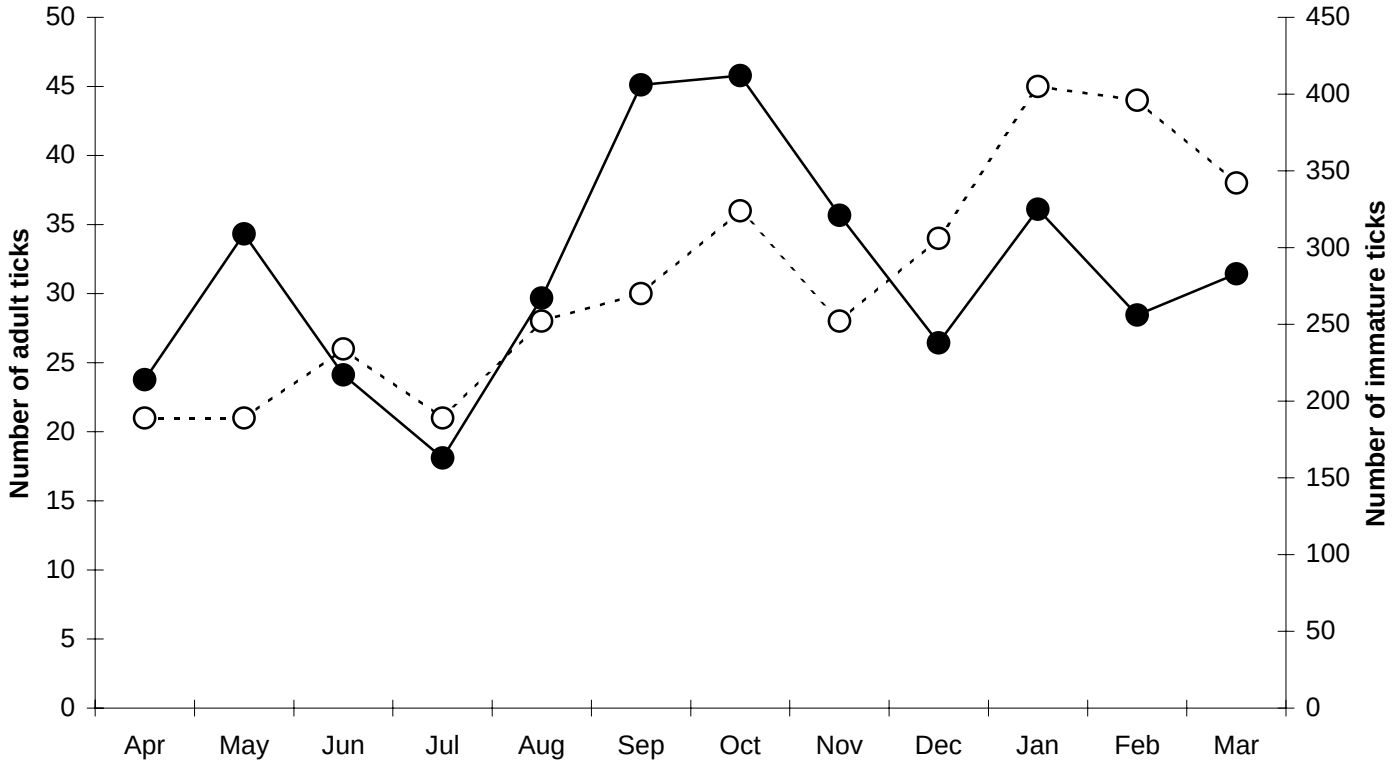


Fig 3:Adult ticks collected from intensively (–■–) and strategically (--□--) dipped adult cattle and calves from April 2002 to March 2003 a) *R.* (*B.) microplus* b) R. (*B.) decoloratus* c) *R. appendiculatus* d) *H. marginatum* e) *A. hebraeum*

