A SERO-EPIDEMIOLOGICAL SURVEY OF PARASITES IN SMALL STOCK IN THE NORTH EASTERN REGION OF THE FREE STATE PROVINCE, SOUTH AFRICA

By

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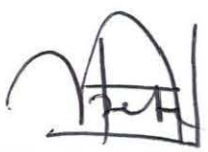
DECLARATION

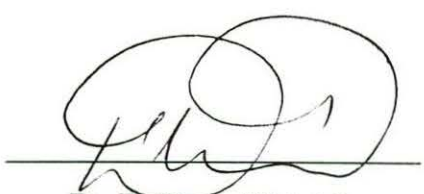
I, **Kagiso Hendrick Ramabowa Mogaswane**, hereby declare that this research project is my original work and has not been presented for a degree in any other university.

KAGISO H.R. MOGASWANE

This thesis has been submitted for examination with our approval as University supervisors.



PROF. P.A. MBATI



Dr D.T. de WAAL

DEDICATION

This thesis is dedicated to my two beloved parents Peter Shimanyana and Eunice Nokufa Mogaswane for the love and trust they always had in me and my brothers and sister, and above all to the almighty for the strength, courage and wisdom he has given me till this far.

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GLOSSARY OF ABBREVIATIONS

CI-ELISA	Competition Inhibition Enzymed Linked Immunosorbent Assay
DNA	Deoxyribo Nucleic Acid
ECF	East Coast fever
EDTA	Ethylene Diamine Tetraacetic Acid
FAO	Food and Agricultural Organization
FITC	Flourescein isothiocyanate
TTBD	Tick and tick-borne disease
IFAT	Indirect Fluorescent Antibody Test
MSP5	Major Surface Protein 5
MoAB	Monoclonal Antibody
OD Values	Optical Density Values
OVI	Onderstepoort Veterinary Institute
PBS	Phosphate Buffer Solution
PCV	Packed Cell Volume
SAWB	South African Weather Bureau
SD	Standard Deviation
TRITC	Tetramethyl rhodaine isothiocyanate
USA	United States of America

ABSTRACT

A sero-epidemiological survey was conducted in the north-eastern Free State region of South Africa to determine the parasites of veterinary importance infecting sheep and goats. Blood smears from sheep (n=371) and goats (n=188) were negative for *Anaplasma* and *Theileria* or any other blood parasites. All the sheep and goats were seropositive for *Theileria* species by IFAT while 85% of sheep and 100% of goats tested seropositive for *Anaplasma* species by competition inhibition ELISA. The observation of the negative blood smears but high incidence of positive serological results for *Anaplasma* and *Theileria* species for the two animal groups indicates that this area is endemic but with a stable disease condition. Because the animals did not show any clinical signs of infection the sheep and goats in the north eastern Free State are probably healthy carriers of *Anaplasma* and *Theileria*.

Two tick species found to infect sheep and goats in the three study sites of Harrismith, Kestell and Qwa-Qwa were *Rhipicephalus evertsi evertsi* and *Boophilus decoloratus*. These two are known vectors of *Anaplasma* species and *Theileria* species, the two diseases found to infest sheep and goats in this region. *Rhipicephalus evertsi evertsi* was the dominant tick species across the study sites. A seasonal pattern in tick infestation was observed, whereby there is a high peak during the warmer months and a very low peak during the colder months of the season. Results from this study will form an integral component in the development of disease control programs in the region.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Preamble

There are probably few domestic animals, not to mention humans in the whole world that have not at one time or another suffered from the unwelcome attention of arthropod parasites. Wild animals too are frequently infected by them, sometimes by the same species that feed on their domesticated relations (Howell *et al.*, 1978). With its topographical and geographical differences, a large country ranging from tropical to temperate climates, and from rain forests to the oldest desert on the planet, South Africa offers an infinite variety of inhabitants to an overwhelming variety of animal species, and an equally diverse parasitic fauna. Parasites of domestic animals were brought into South Africa by successive waves of immigrants; first the sheep herding Khoi and later, in the early age, the Bantu with their cattle, goats, dogs and chickens. Western Europeans added horses, donkeys, pigs and various species of poultry (Penzhorn and Krecek, 1997).

1.2 Tick-borne diseases

Vector-borne animal parasitic diseases are widespread in the entire globe and particularly in Africa because the climate and habitats are ideal for their survival and that of their vectors. Livestock diseases, especially tick-borne diseases, are among the major factors that impede the development of rural economies in many African countries (Saunderson, 1995; Norval *et al.*, 1992a). They cause high morbidity and mortality in infected animals, resulting in considerable losses in livestock productivity. The diseases and their tick vectors are widespread in the highly diversified agro-ecological zones of Africa. They are adapted to different climatic conditions prevailing within their habitat, giving

rise to deficiencies (Fourie and Horak, 1990). Moreover, these climatic conditions vary from tropical to temperate, so that whereas the humidity determines survival and activity of tick instars, temperature affects the rate of development and survival of both ticks and their parasites (Kaufmann, 1996; Ewang, 1995). This has important implications on the epidemiology of disease, and hence strategies for its management are a must. One of the major challenges is the designing of suitable sustainable control strategies for ticks in these diverse climatic zones (Whitehead and Gibson, 1981). It has been shown that different stock of parasites and tick species have great genetic diversity. Knowledge and application of this information is very valuable in formulation of tick and tick-borne disease control strategies (Connor, 1994; Barriga, 1994).

1.3 Protozoal diseases

1.3.1 Babesiosis

Babesiosis is caused by infection with species of tick-borne, intra-erythrocytic and generally host specific protozoan parasites of the genus *Babesia* classified as follows (Levine *et al.*, 1985):

Phylum : Apicomplexa
Class : Sporozoasida
Order : Eucoccidiorida
Suborder : Piroplasmorina
Family : Babesiidae

It occurs in a wide variety of vertebrate hosts, including human, and has a wide distribution around the world. In southern Africa the resulting disease is often given one or more *colloqiual* names in the regions where it occurs, such as redwater in cattle, and billiary fever or galkoors (Afrikaans) in horses and dogs.

The general disease manifestations are similar in all vertebrate host species and, as the popular names imply, turn to be characterised in varying degrees by intravascular haemolysis, haemoglobinaemia and the development of haemoglobinuria (which is responsible for urine being colored red), and icterus. *Theileria equi* is thought also to have a stage of development in lymphocytes of the equine host (De Vos *et al.*, 1994).

The genus *Babesia* is named after Dr V. Babes who in 1887, established the aetiology of a cattle disease in Romania associated with haemoglobinaemia. The disease in cattle is particularly prevalent in tropical and subtropical countries where the vector ticks (*Boophilus* species) are widespread. In southern Africa, bovine babesiosis and other tick-borne diseases such as heartwater, gallsickness (anaplasmosis) and some of the theileriosis (e.g East Coast fever, Corridor disease and Zimbabwe theileriosis) are of considerable economic importance (De Vos, 1992 and 1981; De Vos and Potgieter, 1983; De Waal and Stoltz, 1998b).

Babesia motasi and *Babesia ovis* infections in sheep and goats have not been recorded in southern Africa (Figure 1) (De Vos *et al.*, 1994). Babesiosis is a zoonosis, but cases of disease in humans caused by *Babesia* species are rare and usually occur in immunocompromised (e.g splenectomised) patients. However, on Nantucket Island and the islands off the coast of Massachuesetts and New York in the USA, babesiosis caused by the rodent parasite *Babesia microti* has been reported in immunocompetent humans (De Vos, 1979).

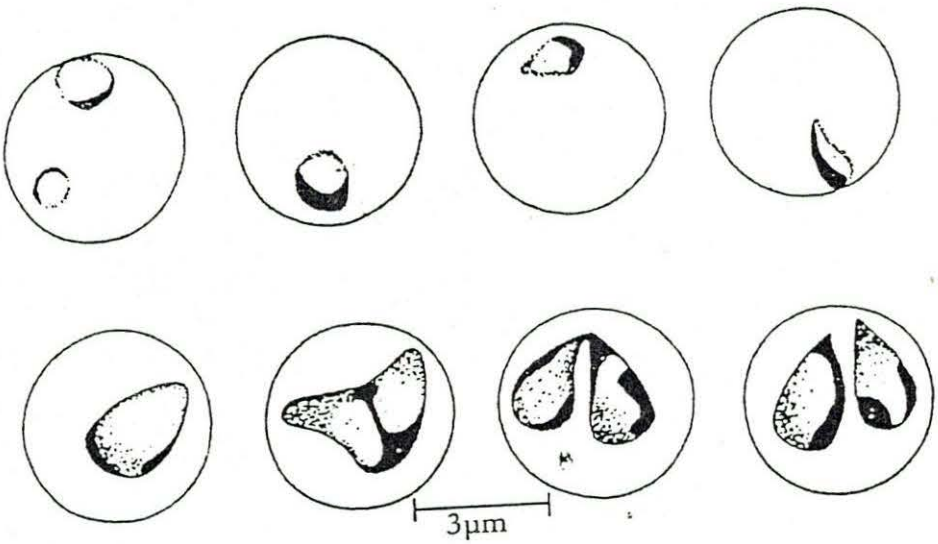

Figure 1: *Babesia* species of sheep; *Babesia ovis* (upper row) and *Babesia motasi* (lower row) (Kaufmann, 1996)

1.3.2 Theileriosis

Theileria is a genus comprising tick-transmitted parasitic protozoa classified as follows (Levine *et al.*, 1985):

Phylum : Apicomplexa
Class : Sporozoa
Subclass : Piroplasmia
Order : Piroplasmida
Family : Theileriidae

Theileria species are a group of tick-borne protozoan parasites of cattle, water buffalo, sheep, goats and wild ruminants. Of greatest importance are *T. parva* and *T. annulata* which are very pathogenic to cattle and *T. lestoquardi* (syn. *T. hirci*), which causes serious disease in sheep and goats, but doesn't occur further south than Sudan (Persson, 1995). According to Uilenberg (1964) only one species occurs in southern Africa, namely *T. separata*, a non-pathogenic *Theileria* which was first described in Tanzania in 1974 as *Haematoxenus separatus*. Nevertheless, non-pathogenic *Theileria* spp. are found worldwide, while the pathogenic species are limited to tropical and subtropical countries e.g Africa, the Middle East and India (Lawrence, 1979). The taxonomy of the genus *Theileria* at species level has been controversial and is still not satisfactorily resolved. Of the species of *Theileria* that infect cattle in the southern Africa, the most important is *T. parva* (Theiler, 1904). Originally described as a single species, it has now been tentatively divided into three subspecies: *T. parva parva* (the cause of classical East Coats fever); *T. parva lawrencei* and *T. parva bovis* (Lawrence, 1979). There is an increasing pressure to reject the trinomial classification of *T. parva* and regard the "subspecies" as types, namely *T. parva* (*parva*), *T. parva* (*lawrencei*) and *T. parva* (*bovis*) (Lawrence, 1979). The

taxonomy of the theilerial parasites of sheep and goats is very confused. According to Uilenberg (1964) only one species occurs in sheep in southern Africa, namely *T. separata*, a non-pathogenic parasite. Uilenberg considers that the name *T. ovis* Rodhan, given in 1916, and which Neitz (1957) used for the southern African parasite, should be reserved for a parasite which so far is known from Zaire, Madagascar and possibly the central African Republic. The differentiating features between the two parasites (*Theileria separata* and *Theileria ovis*) are the structures which are associated with the piroplasms in the erythrocytes, the vectors and the infectivity of the parasites for goats (De Waal, 1998d). If this interpretation is accepted, the first record of *T. separata* is that of Schellase from Tanzania in 1913, as cited by Neitz (1957). When *T. lestoquardi* is injected from the salivary glands of the tick into the blood stream of the animal, sporozoites enter lymphoid mononuclear cells and proliferate as schizonts which are the pathogenic (and immunogenic) form of the parasite. When released from the lymphocytes, the parasite invades erythrocytes to form piroplasms, which in turn are infective for ticks (Hove *et al.*, 1996; Mukhebi *et al.*, 1992).

Theileriosis is characterised by high fever, jaundice, enlargement of lymph nodes, inappetence, emaciation and sometimes anaemia and the mortality in susceptible animals reaches 70%. Post mortem findings are anaemia, enlarged lymph nodes, emaciation and a variable degree of icterus (Sewell and Bradillesby, 1990). Diagnosis of *Theileria* infections is mainly based on clinical symptoms, post-mortem findings and identification of piroplasms in blood smears and schizonts in organ smears. An indirect method of diagnosis is the detection of circulating antibodies to *Theileria* parasites. Several serological tests exist, and Indirect Fluorescent Antibody Test which has found widest usage in the field, indicated that the test can be used for epidemiological studies

since it can detect carrier animals as well (Persson, 1995). Young animals, regularly exposed to the disease agents in their natural environment, may develop immunity without falling victim of the disease. This may result in a situation of so-called endemic stability, where the disease agent is widely present without causing serious disease. However, the introduction of susceptible animals, whether mature indigenous or exotic breeds into tick-borne disease endemic areas, will inevitably lead to massive morbidity and mortality. In general, local breeds will resist infection better than non-indigenous ones (Du Plessis *et al.*, 1994; Evans, 1952).

1.3.3 Sheep Theileriosis

Malignant sheep theileriosis is caused by *T. lestoquardi*, a highly pathogenic tick-borne protozoan. *Theileria lestoquardi* occurs in north Africa, western and central Africa, India and south-eastern Europe (Persson, 1995). It is transmitted trans-stadially but not transovarially by *Hyalomma anatolium*. Although considerable research has been carried out on ticks and tick-borne disease of cattle, much less information is available on tick-borne diseases in sheep and goats. However, serious disease outbreaks with high mortality have been recorded from Sudan for *T. lestoquardi* (Persson, 1995). Another type of *Theileria* species is *T. separata,* a common benign parasite of sheep in the eastern half of southern Africa (Lawrence, 1979). It is of no practical significance but piroplasms are encountered occasionally during examination of blood smears. The parasite does not infect goats. *Theileria separata* shows characteristic morphological and biological features of a *Theileria* parasite and is transmitted by *Rhipicephalus evertsi evertsi* trans-stadially from larva/nymph to adult (Uilenberg, 1982). The piroplasms are characterised by the presence of a clearly defined veil, often situated outside the erythrocytes membrane but lying close to it, opposite the corresponding gap in the erythrocyte cytoplasm

(Young and Mchinja, 1977). By virtue of being a benign parasite it does not have indications of being controlled. *Theileria ovis* found in the southern Africa region, the other *Theileria* species is probably non-pathogenic parasite (Persson, 1995).

1.4 Rickettsial Diseases

Only a few of the rickettsials infecting livestock are well known because of their pathogenicity and the economic importance of the diseases that they cause. Other rickettsias, equally or even more common, have achieved a high degree of equilibrium with their livestock hosts and so are less known, or are unknown because their presence is often unsuspected. Neitz (1968) described some of them as benign rickettsias, but the adjective misleads because some of these organisms may give rise to overt disease and mortality under appropriate circumstances.

1.4.1 Ovine ehlirchiosis

The causative agent of ovine ehrlichiosis, *Herlichia ovina* was the first monocytophilic rickettsia to be recognised. Since then there have been a few serendipitous observations made in the course of their investigation (Khera, 1962). In addition, the same or a similar rickettsia has been linked to three fatal epidemics in sheep in Namibia (Schultz, 1939), Algeria and Turkey. *Erhlichia ovina* is known to cycle in sheep in southern Africa but its economic importance is unknown. *Erhlichia ovina* possess the characteristics of *E. canis* and *E. bovis*, and its mode and site of replication are the same. Neither the host nor the antigenic relationship to the other *Erhlichia* species is known. Neitz reviewed the scanty literature. *Erhlichia ovina* was recorded in Algeria and Turkey, Namibia, South Africa, French, Sudan (Neitz, 1968), Iran (Marchette, 1982), Sri Lanka, and Nigeria. In the fatal epidemics the flocks were suffering from severe

helminthiasis. In addition, Schultz (1939) noted severe tick infestation and malnutrition. The low pathogenicity of *E. ovina* for healthy sheep coupled with are fatal epidemics in flocks in poor condition in which affected animals develop heartwater-like signs, is strikingly analogous to the epidemiological pattern of bovine ehrlichiosis and its fatal exacerbation known as "nofel" in some African countries (Neitz, 1968).

The incubation period in susceptible sheep following infestation with infected adult ticks ranges from 15 to 18 days. The onset of illness is signalled by a sharp rise in the rectal temperature which persists for three to ten days and sometimes for as long as 17 days. Affected animals are listless and stop eating for three or four days. Towards the end of the fever, anaemia is evident. Affected animals were febrile and developed signs of taxia before going into sternal recumbecy. Later, lateral recumbency was followed by unconsciousness, opisthotonus and death (Schultz, 1939).

Overt parasitaemia is at its maximum two or three days after the onset of fever. Relapse parasitaemias may occur several weeks later. A delayed anaemia, eosinopenia and monocytosis have also been reported. A diagnosis depends on detecting within monocytes but not in endothelial cells (Neitz, 1968).

1.4.2 Heartwater

Heartwater (Cowdriosis) is a tick-borne disease of cattle, sheep, goats and some wild ruminants and is caused by a rickettsia, *Cowdria ruminantum* (De Waal and Stoltz, 1998c). Typically, the disease is characterised by high fever, nervous signs, hydropericardium, hydrothorax and oedema of the lungs and brain, and death. It is one of the major causes of stock losses in southern Africa (Bezuidenhout *et al.*, 1994).

The first reference to what could have been heartwater was made by the voortrekker pioneer, Louis Trichardt, in 1838 (Neitz, 1967). While trekking through the north eastern Transvaal many of his sheep succumbed to a disease known locally as "nintas" three weeks after they had suffered massive tick infestation. According to evidence given by a farmer, John Webb, to the Cattle and Sheep Commission of 1876 in Grahamstown, heartwater was observed in 1858 in South Africa in the north eastern Cape Province. Because of its confusion with other local diseases of known aetiology that were prevalent at that time, some of the earlier information regarding the occurrence of heartwater is unreliable (Gruss, 1981).

The first major breakthrough in understanding the disease came in 1898 when Dixon (1899) and Edington (1900) proved that it could be produced experimentally by the intravenous injection into susceptible animals of blood obtained from animals suffering from heartwater. This experiment was successful and led to the conclusion that heartwater was caused by a living microorganism at the time believed to be a virus. However, it was not until 1925 that Cowdry, successfully demonstrated the organism in the tissues of infected animals and ticks, and so confirmed Sir Arnold Theiler's belief that heartwater is caused by a rickettsia (Theiler, 1904). Cowdry named the organism *Rickettsia ruminantum* but this was later changed to *Cowdria ruminantum* (Moshkovski, 1974). Heartwater occurs in all the African countries south of the Sahara Desert, as well as Madagascar, Sao Tome, Reunion, Mauritius, Zanzibar and a number of islands in the Caribbean but apparently it doesn't occur in Namibia, Burkina Faso, Guinea or Sierra Leone (Flach *et al.*, 1990). The actual prevalence of heartwater is unknown as figures are based on broad numbers or estimated mortalities, which at best, can only be regarded as rough indications.

The economic importance of heartwater as a disease of domestic ruminants in Africa is surpassed by East Coast fever and trypanosomosis. In the endemic areas in South Africa, mortalities due to heartwater are three times greater than the combined deaths caused by babesiosis and anaplasmosis (Uilenberg, 1983). North of the equator it is probably the third most important disease of cattle, after rinderspest and schistosomiasis (Provost and Bezuidenhout, 1989).

Heartwater is one of the major obstacles (in some instances the most important) to the introduction of high-producing animals into Africa with the aim of upgrading or replacing local stock. It is also of economic importance when susceptible animals are moved from heartwater-free to heartwater-infested areas, and it remains a cause of mortality even in endemic areas, especially amongst small stock (Uilenberg, 1982). *Cowdria ruminantum* is classified as follows:

Order : Rickettsiales
Family : Rickettsiaceae
Tribe : Ehrlicheae
Genus : *Cowdria*

It is a pleomorphic organism and colonies containing varying numbers of them are found in the cytoplasm of endothelial cells. Small and very large organisms have been identified, while the number of organisms in a colony may vary from one to several thousands. Pleomorphic forms (horseshoe, ring and bacillary shaped) are more frequently seen in those colonies that contain very large organisms. Although there is a lack of information on the development of *C. ruminantum*, there is some evidence that the parasite undergoes a sequential development in both the vertebrates and invertebrates (Du Plessiss *et al.*, 1994).

Transmission electron microscopic studies of *in vitro* cultivated *C. ruminantum* have revealed the presence of intracellular reticulated bodies two to four days after infection and intermediate bodies two or four days after infection. Large numbers of elementary bodies are seen after rupture of endothelial cells five to six days after infection.

Most *Amblyomma* species are three host ticks. Larvae and nymphs become infected when they feed on domestic and wild ruminants, and possibly also on certain game birds and reptiles at a time when *C. ruminantum* is circulating in the blood of these hosts (Bezuidenhout, 1989). The immature stages of the tick commonly feed on smaller species of domestic and wild ruminants and game birds while adults prefer cattle and larger game animals, including buffalo and giraffe as hosts. Nymphs or adult ticks transmit *C. ruminantum* to susceptible host without losing the infection. Intrastadial transmission has been demonstrated, but transovarial transmission has only once been found to occur under laboratory conditions and is probably very rare under natural conditions (Andrew and Norval, 1989). The developmental cycle of *C. ruminantum* in the tick and the infectivity of successive stages of the tick are poorly understood. It is thought that after an infected blood meal, initial replication of organisms in the tick takes place in the intestinal epithelium of the tick, and the salivary glands eventually become parasitised. Colonies of *Cowdria* in tick haemolymph may be intermediate forms in transit from intestinal to salivary gland cells. Transmission of the parasites to the vertebrate hosts probably takes place either by regurgitation of their gut content or through the saliva of the tick while feeding. The minimum period required for *C. ruminantum* to be transmitted after the ticks have attached on susceptible animals is between 27 and 38 hours in nymphs and 15 and 75 hours in adults (Bezuidenhout, 1989).

1.4.2.1 Epidemiology

Factors relating to the tick vector, causative organism (*C. ruminantum*), and the vertebrate host are important in the epidemiology of heartwater. These include possible immunological strain differences of *C. ruminantum*, availability of wild animal reservoir hosts or vectors for heartwater organisms, infection rates of ticks, age and genetic resistance of domestic ruminant populations, seasonal changes influencing tick abundance and activity, and the intensity of tick control (Uilenberg, 1983). *Amblyomma* species are not all equally good vectors, and their importance in the transmission of heartwater depends not only on their vector distribution and adaptation to domestic stock, but their activity and abundance being influenced by temperature and humidity (Petney *et al.*, 1987). An increased incidence of heartwater usually occurs when peak numbers of ticks are present. Good rains are often followed by a transient increase in the prevalence of the disease. Its occurrence is not strictly seasonal, a fact which is particularly true in regions like the Caribbean and certain parts of Africa which have a temperate climate and a poorly-defined rainy season (Camus and Barre, 1987).

In sheep, ticks can acquire the infection from two days after the commencement of the temperature reaction to two days after they have been treated specifically for heartwater (De Waal and Stoltz, 1998c). In Creole goats the period lasts from two days prior to the commencement of the temperature reactions to three days after the temperature has returned to normal whereas infectivity in cattle lasts for 361 days or longer (Andrew and Norval, 1989). As little as 2.7-5.5 µl of blood collected from a host during febrile stage can infect *A. hebraeum* larvae. Since *C. ruminantum* replicates in the tissues of the tick, the infection is amplified so that a single infected tick can transmit the disease to a large ruminant. Ticks may retain their infectivity for life, so that small numbers of

infected ticks could presumably maintain the infection in a particular herd or area. It is generally assumed that the infection rates in tick populations are low, as evidenced by the extended periods (even up to several years) during which susceptible domestic ruminants may escape infection in endemic regions (Brown and Skowronek, 1990). Although the infection rates of ticks may vary according to the season and the locality in which they are collected, limited surveys in South Africa indicate that some 1 to 7% of *A. hebraeum* in endemic areas are infected at any one time. Reasons for this low infection rate could be that not all ticks which feed on animals with a rickettsaemia become infected, and that not all the infected nymphs which fed as larvae on such animals retain the infection to the adult. *Amblyomma* species are also indiscriminate feeders, especially in the immature stages, and many ticks may therefore never acquire the infection, having fed during the larval or nymph stages on a non-susceptible wild animal (Bezuidenhout *et al.*, 1994). In contrast to the low infection rates found in South Africa, considerably higher rates of infection of *A. hebraeum* have been detected in Zimbabwe in the range of 0.0 - 44.9% for males, 20.0-36.1% for females and 0.0 - 13.4% for nymphs (Norval *et al.*, 1990). The discrepancy in infection rates between these two studies can possibly be ascribed to the different methods used in determining the rates. In South African studies the method used to detect infection in ticks was an indirect one which depended on sero-conversion of mice inoculated with homogenates of adult ticks removed from cattle, while in the Zimbabwean study infection rates were derived statistically, based on the frequency with which field-collected unfed nymphs and adults of *Amblyomma hebraeum* infected sheep (Bezuidenhout *et al.*, 1994)

Sheep are more susceptible to heartwater than cattle, but variations in susceptibility between breeds of sheep are less than those in cattle breeds.

Certain sheep breeds such as the black-headed Persian sheep, possess a certain degree of natural resistance to heartwater. Angora goats are highly susceptible to heartwater and their immunity is of short duration. A genetic resistance, which may be due to a recessive sex-linked gene, has been demonstrated in Guadeloupe goats (Matheron *et al.*, 1987). The disease can maintain itself in the absence of a wild ruminant reservoir, as in the case of Madagascar and Sao Tome, where wild ruminants are absent. Little is known about the possible role of wild animals and birds as sources of infection to ticks in endemic areas. Although a variety of indigenous and exotic ruminants may become infected with *C. ruminantum*, so far only blesbok, black wildebeest, helmeted guinea fowl, leopard tortoise and scrub hare have been proven to harbour *C. ruminantum* subclinically for any length of time after artificial infection (Oberem and Bezuidenhout, 1989). Of all the wild ruminants in southern Africa only the eland, blesbok, springbok and black wildebeest have been reported to develop clinical disease. Wild animals may possibly play a role as a source of infection for ticks, particularly in those areas where stringent tick control in domestic animals is practised. In addition, the multimamate mouse (*Mastomys coucha*) and the striped mouse *(Rhabdomys pumilio*) are susceptible to infection, but as wild rodents probably do not serve as hosts of *Amblyomma* species. In heartwater endemic areas, they are unlikely to play a role in the epidemiology of the disease (Howell *et al.*, 1978).

1.4.2 Anaplasmosis

Anaplasmosis is an arthropod-borne disease of cattle, sheep, goats and some wild ruminants species caused by obligate intraerythrocytic rickettsial organisms classified as follows (Potgieter and Stoltsz, 1994):

Order : Rickettsiales

Family : Anaplasmataceae

Genus : *Anaplasma*

The *Anaplasma* parasites are biologically transmitted by ticks, but mechanically by haematophagous insects and iatrogenic means may also occur. Clinicallly, *Anaplasma* species infections in mammalian hosts may range from unapparent infection to severe disease and mortality. Differentiation of species within the genus *Anaplasma* is usually based on one or more of the following criteria: differences in host range; location within the erythrocytes; morphology of the inclusion bodies; and to some extent, pathogenecity of the organism and immunological differences. In the ninth edition of Bergey's Manual of Systematic Bacteriology only four species of *Anaplasma* are recognised; three which are *A. centrale*, *A. marginale* and *A. caudatum* occur in cattle, while the other *A. ovis* occurs in sheep and goats (Ristic and Kreier, 1984).

Anaplasmosis of sheep and goats is an arthropod-borne disease caused by the intraerythrocytic rikecttsial organism, *Anaplasma ovis*. It is usually a subclinical or mild condition, but moderate to severe clinical disease is generally characterised by fever and a variable of anaemia and ictus that may occasionally lead to death. Recovered animals remain carriers of the organism, resulting in premunity which persists for life. Anaplasmosis in sheep was first reported in 1912 in Zimbabwe (Bevan, 1912). In splenectomised sheep there is an acute clinical infection, however, most animals survive without treatment. Neither intact nor splecnectomised cattle are susceptible to *A. ovis*, but goats often develop a severe clinical response (Bevan, 1912).

Although a number of reports of *Anaplasma* infections in sheep and goats in Africa exist, the report by Di Domizio in 1919 is considered to be the first authentic description of anaplasmosis in non-bovine hosts. An *Anaplasma* infection in sheep which was not transmissible to cattle was reported, and

Lestoquard (1924) differentiated the causative organism from *Anaplasma* species of cattle describing and naming it *A. ovis*. Ovine and caprine anaplasmosis occur in many parts of Africa, North and South America, Asia, the southern and central parts of eastern Europe and Russian (Lu *et al.*, 1987). The condition in southern Africa is of little economic importance. Because of its often insidious nature, the deleterious effects by *A.ovis* infection, such as weight loss and unfrithness, are probably erroneously ascribed to other causes (Anon, 1990). Although mortality due to anaplasmosis in sheep and goats is seldom observed, overt disease, abortion and mortality may occur (Anon, 1990). *Anaplasma ovis* is the main cause of ovine and caprine anaplasmosis. It is more pathogenic for goats than for sheep and only very rarely is it infective to cattle. *Anaplasma marginale* species pathogenic to cattle may also under certain condition, cause latent infection in sheep and goats (Maas and Buening, 1981). In Giemsa-stained smears, *A. ovis* is morphologically indistinguishable from *A. marginale* and appears as irregularly shaped, almost spherical, intraerythrocytic granules staining deep blue-purple colour. The organisms vary in diameter from approximately 0.4μm to 0.8μm, with an average of 0.5μm. Within erythrocytes, 60 to 70% of the inclusion bodies of *A. marginale* are situated marginally and 30 to 40% submarginally or centrally. In comparison, up to 90% of the inclusion bodies of *A. marginale* are situated marginally. Differences between *A. ovis* and *A. marginale* with regard to the intraerythrocytic location of organisms have been found to be statistically significant. Apart from morphological similarities, complete serological cross-reactivity has been demonstrated between *A. ovis* and *A. marginale*. They are nevertheless immunologically distinct, as infection with *A. ovis* does not provide immunity against *A. marginale* or versa verse (Stoltsz, 1994).

1.4.2.1 Epidemiology

Anaplasmosis in sheep and goats has been reported from most tropical and subtropical parts of the world. With the exception of Namibia, *A. ovis* has been reported from all the countries in southern Africa and is believed to be prevalent in most sheep and goat farming areas. The prevalence rates of *A. ovis* infection in sheep or goats vary considerably within endemic areas (Anon, 1990) and may be influenced by management factors such as control measures employed and the movement of livestock from non-endemic to endemic areas. Although no extensive survey has been conducted to determined the prevalence of *A. ovis* in South Africa, limited microscopic observations in sheep have revealed their prevalence ranging from 33 to 90% in sheep and 0 to 90% in goats. Similarly, in Mozambique, its prevalence ranges from 0 to 85% in sheep and from 0 to 90% in goats. In India, it has been shown that approximately 60% of sheep and 70% of goats are infected, while in Kenya the prevalence of infection in goats from different localities ranges from 22 to 87% (Shompole *et al.*, 1989). Although sheep and goats of all ages are susceptible to *A. ovis* infection, older animals suffer from greater reduction in packed cell volume.

The tick vectors of *A. ovis* in southern Africa have not been determined. Such studies are complicated by the low parasitaemias found in carriers which makes their identification difficult (Visser *et al.*, 1991). Based on the observations elsewhere that *A. ovis* and *A. marginale* may share common tick vectors, one or more of the tick vectors of *A. marginale* from South Africa may also prove to be vectors of *A. ovis* (Potgieter, 1979). However, during the experimental studies on the tick transmission of *Theileria separata*, *Rhipicephalus evertsi evertsi* and *Rhipicephalus evertsi mimeticus* transmitted only *T. separata* from splenectomised sheep infected with *T. separata* and *A. ovis* (Barry and Van Niekerk, 1990). Intra-uterine transmission has been reported in sheep and in

goats (Barry and Van Niekerk, 1990). In sheep, transplacental infection has been found to occur during the second and third trimester of pregnancy, but no lesions have been observed in foetuses or neonatal lambs (Zaug, 1987). In contrast, a high percentage of artificially infected female goats aborted their foetuses following acute infection (Barry and Van Niekerk, 1990). Sheep and goats that have recovered from *A. ovis* infection remain carriers of the parasite and in areas where both sheep and goats occur, sheep may be particularly important as reservoir hosts because of the lower pathogenicity of *A. ovis* in this host (Sinha and Pathale, 1966).

1.5 General perspective of ticks

Ticks are one of the most important vectors of life threatening or debilitating human and animal diseases. Moreover, ticks transmit a greater variety of infectious agents than any other arthropod group. They are also important pests, affecting man, livestock and wildlife. In addition ticks cause several toxic conditions, e.g tick paralysis and various tick toxicoses, irritation, tick bite allergies and immune responses and blood loss (Botha, 1994). The wounds caused by tick bites are often painful, of long duration and frequently lead to secondary infection by microbial pathogens. Disease causing agents transmitted by ticks, several of which have increased to epidemic proportions (e.g Lyme disease, tick borne encephalitis) constitute an important cause of morbidity and mortality (Perry *et al.*, 1986; Neitz, 1956). Despite their importance, our knowledge of the zoonotic associations that perpetuate and disseminate these diseases is incomplete. In addition to the diseases of health importance, tick-borne protozoan, rickettsial, viral, bacterial and even fungal disease organisms continue to plague livestock production and affect wildlife in most areas of the world. Failure to control ticks and tick-borne diseases is a major factor limiting livestock production. The cost of world wide economic losses and the

traditional burden of protecting livestock against ticks and the tick-borne diseases is now estimated to be in billions of dollars annually (Dargie, 1989).

1.6 Importance of ticks

Ticks are obligate blood sucking arthropods found in almost every region of the world. They are Acarines (Subclass Acari), the taxon that also includes the mites. Approximately 850 species of ticks are known, subdivided into two major families, the "Hard Ticks" or Ixodidae so called because of their sclerotized dorsal scutal plate, and the "Soft Ticks" or Argasidae, so called because of their flexible, leathery cuticle. Ticks infest every class of terrestrial vertebrates, mammals, birds, various reptiles and every amphibian. Many tick-borne diseases remain prevalent today, despite the impressive advances in medicine that have produce partial control of arthropod borne diseases in different regions of the world (Evans, 1952).