a) Adult and immature ticks collected off intensively dipped cattle/calves



b) Adult and immature ticks collected off strategically dipped cattle/calves

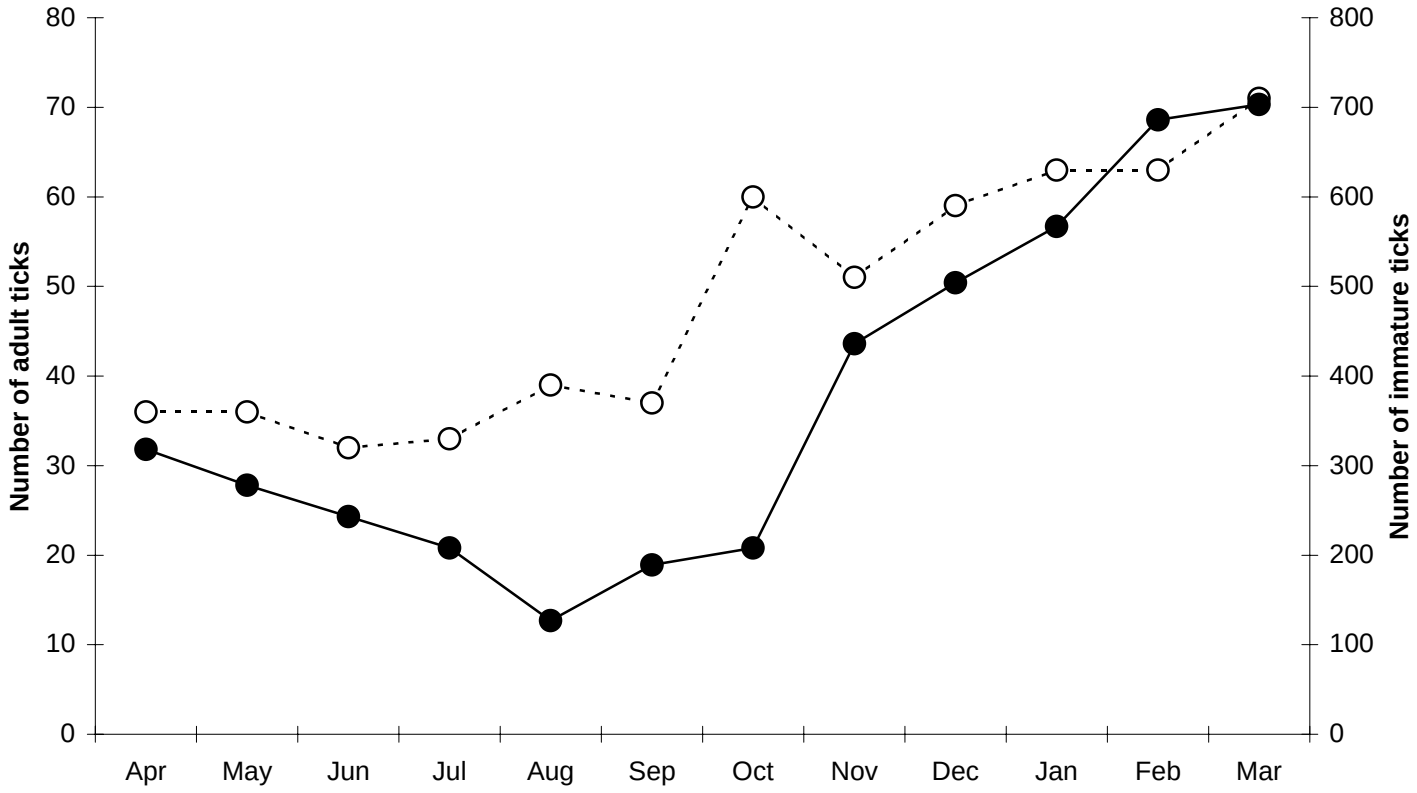
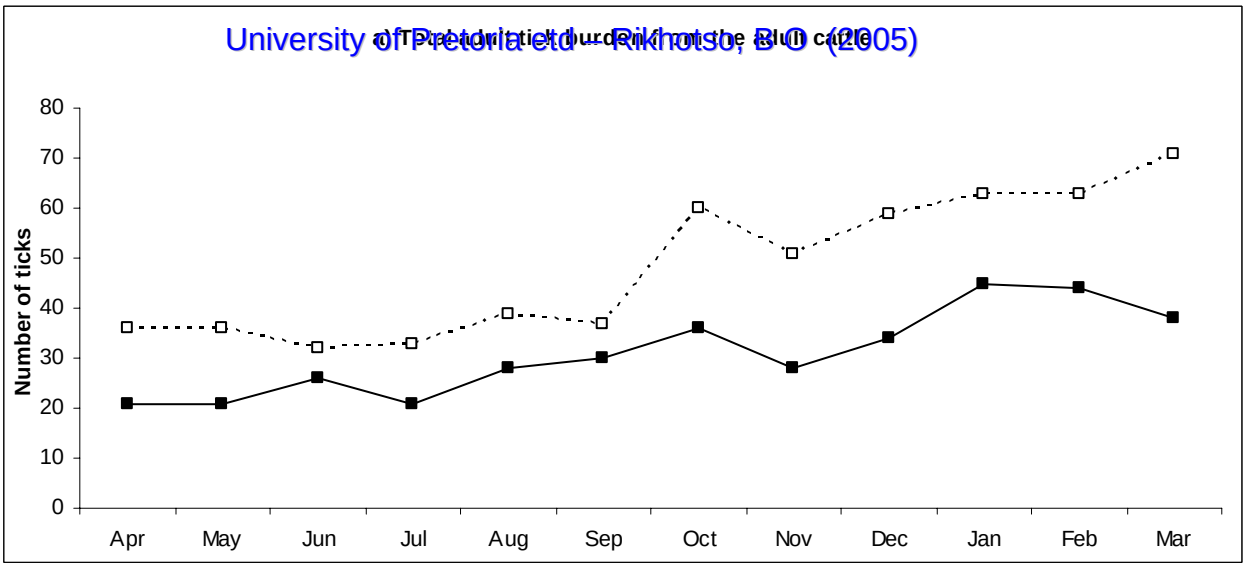
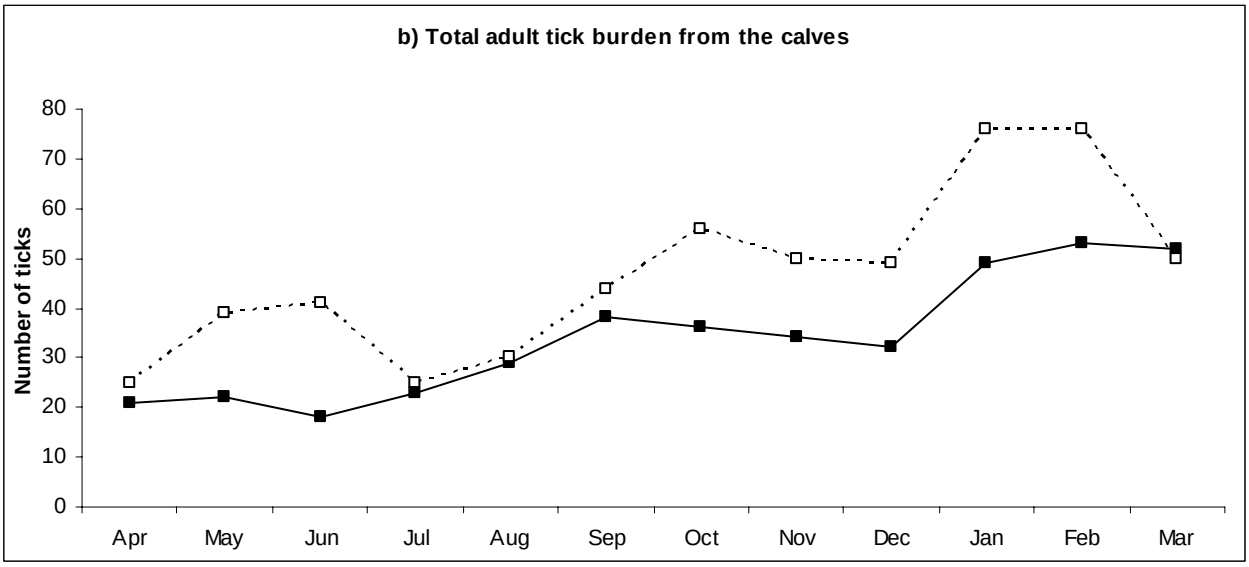