Disease of importance transmitted by African ticks are babesiosis, theileriosis, heartwater and anaplasmosis (Walker *et al.*, 1978). Important ticks in Africa are *Amblyomma*, transmitting *Cowdria; Boophilus* species, the most important vector of *Babesia* species and *Anaplasma*; *Rhipicephalus* species, transmits *Theileria* species and *Babesia* species, and *Hyalomma* species, which are the vectors of several theilerial, babesial and rickettsial infections. Ticks and tick-borne diseases are of great importance in South Africa (Krecek *et al.*, 1995). Although ticks had long been suspected by south African farmers of being implicated in disease outbreaks, primarily heartwater (cowdriosis), it was the discovery of Dixon in 1899 that bovine babesiosis "Texas Fever" was transmitted by ticks that opened up a vast new field of research. In south Africa, the pioneering studies of Lounsbury between 1899 and 1900 on the life cycle of *A. hebraeum* were paramount when he identified *A. hebraeum* as the heartwater

vector (Penzhorn and Krecek, 1997). Diseases like East Coast fever, couping illness, anaplasmosis, babesiosis and cattle fever, destroy vitally needed protein resources. In Zimbabwe, the cessation of tick control measures during the country's seven year liberation war resulted in the death of an estimated one million cattle and half a million sheep and goats from tick-borne diseases (Lawrence *et al.,* 1994). In South Africa and Britain, skin conditions caused by a tick borne fungus *Dermatophilus congolensis* affects livestock and even man. In addition to pathogenesis they transmit, ticks induce lethal paralysis or severe toxemia as a result of their bites or substances they ingest. Blood loss is another important cause of injury, since Ixodid ticks consume large amount of blood as their meals e.g *Hyalomma asiaticum* females consume more than eight millilitre of blood per tick. In some instances, infestation with ticks may reach such extreme magnitudes that animals die of exsanguination or are susceptible to other illnesses due to their weakened conditions (Fourie *et al.*, 1988; Howell *et al.*, 1989).

Tick-borne diseases of major economic importance which affect cattle are heartwater, caused by *C. ruminantum*, babesiosis caused *Babesia bovis* and *B. bigemia*, anaplasmosis, caused by *A. marginale* and the theileriosis caused by *Theileria parva* complex. Of the lesser importance in cattle are generally non-pathogenic mild theileriosis caused by *Theileria mutans*, *T. velifera* and *T. taurotragi;* spirochaetosis (Borreliossis), caused by *Borrelia theileria*, benign anaplasmosis caused by *A. centrale,* benign babesiosis caused by *Babesia accultans* and bovine ehrilichiosis caused by *Ehrilichia bovis*. Horses, mules and donkeys suffer from Equine biliary fever that is caused by *Theileria equi* and *Babesia caballi*, and are also affected by spirochaetosis. Sheep and goats are susceptible to heartwater, to infections with *Anaplasma ovis* and *Theileria*

separata, and to spirochaetosis; and pigs to *Babesia trautmanni* and African swine fever virus (Horak, 1982).

1.7 Ticks as vectors

1.7.1 *Amblyomma hebraeum* (South African bont tick)

This tick transmits heartwater (*C. ruminantum*) and benign bovine theileriosis (*T. mutans*). Adults of *A. hebraeum* are large conspicuous ticks with long mouth parts, brightly coloured patterns on the scutum and brown and white banded legs. The preferred hosts of the adult are the larger domestic and wild angulates, to which they attach in clusters in the groin and axillae as well as on the dewlap, belly, perinium and perianal region (Baker and Ducasse, 1967). *Amblyomma hebraeum* has a three-host life cycle with larvae, nymphs and adults feeding on separate hosts. On completion of feeding, engorged ticks leave the host and seek shaded microhabitats in which to moult or lay eggs. The developmental periods off the host are long and the life cycle may last from one to three years under field conditions. The larval and nymphal stages feed on a wide variety of large and small mammals, birds and even reptiles (tortoises) (Norval *et al.*, 1983a). On domestic livestock nymphs are attached most commonly to the feet, and larva to the face, dewlap, neck and legs. The pattern of seasonal occurrence is dependent on climate and varies considerably throughout the distributional range of the tick. In general, adults tend to be most numerous during the wet season, and larvae and nymphs during the dry season but all stages are usually found on hosts throughout the year. A fully engorged female can lay a batch of up to 18 000 eggs. *Amblyomma hebraeum* is exclusively a southern African tick occurring in the warm, moist coastal areas of the eastern Cape and Natal, Transkei. Its distribution also extends inland through the lowveld areas of Swaziland and the northern Province to southern Zimbabwe and eastern Botswana. The species is absent from the highveld areas of South Africa and

Zimbabwe, probably because these are too cold (Norval, 1983b). Adults of *A. hebraeum* have long feeding periods and consequently good control of the tick can be achieved by treatment of cattle at two-weekly intervals with conventional acaricides. As the ticks attach predominantly to the undersides of cattle, control can be achieved by localised acaricide application. It should be noted that in endemic heartwater areas, control of *A.hebraeum* is only necessary when larger clumps of adults, forming potential sites for strike by screw-worm *Chrysomya bezziana*, occur in cattle. Over vigorous control of tick is likely to have a negative effect of disrupting the natural transmission of heartwater to young animals and so reducing levels naturally acquired immunity to the disease in domestic ruminant population (Norval, 1979 and 1981).

1.7.2 *Boophilus* species

Its common name is African blue tick, transmitting diseases like bovine babesiosis (*B. bigemia*), anaplasmosis, spirochaetosis and porcine babesiosis. Adults of *B. decoloratus* are slightly smaller than those of *B. microplus*, but are otherwise very similar in general appearance. *Boophilus decoloratus* (Figure 2) is an African tick that, like *B. microplus* has one host life cycle and passes through several generations per annum (Anon, 1990). It is usually most evident from September until the end of June, and may breed throughout the year in warmer areas. In colder climates, however, it is inactive in winter and early spring. The engorged female usually lays about 2 500 eggs, which take from 3-6 weeks to hatch, depending on environmental conditions. From the larval attachment to complete engorgement of the female takes approximately three weeks. Thus under optimum conditions the blue tick can complete its life cycle in about two months. The major differences between *B. decoloratus* and *B. microplus* in southern Africa are that it has a wider host range and a wider

distribution. In addition to cattle, *B. decoloratus* frequently parasitise horses, donkeys, sheep

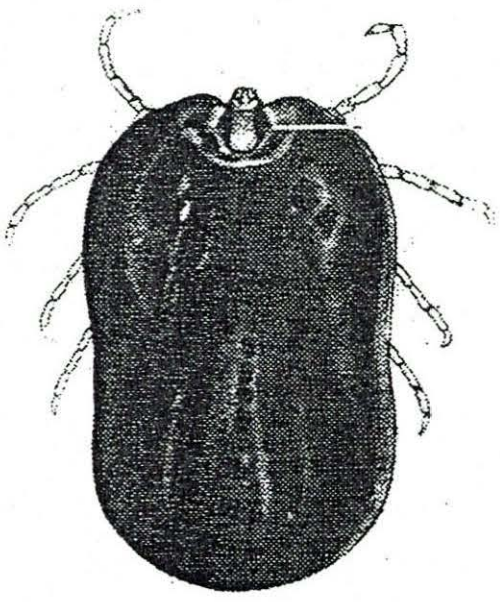

Figure 2: *Boophilus decoloratus* (engorged female) (Howell *et al.*, 1978)

and goats and is commonly found on several wild angulates species such as impala (*Aedycereos mulampus*), kudu (*Tragelaphus strepgiceros*), eland (*Tortragus oryx*) and sable (*Hippotragus niger*). The species is distributed through most of the wetter parts of southern Africa except for those areas where it has been replaced by *B. microplus* and cold mountainous areas such as the Drakensberg range and part of Lesotho. It is absent from those parts of South Africa which receive less than an average annual rainfall of 380mm, including Free State, the central karoo, bushmanland and little Namaqualand (Gothe and Bezuidenhout, 1989). In the generally arid territory of Namibia it is present only in localised areas in the north, and in Botswana it is restricted to the higher rainfall eastern boarder areas and a few scattered localities in the north (Walker *et al.*, 1978).

1.7.3 *Rhipicephalus evertsi evertsi*

Its common name is red legged tick, transmitting diseases like anaplasmosis, equine piroplasmosis, ovine theileriosis, spirochaetosis and spring lamb paralysis. Adults of *R. evertsi evertsi* are easily recognised by their eyes and red legs (Figure 3). They are usually attached in the peri-anal area of their ungulates and hosts. Larvae and nymphs feed deep in the ear canal and are found most commonly on ungulates and hares (De Vos, 1981). The preferred host of the adults are Equidae (horses, donkeys, mules and zebra) although cattle, sheep, goats and a number of wild antelope species are also frequently parasitised. *Rhipicephalus evertsi evertsi* has a two host life cycle in which the larva to nymph moult occurs on one host. The adults feed for about 4-6 days, sometimes longer, the female becoming bluish with a brownish tinge as she engorges (Gothe and Bezuidenhout, 1989). In summer she starts to lay five days after dropping from the host and produces up to 7 00 eggs, which hatch in about a month. In winter a month may elapse before she even begins laying and the

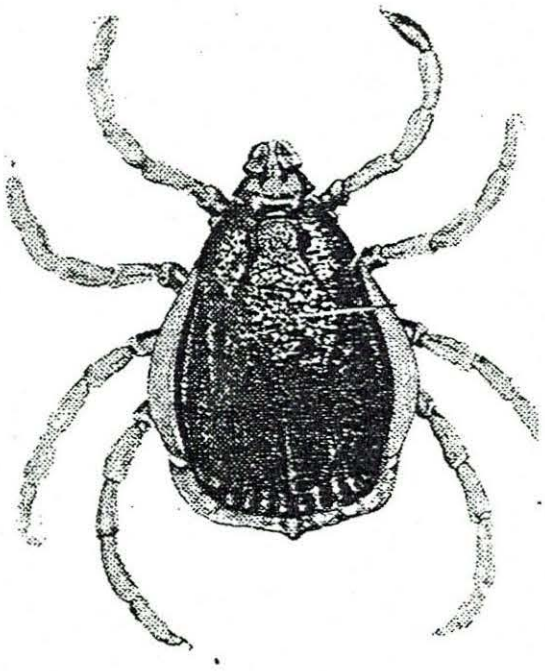

Figure 3: *Rhipicephalus evertsi evertsi* (male) (Howell *et al.*, 1978)

eggs take up to two months to hatch. The larval-nymphal feeding period in the ear lasts about 10-14 days and the nymphae subsequently take 3-4 weeks to develop into adults. Unfed larvae can withstand starvation for seven months and adults for over a year. All stages are on hosts throughout the year, although their abundance may vary from season to season. The tick is tolerant to a wide range of climatic conditions and is present throughout most of the eastern part of southern Africa. The main factor limiting its distribution in the west is increasing aridity, with the critical rainfall level being about 250 to 280 mm per annum (Howell *et al.*, 1978). Adults of *R. evertsi evertsi* are easily controlled by localised application of acaricides to the perianal area. It is probably only on horses that sufficient numbers of adults would ever be present to justify the costs of control. No specific control measures are usually taken against the immature stages (Howell *et al.*, 1978).

1.8 Control of ticks and tick-borne diseases

Food security, sustainable and environmentally acceptable agricultural production systems and trade are closely related. In the long term, global food security depends on maintaining and conserving the natural resource base for food production of which livestock is an important component. There can be no sustainable disease control strategy independent of livestock agriculture's sustainable development (De Castro, 1997). The latter is defined by Food and Agricultural Organisation (FAO) as "The management and conservation of the natural resource base and orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations (Bachman, 1990; Latif, 1993 and Latif *et al.*, 1979). Such sustainable development in the agriculture, forestry and fisheries sectors, conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable

and socially acceptable". It is estimated that the world population will increase by 72% between 1995 and 2050 and then gradually stabilise (FAO, 1996). Nevertheless, food requirements of the developing countries will double and triple in inter-tropical Africa. With growing urbanisation and increased income, demand for animal protein usually goes up. However, it is expected that the livestock sector products in the developing nations will still be well below that of the developed world. Further, in areas of sub-Saharan Africa and South Asia, consumption will remain at very low levels, particularly through lack of milk (De Castro, 1997). Cattle have been present in southern Africa for more than a thousand years and they survived without specific control measures against ticks and tick-borne diseases. It is now known that the indigenous Sanga and Zebu breeds can become very resistant to ticks (Spickett and Heyne, 1988) and acquire immunity to tick-borne diseases if exposed at an early age (Norval, 1982). Most losses due to tick-borne diseases occur in endemically unstable situations where there are insufficient ticks to ensure that all calves receive challenge. Endemic instability exists in areas which are only marginally suitable for the survival of the tick vectors or where tick populations have been suppressed but not eradicated through the use of acaricides. Tick-borne diseases also cause severe losses if they are introduced, together with their vectors, to new areas or regions and they will spread through susceptible livestock population or if susceptible animals, especially of exotic breeds, are moved to endemic areas.

1.8.1 Historical perspective

Control measures against tick and tick-borne diseases were only applied on a large scale in southern Africa following the introduction of East Coast fever (ECF) caused by *T. parva* from east Africa. Prior to that, tick-borne diseases had not been reported as problematic in indigenous cattle, although losses

caused by babesiosis and heartwater had been experienced in imported exotic cattle (Lawrence, 1979). The most serious tick-borne disease problems of the nineteenth century were the spread of heartwater throughout the coastal areas of the eastern Cape Province due to the spread of the vector *A. hebraeum* and babesiosis in cattle imported for stocking following the rinderpest pandemic of 1896 (Fourie and Horak, 1987). East Coast fever, which was introduced to Mozambique, Zimbabwe and South Africa in 1901/1902, caused a mortality rate of around 59% in the herds of susceptible cattle (indigenous and exotic) to which it spread. It caused serious economic losses, affecting agriculture, mining and commerce, all of which still used ox-drawn transport (Lawrence, 1979). Considerable effort was therefore devoted to the development of control measures. Early attempts at vaccination by the eminent microbiologist Robert Koch and later Sir Arnold Theiler were largely unsuccessful. However, it was shown by Lounsbury (1904) that the disease was transmitted by the brown ear tick, *Rhipicephalus appendiculatus*. This knowledge led to more successful control measures which were based on tick control, quarantine procedures, pasture spelling, slaughter and dipping in arsenic solution (Norval, 1977). Dipping proved to be the most practical and effective solution. It was widely adopted and later made compulsory. As a result the disease was brought under control fairly rapidly and then progressively eradicated. Complete eradication of East Coast fever from southern Africa was considered to have been achieved by 1960 (Lawrence, 1979; Ewang, 1995). After the eradication of East Coast fever two options were open to southern African countries. They could either control the remaining major tick-borne diseases (babesiosis, anaplasmosis and heartwater) by vaccination and move towards reduced tick control and endemic stability, which had previously existed, or they could continue to control the diseases by intensive dipping. In South Africa, compulsory dipping was abolished in 1989 and the choice was left on the individual farmers. In

Swaziland and Zimbabwe compulsory dipping was enforced. The dipping policy in South Africa had mixed results. Some progressive farmers took advantage of the availability of vaccines (from Ondestepoort Veterinary Institute) against babesiosis, anaplasmosis and heartwater, used them effectively, and were able to adopt flexible regimes. Others, for reasons that were probably only partially understood, practised little tick control and in a way achieved control of tick-borne disease through the maintenance of endemic stability (De Waal, 1998c).

In Zimbabwe, tick-borne diseases were effectively controlled by dipping for 20 years after the eradication of East Coast fever. Control was particularly good in the traditional (communal) farming areas, where dipping, together with overgrazing which rendered the environment less suitable for the tick survival, frequently resulted in the localised eradication of ticks (Norval *et al.*, 1992b). However this progressive scenario came to hault in the mid 1970's with disruption of dipping due to pre-independence war. Large epidemics of tick-borne disease occurred and losses over a five year period amounted to more than a million head, which was equivalent to one third of cattle owned by communal farmers. This necessitated the reassessment of the national policy on tick and tick-borne disease control. In 1980 dipping as a measure of tick and tick-borne disease control was reintroduced but this time around it was not reinforced. In countries like Botswana, Namibia and Angola intensive dipping is not widely practised. It has been more widely used in Malawi, Zambia and Mozambique where it is used for the control of theileriosis and heartwater but not over the entire countries. For epidemiological and diagnostic studies exposure of *Anaplasma, Babesiosis, Cowdriosis* and *Theileria* is best determined by the detection of antibodies in the sera of animal species. Two methods are used (Buridge and Kimber, 1972), the direct method that involves

the preparation of blood smears and the indirect method which most widely use serological test for this purpose is the Indirect Florescent Antibody Test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA) for detecting haemoparasites. This study is essential to be conducted in this area so that data collected and analysed can be used to give baseline information on the prevalence and status of anaplasmosis and theileriosis on goats and sheep in this area.

CHAPTER 2

OBJECTIVES OF THE THESIS

2.1 RATIONALE

Vector-borne diseases cause economic hardships throughout the tropical and subtropical regions of the world (Kok and Fourie, 1995). Ticks are of considerable importance as vectors of infectious diseases of domestic livestock in southern Africa (Fourie *et al.*, 1988). They are sessile ectoparasites with life cycles consisting mainly of a parasitic phase on the host and a non-parasitic phase away from the host. The devastating effects on livestock production in South Africa has made tick control a necessity in the country's livestock sector. Their presence is always to a greater or lesser degree harmful to its host. Some may cause a slight irritation but they often produce far more serious effects, either because they themselves injure or disturb the host in some way or because they transmit pathogens. Sheep and goat ticks are of great importance but not to the extent that they are in cattle. These parasites cause tick worry, blood loss and damage at feeding sites in small stock. Wounds left from tick bites are susceptible to additional attack by blowflies (Kok and Fourie, 1995).

Infested sheep itch, bite and scratch causing self-inflicted wool damage and skin trauma which may become secondarily infected. Heavy tick burdens may result in anaemia, loss of appetite and weight loss. Since the inception of the concept of the livestock production in South Africa, ticks have established themselves as a major threat to livestock, and their infestation is a serious economic constraint (Norval *et al.*, 1992a). Because resource-poor farming is overwhelming predominant in most parts of Africa, livestock production losses through tick infestation are very high in the continent. Other factors which have influenced the endemicity of various tick infections in Africa are favourable conditions

especially temperature and rainfall, vegetational cover, soil types, socio-cultural behaviour and socio-economic status (Dipeolu *et al.*, 1992). There is neither published nor reliable information of small livestock diseases in the north eastern Free State (Mbati, personal communication). This study therefore aims in documenting such parasitoses in goats and sheep. Data obtained from this study will be invaluable in developing disease control strategies for livestock in the area.

2.2 General objective

1. To document parasites of economic importance infesting small stock in the north eastern Free State, South Africa.

2.3 Specific objectives

1. To use Giemsa-stained blood smears to investigate the haemoparasites infesting sheep and goats in the north-eastern Free State;
2. To identify sheep and goats exposed to *Anaplasma* and *Theileria* infections using indirect methods such as Enzyme Linked Immunosorbent Assay (ELISA) and Indirect Fluorescent Antibody Test (IFAT), respectively.
3. To identify tick species infesting sheep and goats in the same region.
4. To determine the density, distribution, relative abundance and seasonal dynamics of these parasitic infections.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of study area

3.2 Experimental location

The study was carried out from September 1999 to August 2000 in the north-eastern Free State Province in three areas, namely Harrismith, Kestell and Qwa-Qwa, which are located 40, 28 and 5 km respectively away from the University of the North Qwa-Qwa Campus (Vrey and Smith, 1980). The geographical position of the Free State Province in South Africa is indicated in Figure 4. The location of Harrismith, Kestell and Qwa-Qwa in the Free State Province is shown in Figure 5.

3.3 Topography and altitude

The eastern Free State is a predominantly hilly and mountainous area situated between latitude 20° and 30°S, and 28° and 30°E. It is bounded to the south by Lesotho and to the east by KwaZulu-Natal Province. It is situated at a height of about 1 500 to more than 3 000 m above sea level (Kritzinger and Pieterse, 1987).

3.4 Geology

The eastern Free State Province with its beautiful landscape consists of the rocks of the Karoo super-group, of the Beaufort and Stormberg groups, with the soil types belonging to the Clovelly-Avalon, Katspruit, Kroonstad-Katspruit and Sterkspruit. Numerous dolerite dykes and occasional sills have intruded the Karoo formations and a few small dolerite diatremas have also been recorded in Qwa-Qwa which contain sedimentary breccias (Kritzinger and Pieterse, 1987).

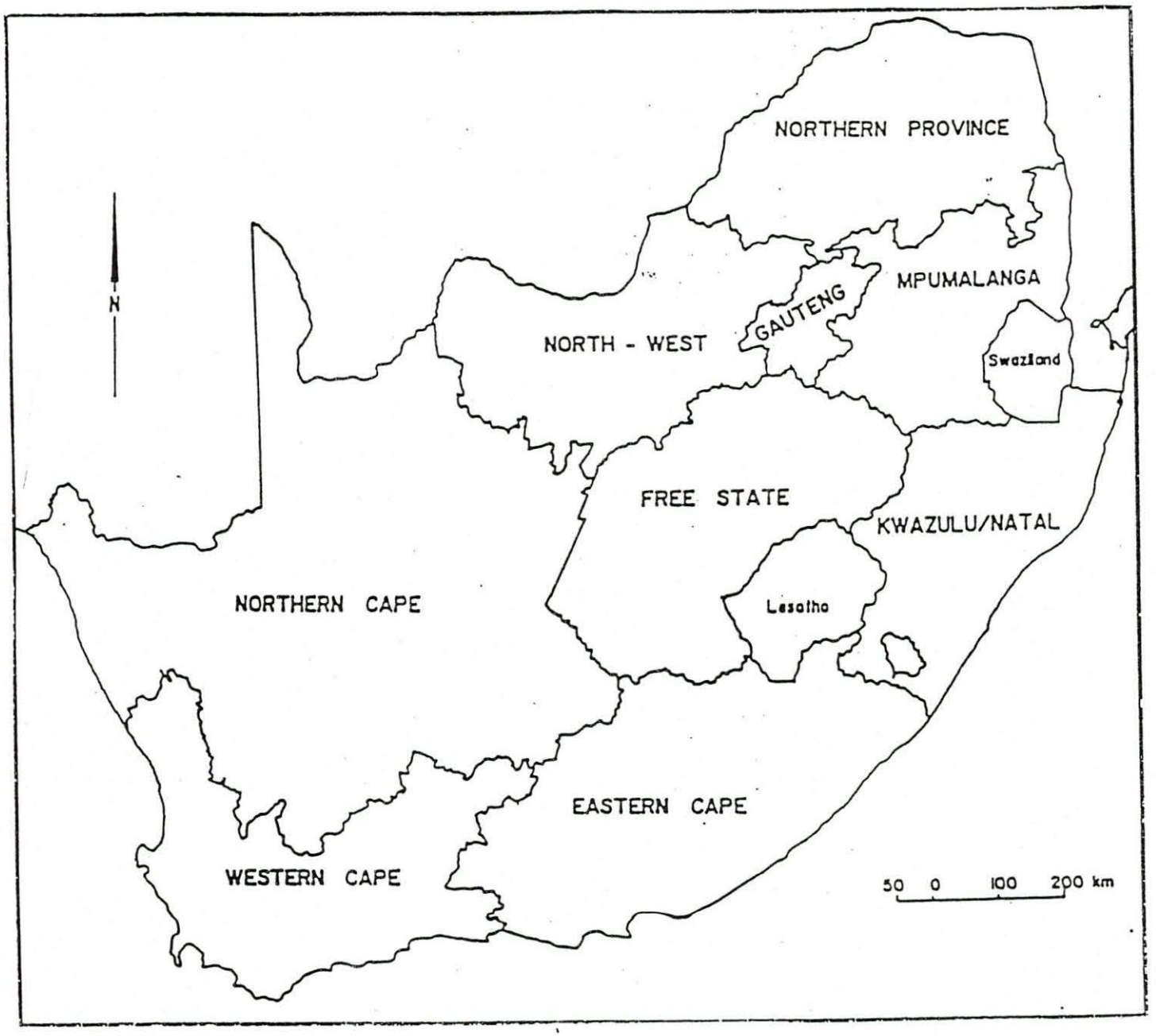

Figure 4: Location of the Free State Province in the National South African context (Adapted from Krige, 1995)

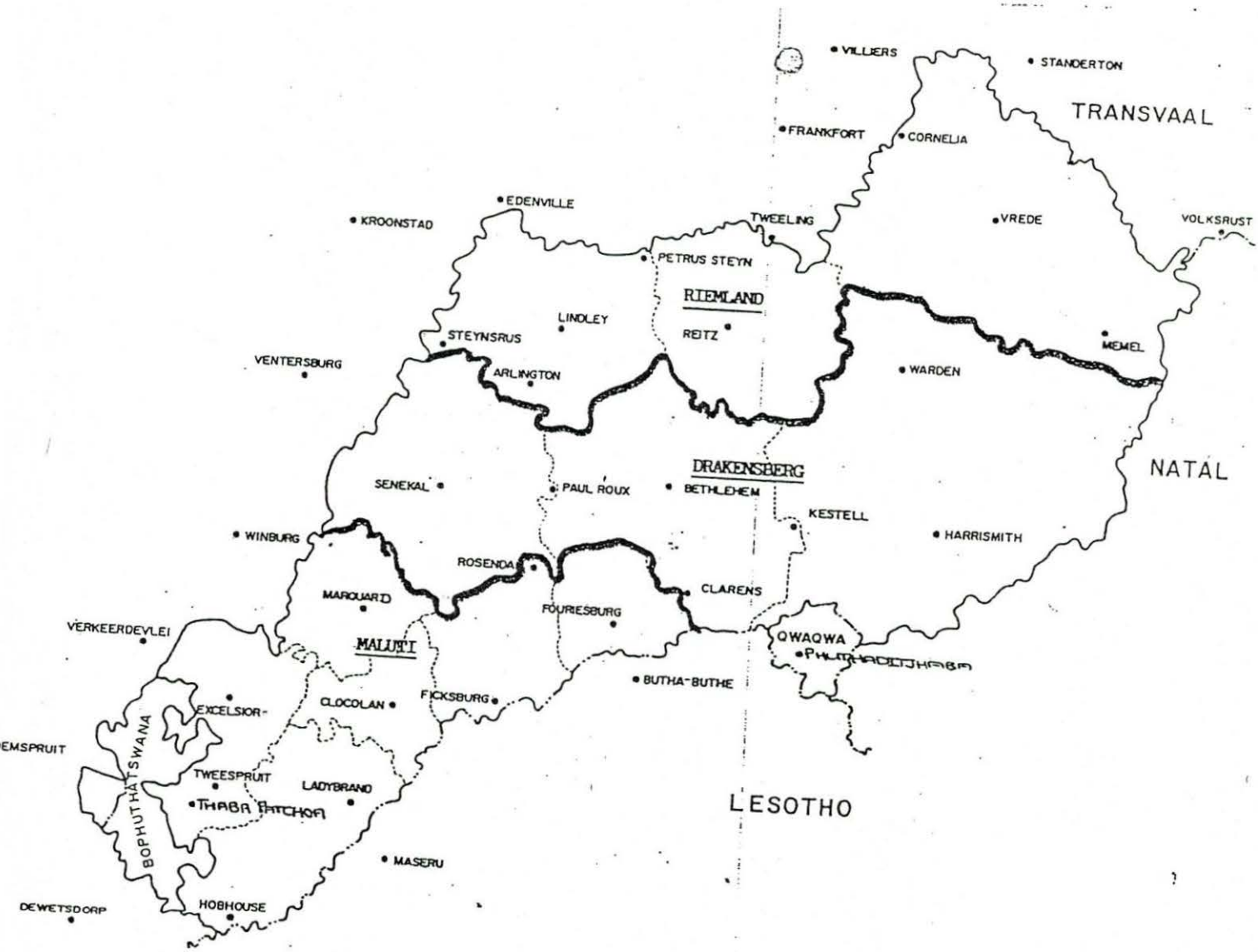

Figure 5: Location of study sites, Harrismith, Kestell and Qwa-Qwa

3.5 Climate

Climate is one of the most significant and unalterable sources of uncertainty influencing African farming systems. In attempting to develop technologies or frame policies for African farming, it is very crucial to understand the influence of climate in African ecosystems (Ellis, 1994).

3.5.1 Rainfall

The eastern Free State lies within the summer rainfall region of South Africa with more than 85% of the precipitation occurring during the period of September to March, mainly in the form of thunderstorms. The annual precipitation on the lower lying plateau, occurring in the Harrismith and Kestell localities is approximately 700 mm per annum, increasing gradually to approximately 1340 mm per annum in Qwa-Qwa (Drakensberg mountains) as one progresses in a southerly direction. Hail storms are common, occurring three to five times per annum. Snowfall occurs in the high-lying south with the heaviest fall occurring towards the end of June (Kritzinger and Pieterse, 1987).

3.5.2 Atmospheric temperature

The temperature in the eastern Free State Province may be described as being cool to moderately warm. The average temperature during mid-winter is 7°C and during mid-summer is 17.9°C. The average daily minimum temperature during winter is 4.6°C and the average daily maximum temperature during summer is 29.9°C. Cold spell temperature decreases in excess of 5°C can occur many times a year, lasting two to three days per year. Hot spells with temperatures in excess of 35°C also occur for four to five days at a time. During September and October temperatures are regarded to be unstable, but during April and May are quite stable (Kritzinger and Pieterse, 1987). The atmospheric temperatures for 1999-2000, obtained from South African Weather Bureau

(SAWB), ranged from a mean monthly of about 6°C to a mean monthly of 19°C in summer.

3.6 Vegetation

The eastern Free State Province of South Africa belongs to the grassland biome, with five vegetation types namely: moist cool highveld, moist cold highveld, wet cold highveld, afro-mountain and alti-mountain grassvelds (Moffet, 1997). The vegetation can also be described as highland sourveld which changes into Themeda Festuca veld at heights in excess of approximately 2 200 m. In the almost pure sourveld, red grass (*Heteropogon contortus*) predominates and these are easily reduced, through over-grazing, to tough grass (*Eragrotis plana*) and wiregrass (*Elionums argenteus*) (Kritzinger and Pieterse, 1987).

Trees and grasses are fairly rare while *Leucosidea sevicea*, *Trimeria grandifolia* and *Hereomorpha arborescens* are most common. When overgrazing occurs, the first mentioned type (*Leucisidea sevicea*) tends to become hardy. A few examples of upright yellow wood (*Podocarpus latifolius*) occur in sheltered ravines (Kritzinger and Pieterse, 1987). In the Themeda-Festuca alpine veld, mostly red and wiregrass and *Festuca costata* is to be found. The last two grass types are unpalatable grasses. The shrubs are limited to mainly *Leucosidea sericea* and *Erica-Helichrysum* (Kritzinger and Pieterse, 1987).

3.7 Collection of blood specimens from small stock

Blood samples were collected from sheep (Plate 1) and goats (Plate 2) from the jugular vein. The study was conducted over a period of twelve months from September 1999 to August 2000, with estimated sample size of ten sheep and ten goats randomly sampled monthly from all three study sites. Thus ten sheep

plus ten goats per month multiply by the three regions (10x12x3) will give 360 sheep and 360 goats.

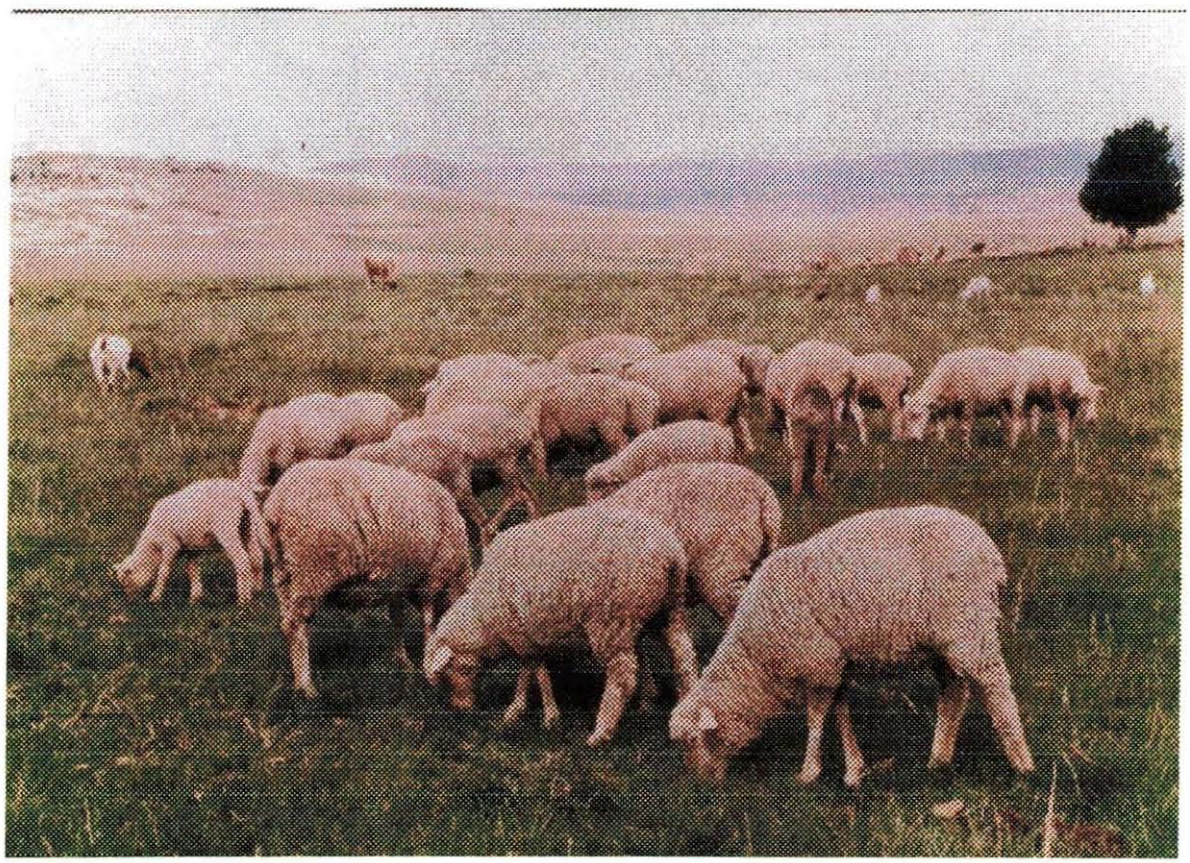

Plate 1: A flock of sheep grazing on a farm in Qwa-Qwa

Plate 2: A flock of goats grazing on a farm in Qwa-Qwa

During the course of the project it was established that most of the farmers around the north eastern region of Free State Province keep more sheep than goats, thus the imbalances in terms of sampled of number between sheep and goats.