Fig 4: Comparison of the total adult (--○--) and immature (—●—) ticks collected off intensively (a) and strategically (b) dipped cattle and calves from April 2002 to March 2003





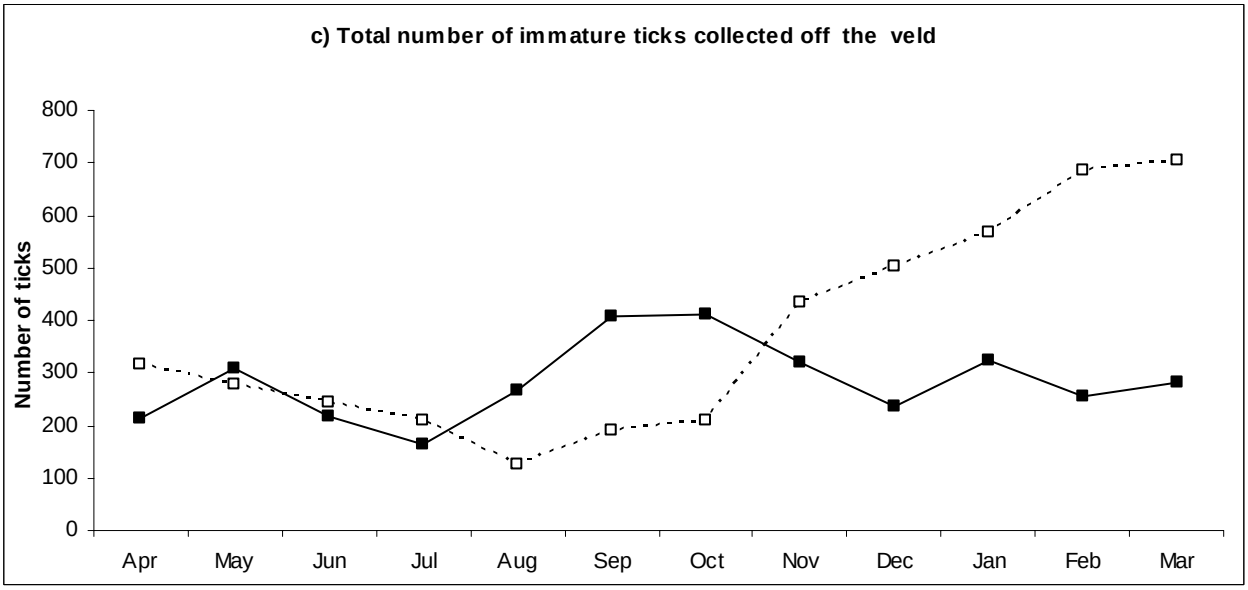


Fig 5: Total adult and immature ticks collected off: adult cattle (a) and calves (b) which had been intensively (–■–) and strategically (--□--) dipped from April 2002 to March 2003. The total number of immature ticks collected off the veld (c) is also included

4.3 **Clinical disease, mortality and abscessation (Table 4)**

Three clinical cases of bovine babesiosis (*B. bigemina*) and nine cases of anaplasmosis were recorded in the strategically dipped group whilst only one case of anaplasmosis was reported in the intensively dipped group during the survey. A further three mortalities due to anaplasmosis was recorded in the strategically dipped group and one in the intensively dipped group. Seventeen abscesses were also recorded in the strategic group and only two in the intensive group. The diagnoses of the TBD were made by a veterinarian, with a combination of clinical signs and the microscopical examination of blood smears.