During the day these animals graze on communal pastures for between 7 to 9 hours, depending on the season. At night animals are herded and kept in kraals with manure floor and are individually restrained within the kraal.

Using a nineteen gauge needle, approximately 2 ml of blood was collected from the jugular vein situated in the neck region for both sheep and goats into a sterile 7 ml EDTA coated vacutainer tube. The blood sample was mixed with EDTA to prevent clotting. The vacutainer was labelled and put into a dust-proof box to prevent direct contact with sunlight and dust. Another clean nineteen gauge needle was used to draw 10 ml of blood into a sterile non-silicone coated vacutainer containing no anti-coagulants. The vacutainer was labelled and stored in a dust-proof box. The vacutainers were transported to the laboratory for further analysis.

3.8 Preparation of thin smears

In preparing thin and thick smears blood samples from the EDTA vacutainer were used. Standard glass microscope slides were used for preparation of smears. The slides were initially washed with a mixture of 50% Ethanol: 50% Acetone and wiped with an absorbent material before use.
A drop of blood was picked up with the corner of a microscope slide (the spreader slide) from the vacutainer. Another clean microscope slide was held firmly on a flat surface and the drop of blood on the corner of the spreader slide was transferred to the centre of the second slide but about 1cm from its frosty

edge. The drop of blood was spread by touching the edge of the spreader to the other slide at an angle of 30°, slightly in front of the droplet, and then gently drawing the spreader backwards until it touches the droplet. The blood was allowed to flow along the width of the spreader. The spreader slide is, whilst held against the smear slide, moved forward quickly and smoothly away from the drop of blood towards the far end of the microscope slide, drawing the blood with it. This left a characteristic-shaped smear which tails off towards its end. The slide with the smear was left to air-dry. The slide was fixed with pure methanol to remove any impurities and left to air-dry. The slide was put into an immersion tank containing 10% Giemsa stain (Appendix 1-2) solution and stained for thirty five minutes. The slide was then removed from the Giemsa stain and washed with double distilled water and was allowed to air dry in a slide rack (Ondesrtaepoort Veterinary Institute, (OVI), 1999).

3.9 Preparation of thick smears

With the thick blood smear, a fairly big drop of blood was placed on the other side of the thin smear. Thick smears differ from the thin ones in that the blood is not spread over a large area and is not fixed before staining, thus allowing lysis of the red blood cells and concentration of the parasites. The slide was left to air dry. The slide was put into an immersion tank containing 10% Giemsa stain solution (Appendix 1-2) and stained for thirty five minutes. The slide was then removed from the Giemsa stain and washed with double distilled water and slide was allowed to air dry in a slide rack. Then a drop of 518C immersion oil (Zeiss Germany) was put on both thick and thin smears. The slides were then examined using a standard light microscope at x1000 magnification (eyepiece, x10; objective x100) (De Waal, 1999).

3.10 Determination of anaemia by Packed Cell Volume (PCV)

Micro-haematocrit tubes (Brand, PCS100) were filled with blood samples from EDTA tubes collected from sheep and goats and centrifuged in a micro-haematocrit (KHT 400) for fifteen minutes. Using a micro-capillary reader (KHT 400), the PCV of both sheep and goats was measured and recorded.

3.11 Serodiagnosis of serum samples

The serum samples obtained from the non-silicone coated vacutainer was used to determine presence of antibodies against various haemoparasites. The labelled vacutainer was centrifuged for thirty minutes at 2000 rpm. The serum was put into sterile cryovial bottle (Simport Plastics, PK 100), labelled and stored at -34^{O}C until used for serological assays for the different parasitoses.

Techniques used for sero-diagnosis were:

3.11.1 Competition Inhibition Enzymed Linked Immunosorbent Assay (ELISA)

Competition inhibition type of ELISA was used as a diagnostic tool to determine *Anaplasma* antibodies in both sheep and goats (Molloy *et al.*, 1998; Ndungu *et al.*, 1995; Voller *et al.,* 1976). A lisate made from a recombinant *Entamoeba coli* that expresses an immunogenic major surface protein 5 (MSP5) *of Anaplasma marginale* was used as an antigen (Appendix 1-1). The test sera and monoclonal antibody (MoAb) that is specific against the MSP 5 antigen compete for binding sites to the antigen on the ELISA plate. The conjugated anti-mouse antibody bind to the monoclonal and after addition of the substrate there is a colour reaction that can be read with an ELISA reader. The higher the concentration of *Anaplasma* antibodies in the test sera the lower the absorption reading, due to a lower concentration of MoAb binding to the antigen. High

absorbance values therefore indicate high concentration of mouse MoAb to the antigen, indicating absence of *Anaplasma* antibodies in test sera (OVI, 1999).

Binding: 4 µl of antigen was diluted in 11ml PBS for each ELISA plate (Figure 6) that was used. The plates were covered and incubated overnight at 4°C. Undiluted antigen was stored at -20°C.

Washing: The following day, the plate was rinsed-filled with PBS containing 0.1% Tween 20 using a strip washer and flipped empty. The plate was then washed-filled with PBS plus 0.1 % Tween 20 and put in the orbital shaker for five minutes, flipped empty and tap dried.

Blocking: The plate was filled with 225µl of PBS containing 5 % Elite milk powder and covered with lids. The plate was placed in an incubator at 37°C for 1-2 hours. NOTE: one hour is sufficient, the reason for 1-2 hours was only to allow enough time to arrange the sera numerically and make dilutions.

Sera dilutions: The blocking buffer was expelled (no rinsing or washing was done after blocking step just slapping the inverted ELISA plate dry on a towel. Each well on the plate was filled with 90 µl of PBS containing 1% Elite milk powder and 0.1 % Tween 20. Sera was added into the wells according to the plate lay-out, 10 µl into each well. The plate was incubated for thirty minutes at 37°C. NOTE: The plate contents must not be thrown away after incubation because monoclonal antibody must be added to the serum in the plate.

Monoclonal antibody: 2 µl of monoclonal antibody was diluted in 5 ml of PBS containing 1% Elite milk powder solution and 0.1% Tween 20 for each plate. 25 µl of monoclonal was added to each well without removing the diluted sera

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	1		9		LPC 1 %	17		25		33	
B	NC	2		10		LPC 0,5 %	18		26		34	
C	NC	3		11		NC	19		27		35	
D	NC	4		12		NC	20		28		36	
E	NC	5		13		NC	21		29		37	
F	NC	6		14		PC	22		30		38	
G	*PC*	7		15		*NC*	23		31		39	
H	*NC*	8		16		NC	24		32		40	

Figure 6: Diagram of a 96 well ELISA plate used for serodiagnosis of haemoparasites

Figure 6: Diagram of a 96 well ELISA plate used for serodiagnosis of haemoparasites

already in the plate. The content of the plate was mixed using the orbital shaker and incubated for sixty minutes at 37^0C.

Washing: The plate was rinsed with PBS plus 1% Tween 20 and flipped empty. It was washed for the second time with PBS plus 0.1% Tween 20 and placed on the orbital shaker for five minutes. The plate was flipped empty and tap dried.

Conjugate: 4 µl of antigoat (Ig) was diluted in 11 ml PBS containing 1% milk powder and 0.1 % Tween 20 and thereafter 100 µl was added to each well. The plate was incubated for thirty minutes at 37^0C. NOTE: It should be noted that Elite milk powder must not be used with the Avidin and Biotin part. Dilute the Avidin and Biotin part of the conjugate as follows: 8 µl of part A was added to 11 ml of PBS with 0.1 % Tween 20 and 8 µl of part B to the same 11 ml. [Part A (Avidin) and B (Biotin)] were mixed in the same 11 ml). The plate was incubated at 37^0C for thirty minutes. Positive and negative controls were put into the first two rows of wells because all the samples were tested in duplicate.

Washing: The plate was rinsed once and washed twice with PBS 0.1 % Tween 20. 100 µl of A+B conjugate per well was added into the plate and incubated for thirty minutes at 37^0C. The plate was once again rinsed and washed twice with PBS 0.1% Tween 20.

Substrate: 0.01g of P-nitrophenol phosphate was weighed and added to 11 ml of substrate buffer, mixed and 100 µl added to each well. The plate was incubated for thirty minutes at 37^0C and read at 405 nm (OVI, 1999).

Interpretation of the test

The mean and standard deviation of seven negative control samples each in duplicate were determined. The cut-off point for positive samples was the mean value of the negative samples minus 3 times the standard deviation [x (negative sample) – 2x(Standard Deviation of the x) = cut-off point]. Values higher than the cut-off point are negative and lower are positive. One positive control serum in duplicate was built in with the negative controls (Ross and Lohr, 1968).

3.11.2 Indirect Fluorescent Antibody Test (IFAT)

3.11.2.1 Introduction

What is fluorescence? Certain chemicals called fluorochromes are excited by short wavelength light (ultra-violet) which cause rearrangement of their structure. Consequently, these fluorochromes emit visible light of longer wavelength which is called fluorescent light. The fluorochrome in its ground state (unexcited state) absorbs energy. The amount of energy absorbed depends on the characteristics of the molecule. Light is also a form of energy which implies that the wavelength (amount of energy) used is also depending on the molecule used. With the absorption of energy the fluorochrome is excited to a higher level. The excited molecule is unstable and looses some of its energy, for example as heat. The still excited (but at a lower energy level) molecule then returns to its ground state emitting light. The wavelength of emitted light is always longer than the wavelength of the absorbed light because of the energy lost in the beginning. Examples of fluorochromes in common usage are fluorescein isothiocyanate (FITC) and tetramethyl rhodaine isothiocyanate (TRITC) (Price and Newman, 1991; Harith *et al.*, 1986).

The Indirect Fluorescent Antibody Test makes use of a known antigen to determine the presence of antibody in the serum (Joyner *et al.*, 1972). The

antigen/antibody complex is visualised by staining the complex with secondary antibody conjugated with a fluorochrome. The secondary antibodies for the conjugate are prepared in an unrelated animal species. An advantage of this test is that a batch of antigen can be prepared and frozen away for future use. This test only indicates that the animal has been exposed to the particular parasite, but does not necessarily represent immunity to the parasite (OVI, 1999).

Indirect Fluorescent Assay Test was used to identify the presence of *Theileria* antibodies in both sheep and goats sera. Antigen slides (Figure 7) contained in plastic pockets, test and control sera were taken from the freezer at -20^{0}C and placed in the incubator at 37^{0}C for ten minutes (OVI, 1999). Serum samples were marked numerically and annotated in serological result sheets. Test tubes were placed in the test tube rack and marked from the left to the right side numerically to correspond to serum numbers. 2 ml of PBS was placed into the test tubes for the 1/80 dilution *i.e* 1 ml serum + 79 ml PBS. The antigen slides were removed from their protective covering and fixed in cold acetone for one minute. The slides were marked *T. separata*. A drop of diluted serum was placed on the antigen slide. The antigen slide was incubated in a humid chamber at 37^{0}C for one hour. After incubation, the antigen slide with serum dilutions was rinsed by dipping the slides into a container containing +/- 200 ml PBS.

After rinsing, the slide was placed into another container with 1L PBS and washed on a magnetic stirrer at very low revolutions for ten minutes. The slide was then washed again in distilled water for five minutes using a magnetic stirrer as above. A conjugate was prepared at the dilution of 1/80, 0.5 ml/slide. Excessive distilled water was shaken off the slide and the slide was covered completely with the conjugate dilution. The antigen slide was incubated in a humid chamber at 37^{0}C for one hour. After incubation, the slide was rinsed in

fresh PBS and washed in PBS for ten minutes using a magnetic stirrer. The slide was left to air dry. One drop of 50% glycerine in PBS was placed on the slide and the slide was covered with a 24x50 mm cover slip. The slide was examined

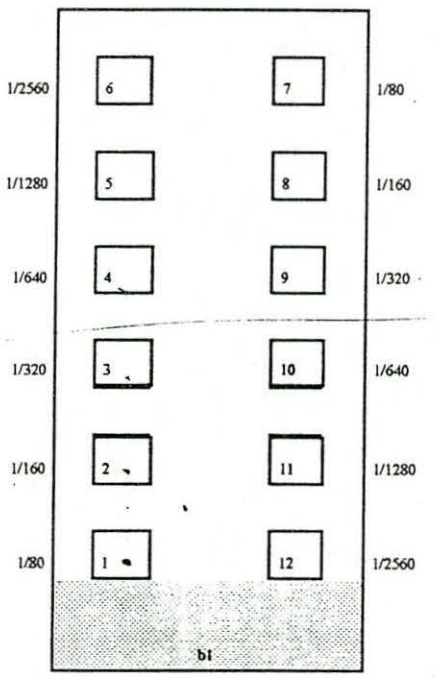

Figure 7: A diagrammatical representation of an antigen slide used for IFAT

under a fluorescent microscope at 500x magnification (eyepiece 5x; objective x10). The slides was stored at 4^0C in the dark for 4-5 days before reading.

3.11.2.2 Interpretation of IFA Test

Test readings were based only on specific fluoresence in the parasites and were graded as – (negative), ± (positive/negative) and + (positive). A negative reading was made when no specific fluorescence was recognisable in parasites, although their outlines were sometimes discernible. Doubtful readings (±) were those where the presence of weak fluorescence was arguable. The strong positive reading (+) at serum dilution of 1/80 was one with fluorescence so bright that parasitic cellular structure was indistinguishable, also most host-cells showed varying degrees of staining (OVI, 1999).

3.12 Identification of ticks

The three study sites (Harrismith, Kestel and Qwa-Qwa) were each visited on a monthly basis for tick sampling between September 1999 and August 2000. Ticks were simultaneously collected from the same group of sheep and goats from which blood had been taken for sero-diagnosis of haemoparasites. Adult female and male ticks in all stages of engorgement were collected from selected body regions of the hosts. This method of counting ticks on selected regions is generally used to estimate the overall level of tick infestation on animals (Hermans *et al.*, 1994; Dreyer *et al.*, 1998)

Animals were individually physically restrained within the kraal. The body was divided into three different regions and ticks, from each region, were collected separately (Dreyer *et al.*, 1998). These regions corresponded to anatomical regions namely the ear pinna, anal area and scrotum or udder. The position of attached adult ticks was first checked by visual examination and secondly

determined by means of palpation with the palm of the hand and fingers through the hair coat on the body of animals. The ticks were removed by entomological forceps from each of the defined body regions and placed in labelled containers filled with 70% alcohol (Appendix 1-3). Only adult ticks were collected over the 12 month period and no larval-nymphal stages were collected from any of the three body regions.

The collected ticks were counted, identified and recorded to species level using a standard stereo microscope. Identification was done according to methods of Hoogstraal (1956) and Walker (1961). The data for each body region of each individual animal was recorded separately.

3.12.1 Data presentation and analysis

The data sets were analysed statistically using the appropriate analysis of variance techniques. All statistical data analysis was done on Mercer-desktop computer. The software program used was Statistical Analysis System (SAS). The chi-square distribution was used in testing the hypothesis of independence between different variables of classifications. Any random variable of chi-square distribution is denoted by the statistics χ^2 (Wagner, 1992; Walpole, 1983). The statistics has been squared to emphasize the fact that χ^2 values can't be negative and consequenstly the chi-square distribution can't be sympatric about a vertical line through the origin. An unpaired Student's t-test was used to compare burdens of various tick species across the three study sites (Zar, 1974). A one way analysis of variance (ANOVA) test (Barnard *et al.*, 1993) was used to determine whether significant difference occurred between serodiagnostic tests and tick counts. A significant level of $p<0.05$ was used throughout.

3.13 Physical condition scoring

The condition scoring method as describe by Van Niekerk and Louw (1990) was partially used. The system is based on a 4-point scale with a score of 1 representing an extremely thin animal, and a score of 4 is for an over fat animal (Table 1). Animals were not weighed due to lack of facilities in the area. Since the study was a survey, random physical conditions for the animals were assessed monthly.

Table 1: The condition scoring chart used in the north-eastern region of Free State areas of Harrismith, Kestel and Qwa-Qwa, to assess physical condition of sheep and goats (after Van Niekerk and Louw, 1990).

Area	Score		
	Poor	Fair	Good
	1	2.5 - 3	4
General feature of animals	Animal appear emaciated	Ideal condition	Overfat animal

CHAPTER 4

RESULTS

The study was conducted over a period of 12 months from September 1999 to August 2000 on the grazing areas of Harrismith, Kestell and Qwa-Qwa. A sample size of at least 10 animals of each species was randomly sampled every month. Blood samples were collected from a total of 371 sheep and 188 goats from which *Anaplasma* and *Theileria* infections were screened parasitologically and serologically. The Packed Cell Volume (PCV) of animals was determined and recorded for each individual animal. Ticks were collected from three body regions and identified to species level.

4.1 Serodiagnosis for *Anaplasma* and *Theileria* species in sheep and goats

4.1.1 Serodiagnosis results of sheep

An Indirect Fluorescent Antibody Test was used in the laboratory to detect levels of antibody of *Theileria* species in sera of sheep over a period of 12-months (Table 2). At a dilution titre of 1/80 all the sera for sheep (n=371) were seropositive for *Theileria* species by IFAT, indicating that they had been exposed to *Theileria*. Results of serodiagnosis for *Theileria* by IFAT did not differ across the three areas studied (χ^2: Df=1 at $p<0.05$) (Plate 3).