Table 4: **Summary of the prevalence of clinical disease, mortality and abscessation on the cattle/calves at Bushbuckridge (the number of clinical cases in each case is also indicated)**

Dipping regime	**Clinical disease**	**Mortality**	**Abscessation**
Strategic group	Anaplasmosis (9) Babesiosis (3)	Anaplasmosis (3)	Abscesses (17)
Intensive group	Anaplasmosis (1)	Anaplasmosis (1)	Abscesses (2)

CHAPTER 5

DISCUSSION

The following were the important findings of the study:

5.1 **Serological findings**

5.1.1 ***B. bovis***

It is generally accepted that endemic stability to TBD exists when the number of seropositive animals in a herd reaches 80% (Mahoney & Ross 1972; Norval *et al.* 1983). Mahoney and Ross's model was developed using the serological results from calves up to 9 months old. Older cattle were included in this study to give a more realistic idea of the risk of disease outbreaks in the area (Perry, Musisi, Pegram & Schels 1989). The finding that the cattle populations at Oakley and Ronaldsy, which formed the strategic group, showed a statistically significant increase in seroprevalence to *B. bovis* in both the adult cattle and the calves ($p<0.05$) in 2003 is similar to findings in other surveys in southern Africa where *B. bovis* seroprevalence was high and where *R. (B.) microplus* was common and little tick control was practiced (Norval *et al.* 1983; Perry *et al.* 1989; Regassa, Penzhorn & Bryson 2003). The main reason for the increase in seropositivity was probably due to the reduced number of dippings which allowed more ticks on the cattle, therefore, increasing the rate of transmission of *Babesia* (Perry *et al.* 1989; Regassa *et al.* 2003). The seroprevalence levels of *B. bovis* in both the calves and the adult cattle in the intensive group (Cunningmore and Mkhuhlu) declined during the study and this was probably because this group was still intensively dipped (Meltzer, Norval & Donachie 1995). The decline could have been due to a better compliance by farmers in bringing their cattle for dipping during the study.

5.1.2 ***B. bigemina***

The finding that there was a statistically significant increase in the seroprevalence to *B. bigemina* in the adult cattle in 2003 was consistent with findings on farms with medium tick control in Zimbabwe (Norval *et al.* 1983). The reasons for the increase in seroprevalence to *B. bigemina* in the strategic group was also probably due to the increase in tick burdens on the cattle (Fig. 3) especially the increase in *R. (B.) decoloratus* and *R. (B.) microplus.*

The overall seroprevalence to *B. bovis* was found to be higher than to *B. bigemina* in the strategic group than in the intensive group, whereas, one would expect to find higher transmission rates and higher seroprevalence to *B. bigemina* when compared with *B. bovis* (De Vos 1979; De Vos & Potgieter 1983). However, several studies in Africa (Norval *et al.* 1983; Perry *et al.* 1989; Tonnesen, Penzhorn, Bryson, Stoltsz & Masibigiri 2004) have found a higher prevalence to *B. bovis* when compared to *B. bigemina* in communal herds where both *B. bovis* and *B. bigemina* co-exist. This could be due, at least in part, to the displacement of *R. (B) decoloratus* by *R. (B) microplus* (Tonnesen *et al.* 2004) and the fact that *R. (B) microplus* feeds more efficiently on cattle hosts than *R. (B) decoloratus* (Norval *et al.* 1983). Endemic stability to *B. bovis* and *B. bigemina* was not present in the cattle population in this study if one uses the criteria of Mahoney and Ross (1972).