Table 2: Results of serodiagnosis for *Theileria* species by IFAT across Harrismith, Kestell and Qwa-Qwa for sheep

Sites	Number of sampled animals	Number of samples +ve by IFAT at ≤ 1/80 dilution
Harrismith	105	105/105 (100%)
Kestell	137	137/137 (100%)
Qwa-Qwa	129	129/129 (100%)
Total number of sampled animals	371	371/371 (100%)

Note:

Number in parenthesis indicates percentage value

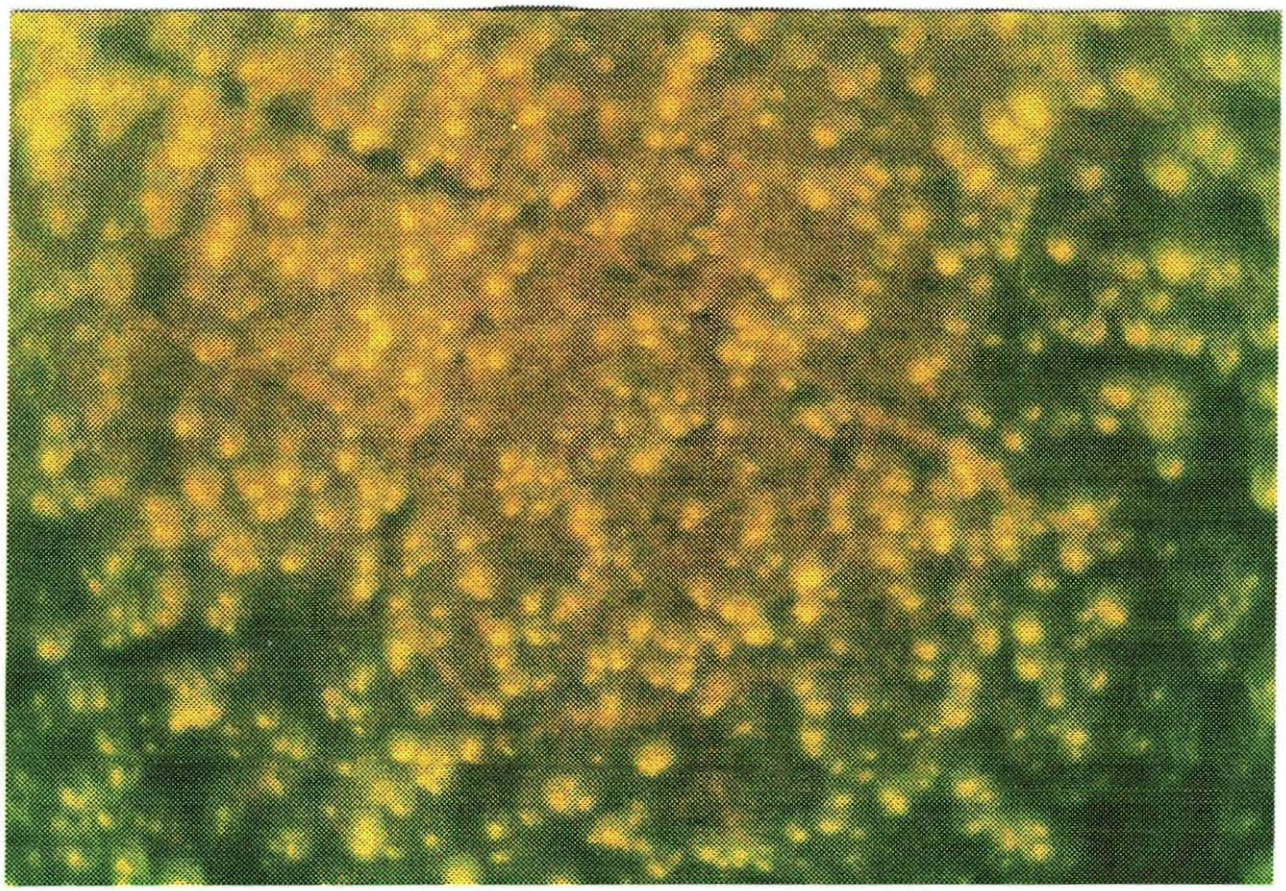

Plate 3: Fluorescence from a positive IFAT result in the diagnosis of theileriosis in sheep

The results of serodiagnosis for *Anaplasma* showed that Harrismith had the highest number of seropositive animals followed by Kestell and Qwa-Qwa (Table 3).

Table 3: Results of serodiagnosis for *Anaplasma* by ELISA across Harrismith, Kestell and Qwa-Qwa on sheep

Study Sites	ELISA *(Anaplasma)*	
	Number of negative animals	Number of positive animals
Harrismith	6/105 (5.7%)	99/105 (94.3%)
Kestell	25/137 (18.25%)	112/137 (81.7%)
Qwa-Qwa	24/129 (18.6%)	105/129 (81.4%)
Total number of sampled animals	55/371 (14.82%)	316/371 (85.18%)

Note:

Number in parenthesis indicates the percentage value

A total of 371 sheep were sampled to determine their physical condition in Harrismith, Kestel and Qwa-Qwa. In Kestell a 100% of the total number of animals sampled were in fair physical condition followed by Qwa-Qwa with 99.2%, whereas Harrismith had 73% of its sheep in good physical condition (Table 4).

Table 4: Comparison of sheep results for physical condition by sites

Study Sites	Physical condition	
	Fair score of 2-3	Good score of 3-4
Harrismith	28/105 (26.6%)	77/105 (73%)
Kestell	137/137 (100%)	0/137 (0%)
Qwa-Qwa	128/129 (99.2%)	1/129 (0.3%)
Total number of sampled animals	293/371 (78.97%)	78/371 (21.03%)

Note:

Number in parenthesis indicates the percentage value

4.1.2 Serodiagnosis results for goats

At a dilution titre of 1/80, all sera of goats (n=188) were seropositive for *Theileria* species by IFAT. There was no significant difference on the IFAT values across the three study sites (χ^2: Df=1, $p<0.05$) (Table 5).

Table 5: Results of serodiagnosis for *Theileria* by IFAT across Harrismith, Kestell and Qwa-Qwa on goats

Sites	Number of sampled animals	Number of samples +ve by IFAT at ≤ 1/80 dilution
Harrismith	65	65/65 (100%)
Kestell	62	62/62 (100%)
Qwa-Qwa	61	61/61 (100%)
Total number of sampled animals	188	188/188 (100%)

Note:

Number in parenthesis indicates percentage value

The results of the ELISA test for *Anaplasma* species for goats over the 12-months period indicated that 100% of sampled animals were seropositive in Harrismith, Kestell and Qwa-Qwa (χ^2: Df=1, $p<0.05$) (Table 6).

Table 6: Results of serodiagnosis for *Anaplasma* by ELISA across Harrismith, Kestell and Qwa-Qwa on goats

Sites	Number of sampled animals	Number of +ve samples by ELISA
Harrismith	65	65/65 (100%)
Kestell	62	62/62 (100%)
Qwa-Qwa	61	61/61 (100%)
Total number of sampled animals	188	188/188 (100%)

Note:

Number in parenthesis indicates percentage value

A total of 188 goats were assessed for their physical condition. Approximately 90% could be described as being in fair physical condition. Kestell was the only site that reported the highest number of goats in good body condition (Table 7).

Table 7: Comparison of results between study sites and the physical condition of goats.

Sites	Physical condition	
	Fair of score 2-3	Good of score 3-4
Harrismith	65/65 (100%)	0/65 (0%)
Kestell	42/62 (67.7%)	20/62 (32.25%)
Qwa-Qwa	61/61 (100%)	0/61 (0%)
Total number of sampled animals	168/188 (89.4%)	20/188 (10.6%)

Note:

Number in parenthesis indicates percentage value

Approximately 79% of sheep (n=371) and 80% goats (n=188) sampled had fair body condition and were seropositive for *Theileria* by IFAT (Table 9).

Table 9: The general association between IFAT for *Theileria* and physical condition of sheep and goats in Harrismith, Kestell and Qwa-Qwa.

Animal species	Number of +ve samples by IFAT at ≤ 1/80 dilution	Physical condition	
		Fair score of 2-3	Good score of 3-4
Sheep	371	293/371 (78.98%)	78/371 (21.02%)
Goats	188	152/188 (80.85%)	36/188 (19.15%)

Note:

Number in parenthesis indicates percentage value

There was no association between *Theileria* infection and age of goats across the three study sites (χ^2: Df=0.873, $p<0.05$) (Table 10).

Table 10: Results of an association between IFAT for *Theileria* and age of goats.

Study sites	Number of +ve samples by IFAT at ≤ 1/80 dilution	Age	
		Adult	Young Adult
Harrismith	65	60/65 (92.3%)	5/65 (7.7%)
Kestell	62	60/62 (96.77%)	2/62 (3.23%)
Qwa-Qwa	61	58/61 (95.1%)	3/61 (4.9%)

Note:

Number in parenthesis indicates percentage value

4.2 Results of the parasitological screening for *Anaplasma* and *Theileria*

Results of parasitological screening of goats and sheep by Giemsa-stained thick and thin smears for *Anaplasma* and *Theileria* species over 12-months period indicated that all blood smears were negative in the three study sites (Tables 11 and 12).

TABLE 11: Results of parasitological screening of goats by Giemsa-stained thick and thin smears for *Anaplasma* and *Theileria* in Harrismith, Kestell and Qwa-Qwa.

Month/Year	Number of animals screened in Harrismith	Number of animals screened in Kestell	Number of animals screened in Qwa-Qwa	Presence of *Anaplasma*, and *Theileria*
September (1999)	5	5	5	-ve
October (1999)	5	5	5	-ve
November (1999)	5	6	5	-ve
December (1999)	5	5	5	-ve
January (2000)	5	5	5	-ve
February (2000)	6	5	5	-ve
March (2000)	5	5	6	-ve
April (2000)	6	5	7	-ve
May (2000)	6	7	4	-ve
June (2000)	5	4	4	-ve
July (2000)	7	5	5	-ve
August (2000)	5	5	5	-ve
TOTAL	n=65	n=62	n= 61	n=188

Key:

-ve means negative for the blood parasites indicated above.

Table 12: Results of parasitological screening of sheep by Giemsa-stained thick and thin smears for *Anaplasma* and *Theileria* in Harrismith, Kestell and Qwa-Qwa.

Month/Year	Number of animals screened in Harrismith	Number of animals screened in Kestell	Number of animals screened in Qwa-qwa	Presence of *Anaplasma* and *Theileria*
September (1999)	10	15	10	-ve
October (1999)	10	10	10	-ve
November (1999)	10	10	10	-ve
December (1999)	10	10	10	-ve
January (2000)	10	10	10	-ve
February (2000)	10	13	14	-ve
March (2000)	14	10	10	-ve
April (2000)	6	10	11	-ve
May (2000)	7	16	13	-ve
June (2000)	5	13	10	-ve
July (2000)	7	10	10	-ve
August (2000)	7	10	11	-ve
TOTAL	n= 106	n=137	n=129	n= 371

Key:

-ve means negative for the blood parasites indicated above.

Results for the analysis of variance for the packed cell volume for sheep and goats in all three study sides over a 12-months period is shown in Appendices 17. The average PCV throughout the study period was 28% for both sheep and goats. The PCV values were not significantly different throughout the study period by months and fell within the normal PCV range of 26-30% for sheep and goats and it was similar to a study conducted in Eastern Cape on small livestock (Horak *et al.*, 1991).

4.3 Results of tick collection and identification

4.3.1 Diversity and relative abundance

A total of 1567 adult ticks belonging to two species were collected over the 12-months investigative period. Tick species, in decreasing order of relative abundance were: *Rhipicephalus evertsi evertsi* (68%) and *Boophilus decoloratus* (32%). The mean *Rhipicephalus evertsi evertsi* burden per host was 8.2±3.4 and that of *Boophilus decoloratus* was 4.1±1.7 on both sheep and goats.

Table 13 indicates the mean and standard deviation of ticks collected monthly in Harrismith, Kestell and Qwa-Qwa from both sheep and goats on three body regions. A seasonal pattern in the distribution of ticks was observed throughout the project and is discussed under section 5.5.

Table 13: The mean and standard deviation of total number of ticks on ear, anal and scrotum/udder regions on monthly collection

Month/Year	Mean and standard deviation	
	Sheep	Goats
September (1999)	8 ± 4.6	4.5 ± 3.18
October (1999)	13 ± 9.16	4.5 ± 3.18
November (1999)	18.3± 10.5	9 ± 2.7
December (1999)	15.3± 8.85	18 ± 13.07
January (2000)	21.3± 12.3	24 ± 6.97
February (2000)	20.3± 11.7	14 ± 9.89
March (2000)	17.6 ±10.2	12.5± 8.83
April (2000)	7.6 ± 4.43	4.5 ± 3.18
May (2000)	4.3 ± 3.3	3 ± 2.12
June (2000)	3 ± 1.73	1 ± 0.707
July (2000)	3.3 ± 1.92	5 ± 3.5
August (2000)	7.3 ± 4.23	7 ± 4.94

4.3.2 Distribution

Rhipicephalus evertsi evertsi was the most prevalent tick species infecting sheep and goats in all three study sites (Table 14).

Table 14: The total number of tick species collected from sheep and goats respectively from all three study sites

Study sites	Sheep		Goats	
	R. e. evertsi	*B. decoloratus*	*R. e. evertsi*	*B. decoloratus*
Harrismith	30/160 (56%)	45/120 (47%)	70/160 (43%)	35/80 (43%)
Kestell	178/298 (60%)	65/120 (55%)	120/298 (40%)	55/120 (45%)
Qwa-Qwa	345/607 (56%)	185/301 (62%)	262/607 (44%)	116/301 (38%)

Note:

Number in parenthesis indicates percentage value

Two tick species were identified from the three study localities over the 12-months period. *Rhipicephalus evertsi evertsi* was the most abundant species on goats and sheep grazing in the north eastern region of the Free State (Table 15).

TABLE 15: The average percentage of ticks collected from both sheep and goats in the north-eastern Free State.

Study sites	Tick species	
	B. decoloratus	*R. e. evertsi*
Harrismith	80/501 (16%)	160/1065 (15%)
Kestell	120/501 (24%)	298/1065 (28%)
Qwa-Qwa	301/501 (60%)	607/1065 (57%)

Note:

Number in parenthesis indicates percentage value

Table 16 indicates the mean and standard deviation of ticks collected monthly from three body parts examined for both sheep and goats. When comparing these tick counts per body region, those of the goat's ears was higher than those of the anal and scrotum/udder regions ($F_{0.05(0.709)}$=0.07 at p>0.05). For the sheep, the number of ticks collected in the anal region was significantly higher (p>0.05) than those of both the ear and scrotum/udder regions and same for the goats.

TABLE 16: The mean and standard deviation of *R. e. evertsi* and *B. decoloratus* ticks counted monthly on sheep and goats in Harrismith, Kestell and Qwa-Qwa.

Animal species	Body regions	Study sites		
		Qwa-Qwa	Kestel	Harrismith
Goats	Ear	1.3±0.29	1.26±0.3	1.3±0.33
	Anal	2.61± 0.44	2±0.4	1.05±0.28
	Scrotum/Udder	1.12 ±0.29	1.03±0.2	1.1±0.31
Sheep	Ear	0.9± 0.25	2.2±0.41	1.125±0.30
	Anal	2.67±0.48	3.45±1.2	2.24±0.42
	Scrotum/Udder	1.3±0.34	1.4±0.37	1.16±0.29

4.4 Distribution of ticks attaching to different body regions on goats

A survey of monthly distribution of tick abundance on goats in the three body regions indicated a rise in tick count between September 1999 and December 1999. The ear region had two peaks observed, the first one between September 1999 and December 1999 and the second between February 2000 and April 2000. The anal region had also two peaks observed between September 1999 and December 1999 and a lesser peak during December 1999 and February 2000. The scrotum or udder area had a low number of tick counts as compared to the anal and ear region (Figure 8).