5.1.3 ***Anaplasma***

Unfortunately a good comparison between the *Anaplasma* 2002 and 2003 serological results could not be made because the OVI laboratory used different antigenic kits for testing during 2002 and 2003. There was, however, a sharp decline in percentage

seroprevalence to *Anaplasma*, despite an increase in the number of *R.* (*Boophilus)* ticks in the strategic group. The following factors may have influenced the results: Firstly the degree of endemic stability to anaplasmosis had previously not been found to correlate with dipping frequency (Biggs & Langerhoven 1984). Norval, Fivaz, Lawrence & Brown (1983) also stated that dipping frequency did not reduce the seropositivity to *Anaplasma.* The presence of non-pathogenic strains of the organisms may have also lead to the absence of clinical disease outbreaks prior to the study (James, Jongejan, Lopez, Melendes & Ristic 1985; Jongejan, Perry, Moorhouse, Musisi, Pegram & Snacken 1998). Cattle in these villages may also have been resistant to TBD after years of exposure to TBD.

5.2 **Tick counts**

The study indicated that the adult ticks occurred seasonally which was similar to previous findings in the area (Fourie & Horak 1990; Horak 1982), peaking in spring and summer (Fig 3). The immature ticks also showed a definite seasonal pattern, peaking in autumn and spring with a statistically significant relationship ($p < 0.05$). There was also a statistically significant relationship between the seasonal counts of the immature ticks and the two dipping treatments ($p<0.05$). There was, however, no statistically significant correlation between the adult tick counts on adult cattle when compared with the calves. The most prevalent tick species collected off the cattle was *A. hebraeum* and this was similar to the findings by Horak (1999) where *R. (B.) decoloratus, R. (B.) microplus*, *R. appendiculatus* and *Hyalomma marginatum rufipes,* were also collected in significant numbers. Horak, Boomker, Spickett & de Vos (1992) collected *A. hebraeum* as the most common tick species on kudus in the Kruger National Park which is adjacent to the study sites. These results are similar to

other recent surveys in this area (Bryson, Tice, Horak, Stewart & du Plessis 2002). *R. (B.) microplus* was also common and this would indicate why there was an increase in seroprevalence to *B. bovis*, a similar finding to that of Tonnesen, Penzhorn, Bryson, Stoltsz & Masibigiri (2004). The number of *Rhipicephalus* (*Boophilus)* ticks collected off the cattle peaked during spring and autumn, which correlates with other studies in the region (Baker, Ducasse, Sutherst, & Maywald 1989; Bryson *et al.* 2002; Rechav 1982).

5.3 **Clinical diseases, mortality and abscessation**

Eleven of the clinical cases due to TBD occurred during the first quarter of the study period and the clinical cases were limited to the strategic group, which had been maintained on a fourteen-day dipping interval prior to the study. The intensively dipped group of cattle had only one case of anaplasmosis during the corresponding period. The low incidence of clinical anaplasmosis may be due to a greater degree of endemic stability as suggested by the 80% positive seroprevalence. All twelve clinical cases were treated and eight recovered.

5.4 **Conclusions**

It was concluded that it was not necessary to dip the cattle intensively at fortnightly intervals in this region especially when one considers the relatively low tick burdens on the cattle and on the local vegetation. In the strategic group there was an increase in seroprevalence to *B. bovis* and *B. bigemina* indicating that if the decline in dipping could be maintained for long enough an endemically stable disease situation should result. Outbreaks of clinical cases of disease could be treated and vaccination could be used to supplement the natural tick challenge if natural exposure is not enough to

maintain endemic stability. The increasing seroprevalence to both *B. bovis* and *B. bigemina* in calves suggested that calf vaccination was not necessary and that tick control should, therefore, be aimed mainly at preventing excessive tick worry.

CHAPTER 6

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