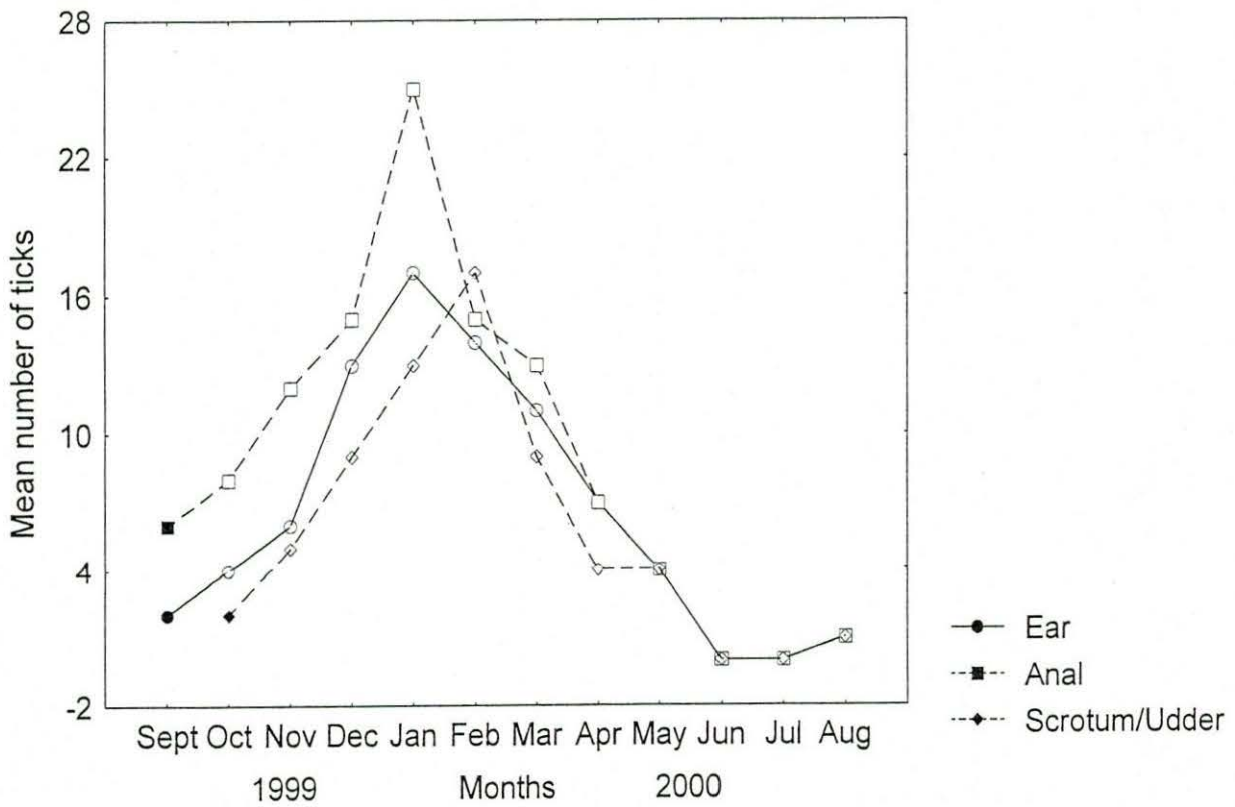

Figure 8: The distribution of *R. e. evertsi* and *B. decoloratus* ticks attaching to different body regions on goats in Harrismith

A gradual rise in the distribution of ticks collected on goats in all three body regions was observed in Kestel. The anal region had the highest number of tick infestation as compared to the other two body regions. A peak was observed between December 1999 and March 2000 (Figure 9).

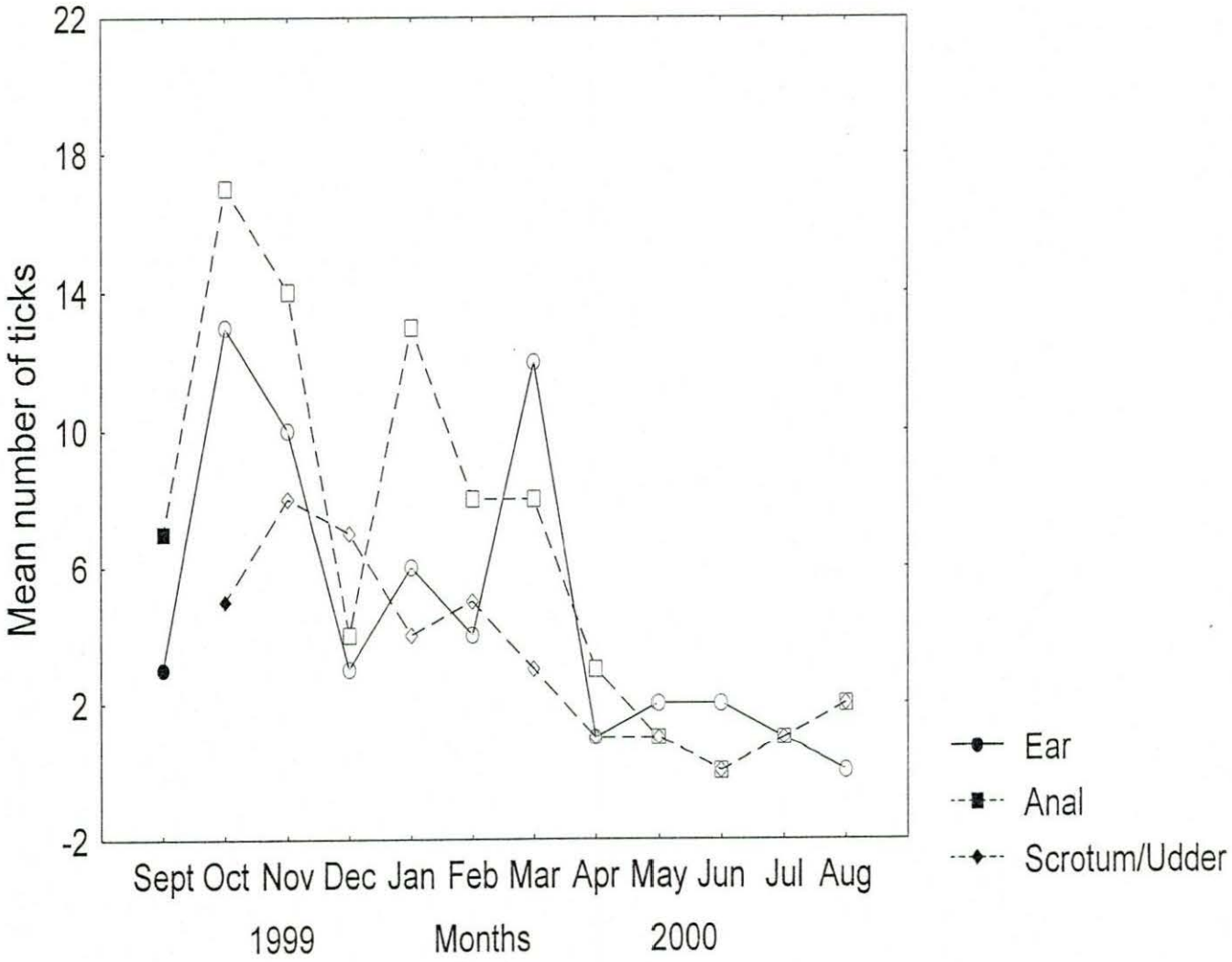

Figure 9: The distribution of *R. e. evertsi* and *B. decoloratus* ticks attaching to different body regions on goats in Kestel

Specificity in the site of attachment of ticks to hosts is common for many species of ticks. The results in the present study confirmed the concept of preferential attachments. Most of the ticks collected from sheep in Qwa-Qwa were found in the anal region. The anal region had a peak observed between October 1999 and April 2000. The scrotum or udder region had two peaks observed, the first one between October 1999 and January 2000 and the second between January 2000 and May 2000. A low number of tick counts was observed between May 2000 and July 200 for both the ear and scrotum or udder region (Figure 10).

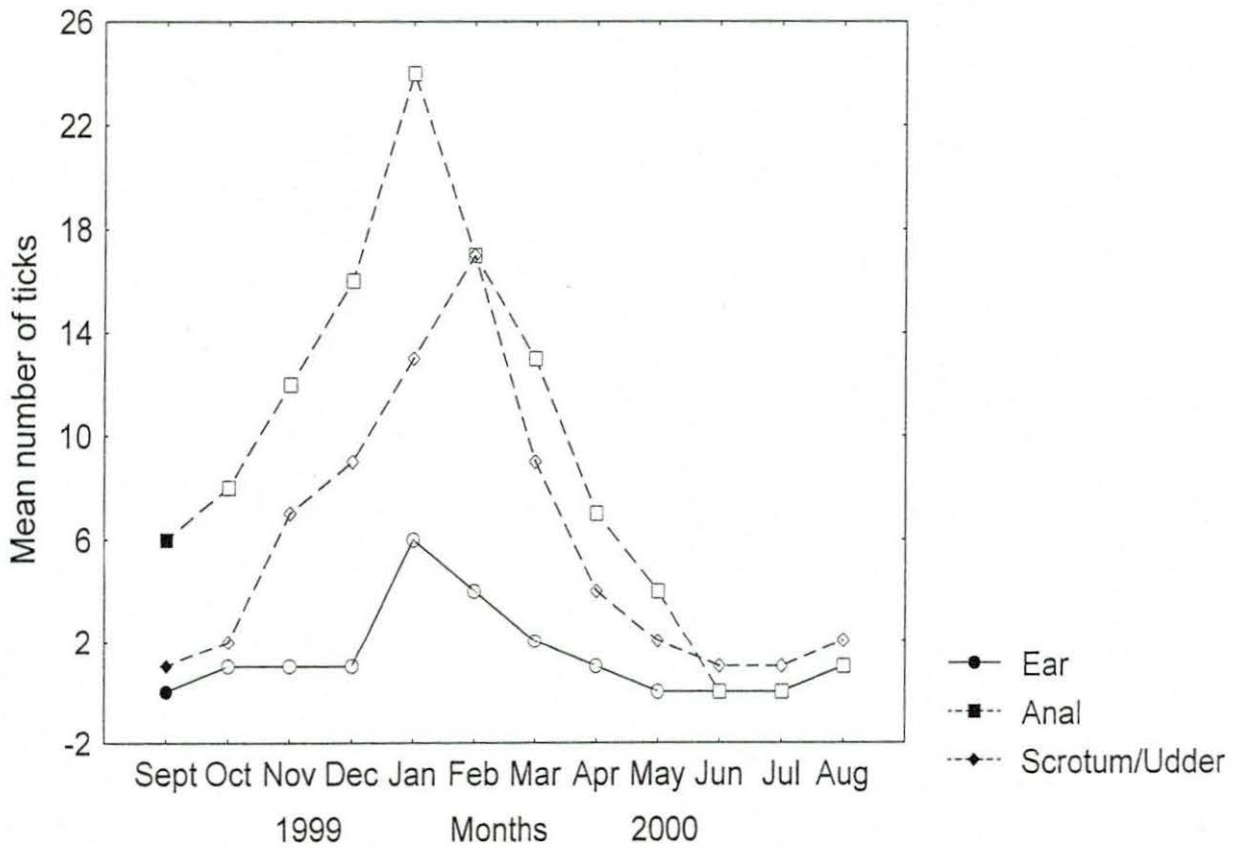

Figure 10: The distribution of *R. e. evertsi* and *B. decoloratus* attaching to different body regions on goats in Qwa-Qwa

4.5 Monthly fluctuation of *R. e. evertsi* and *B. decoloratus* species on goats in Harrismith, Kestel and Qwa-Qwa

The red-legged tick (*R. e. evertsi*) was most active during the summer months (between November 1999 and February 2000), though some specimens could be found right through the year. The number of adult ticks was high between November 1999 and April 2000. A low number of *Boophilus decoloratus* tick was observed throughout the project (Figure 11).

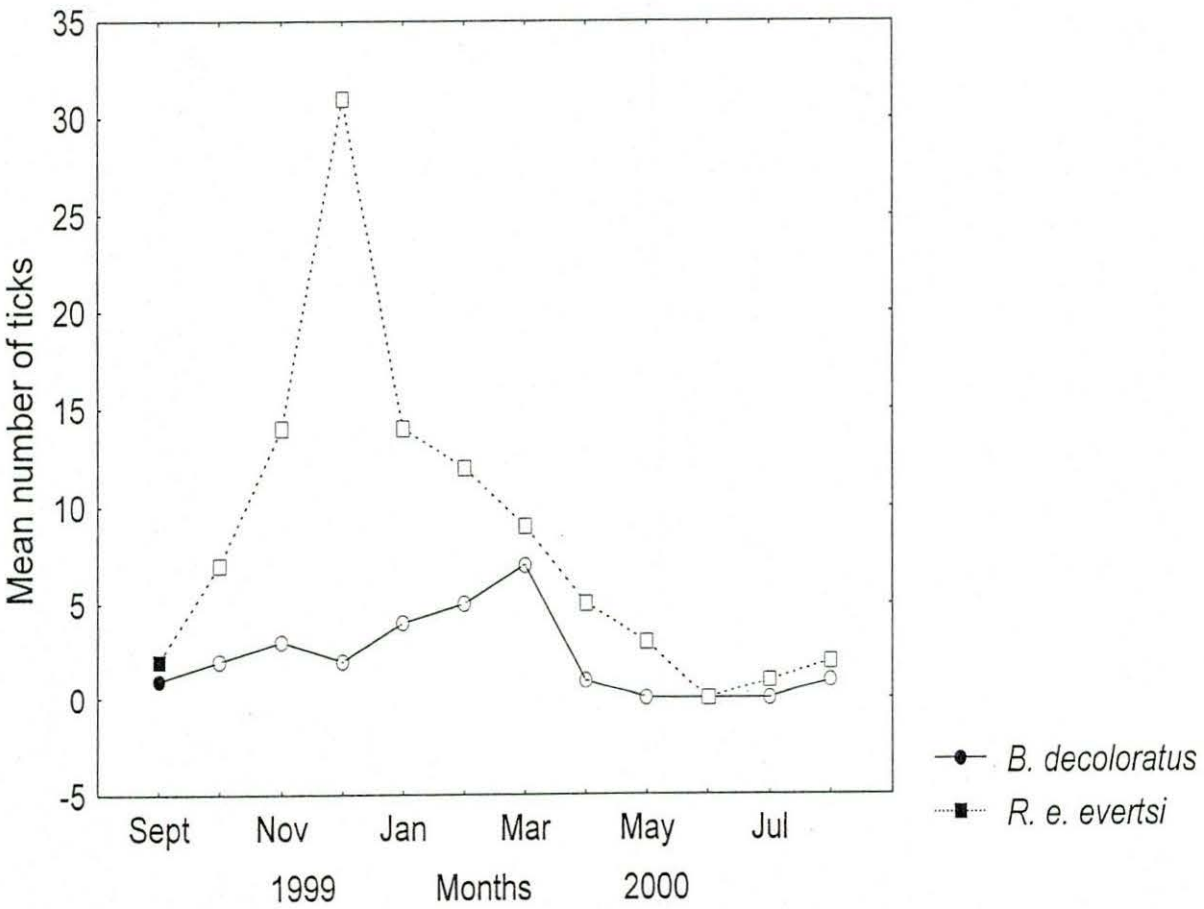

Figure 11: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on goats by months in Harrismith

For the Kestel area, monthly fluctuation in tick abundance on goats indicated that *R. e. evertsi* was the most dominant tick species. Two peaks were observed for adult *R. e. evertsi* tick on goats in Kestel, the first one between September1999 and February 2000 and the second between March 2000 and May 2000 over the 12-months period of tick collection (Figure 12).

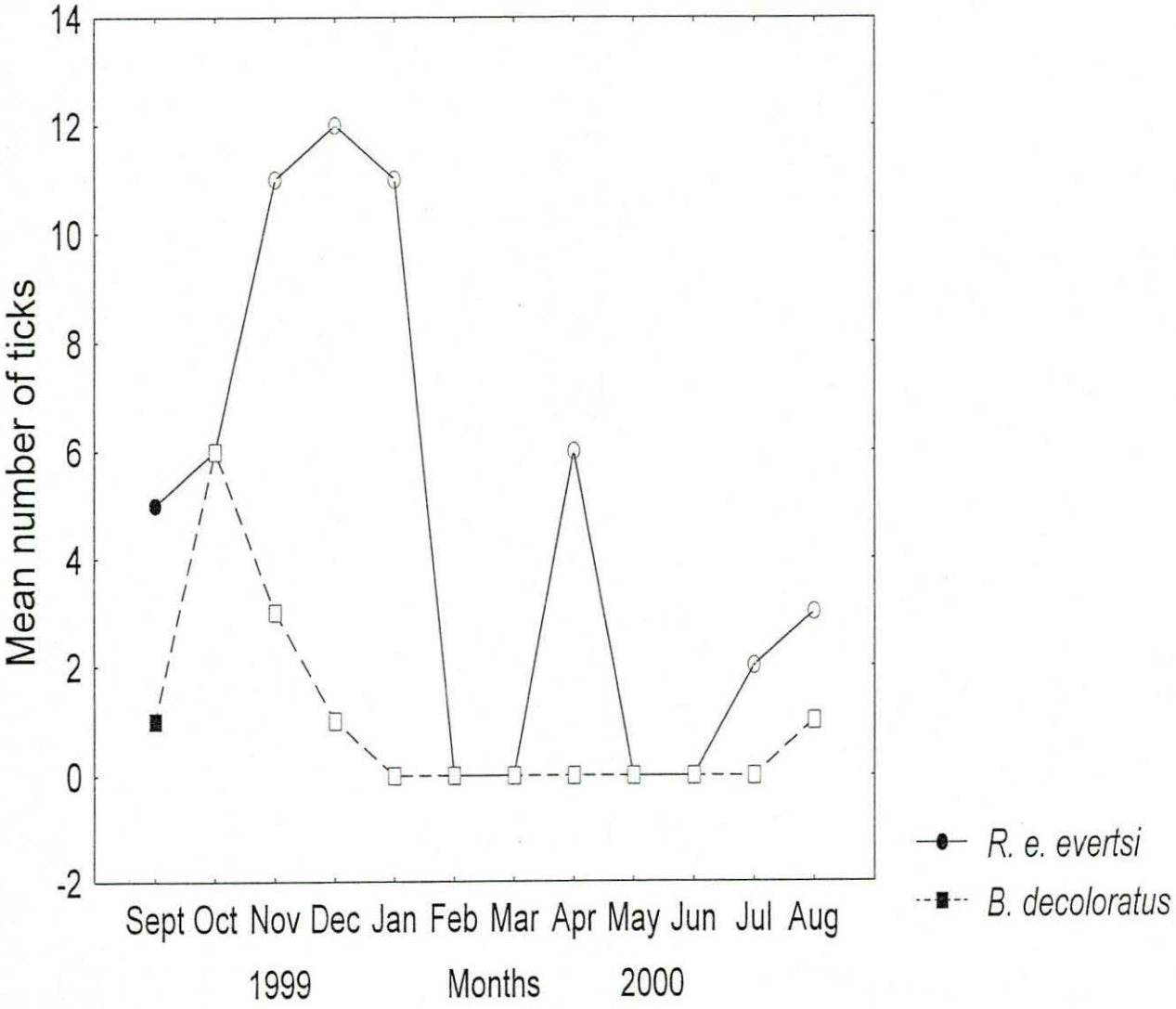

Figure 12: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on goats in Kestel

Rhipicephalus e. evertsi was found to be the abundant tick species on goats in Qwa-Qwa grazing areas with a peak observed between December 1999 and May 2000. With *B. decoloratus* a peak was observed between January 2000 and April 2000, followed by a low number of tick counts between May 200 and July 2000 (Figure 13).

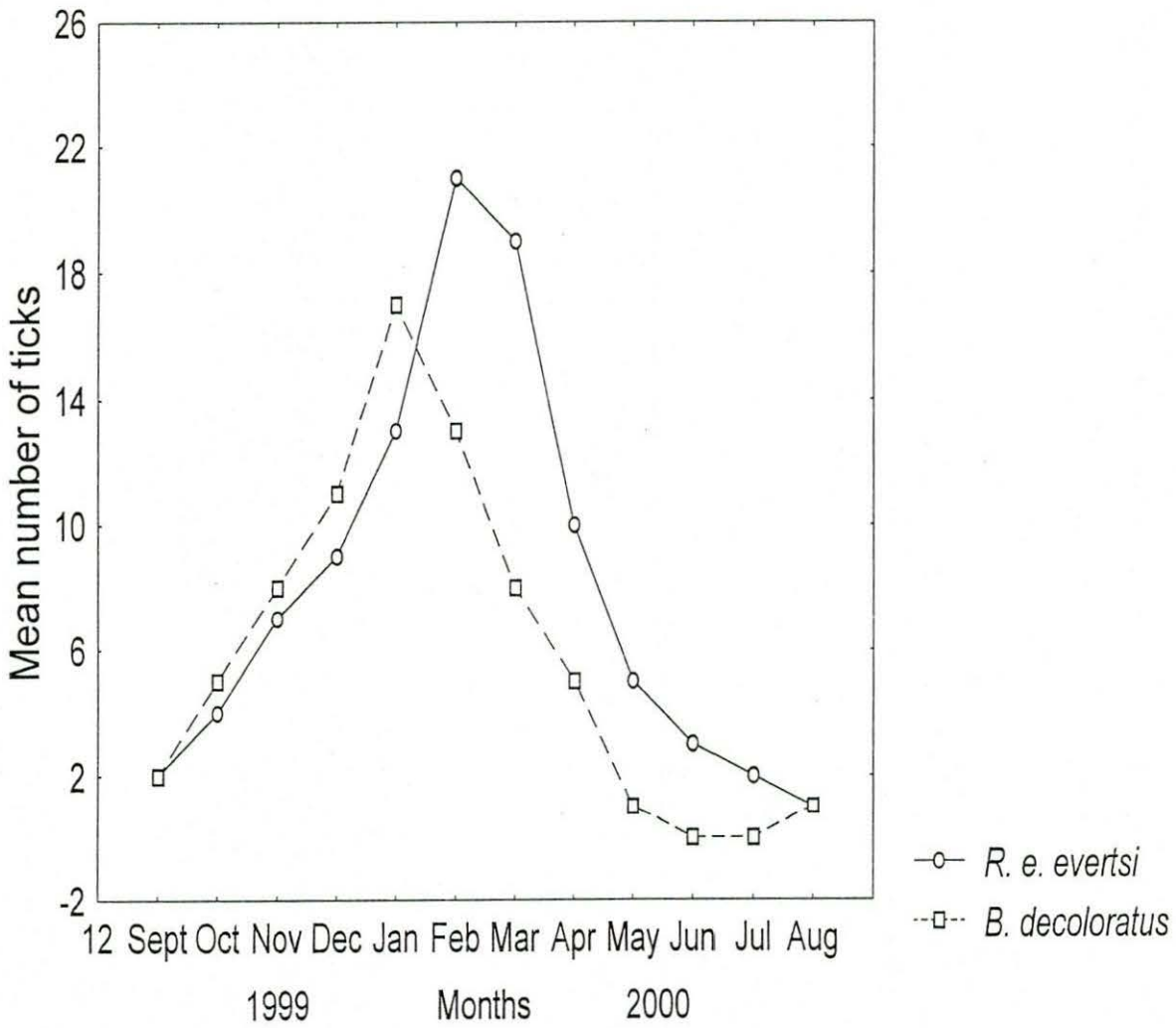

Figure 13: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on goats in Qwa-Qwa

4.6 Distribution of ticks attaching to different body regions on sheep

There was a general distribution of ticks observed throughout the 12-months study period in Harrismith. The ear region had the lowest number of tick counts compared to the other two regions. The anal region had relatively high number of ticks collected as indicated by a peak between January 2000 and May 2000 and it was the most preferred site of attachment. The scrotum/udder region had two peaks observed for the adult ticks, the first one between September 1999 and December 1999 and the second between December 1999 and May 2000 (Figure 14).

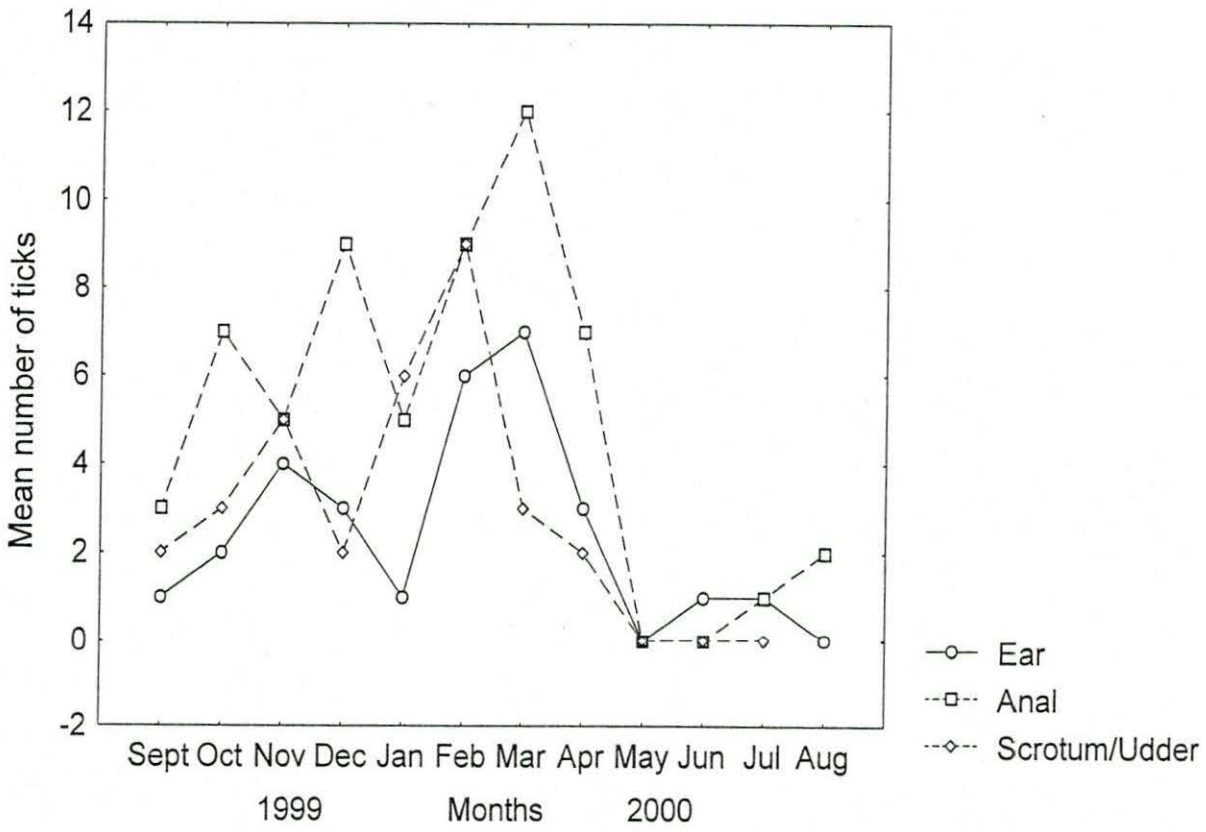

Figure 14: The distribution of *R. e. evertsi* and *B. decoloratus* attaching to different body regions on sheep in Harrismith

Figure 15 shows the distribution of sampled ticks attaching to different body regions on sheep in Kestel. A relatively high number of ticks were collected during the warmer months (between November 1999 and March 2000). The ear region had a peak between November 1999 and March 2000 followed by a low number of tick counts between May 2000 and July 2000. The anal region was the most preferred attachment site with a highest peak between October 1999 and March 2000.

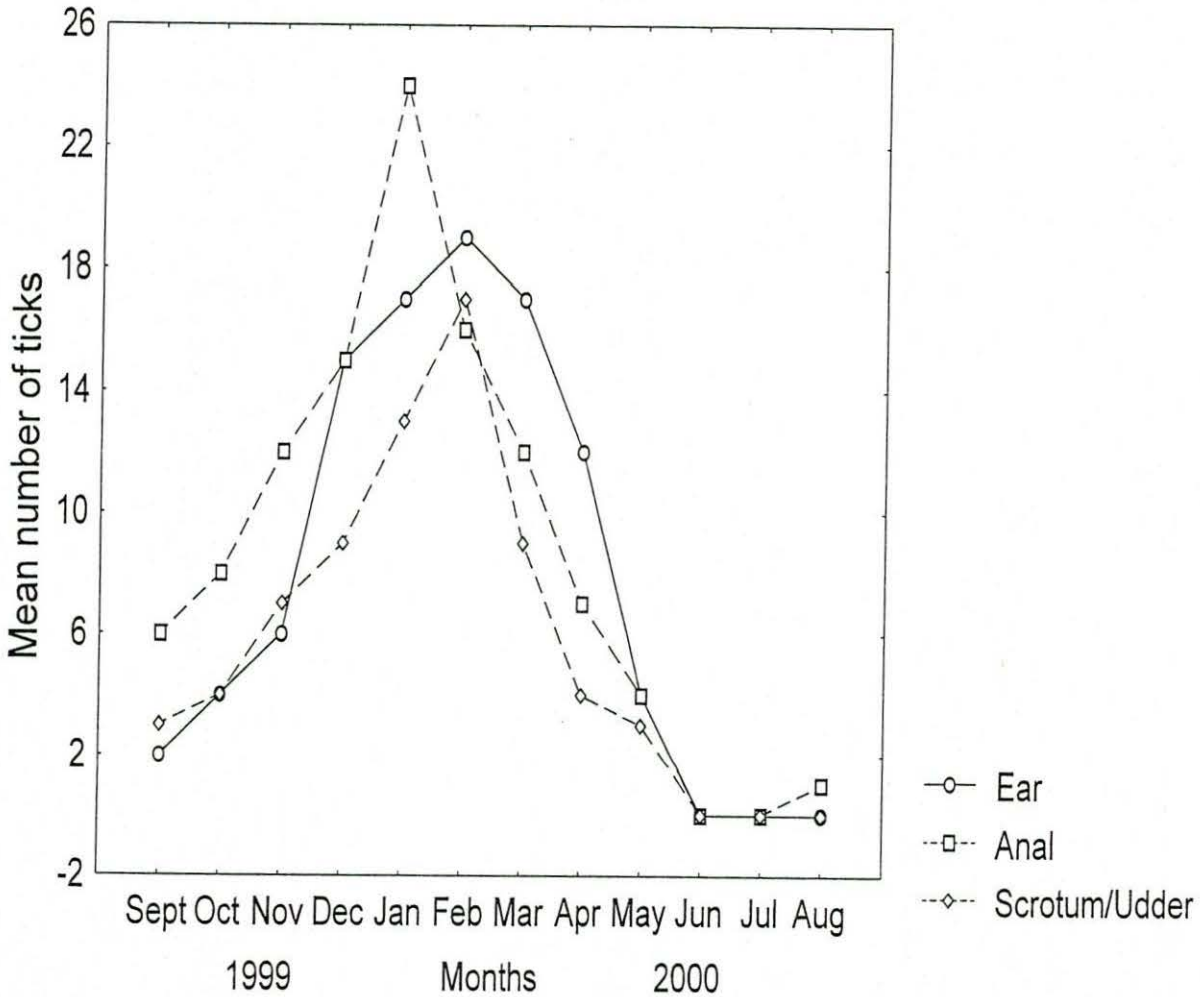

Figure 15: The distribution of *R. e. evertsi* and *B. decoloratus* attaching to different body regions on sheep in Kestel

A seasonal pattern in attachment sites was observed in the anal, ear and udder or scrotum regions. In spring and summer (between October 1999 and March 2000), tick burdens were higher in all three predilection sites with the anal region having the highest number of tick counts. In autumn and winter (between April 2000 and July 2000) lower tick burdens on all three predilection sites was observed and these seasonal differences in tick burdens were statistically different ($p>0.005$) (Figure 16).

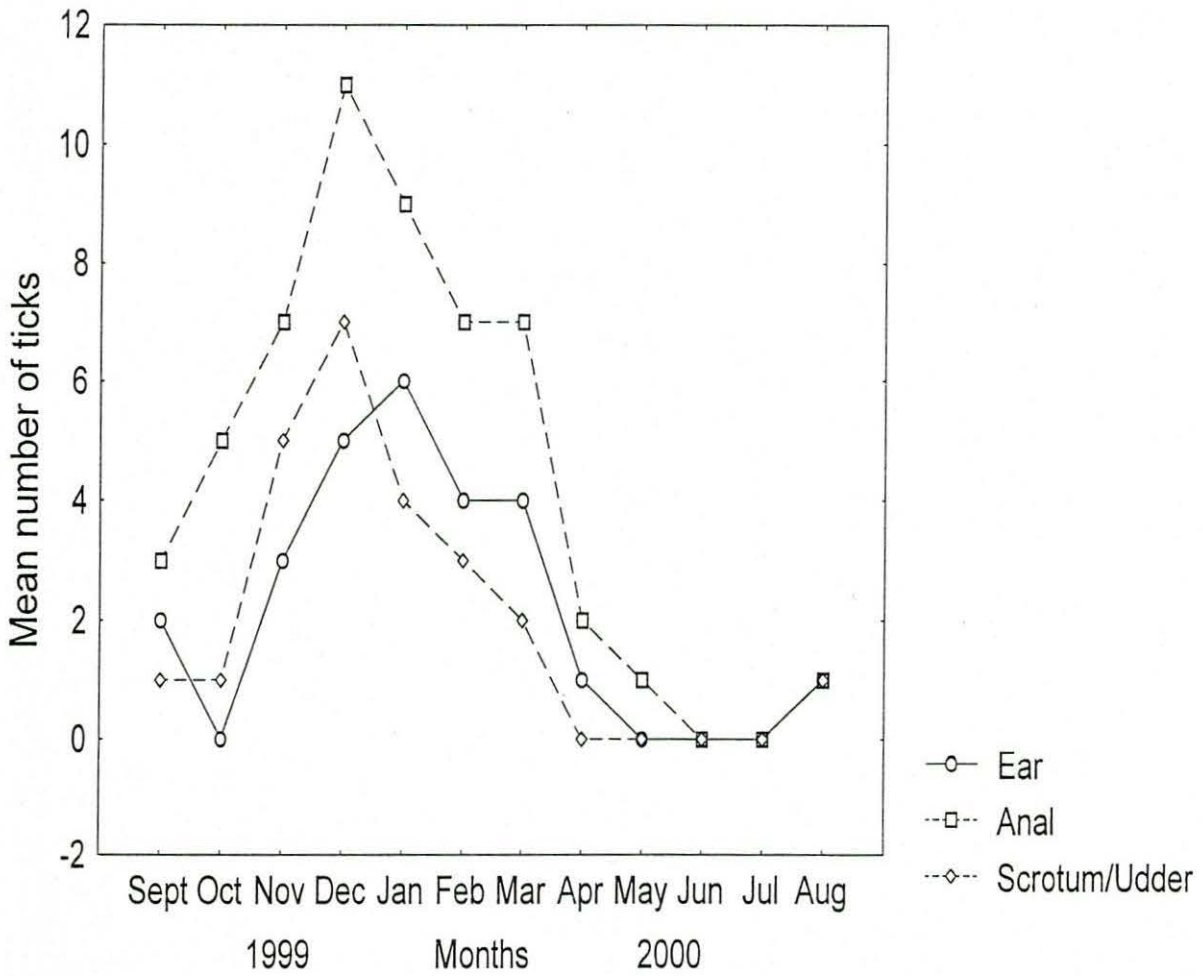

Figure 16: The distribution of *R. e. evertsi* and *B. decoloratus* attaching to different body regions on sheep in Qwa-Qwa

4.7 Monthly fluctuation of *R. e. evertsi* and *B. decolarotus* species on goats in Harrismith, Kestel and Qwa-Qwa

In Harrismith monthly fluctuation in tick abundance indicated that *R. e. evertsi* was the most dominant tick species. Two peaks of adult *R. e. evertsi* were observed between October 1999 and February 2000 and February 2000 and April 2000. A peak was observed for *B. decoloratus* between December 1999 and March 2000 and a very low number of tick counts between April 2000 and July 2000 (Figure 17).

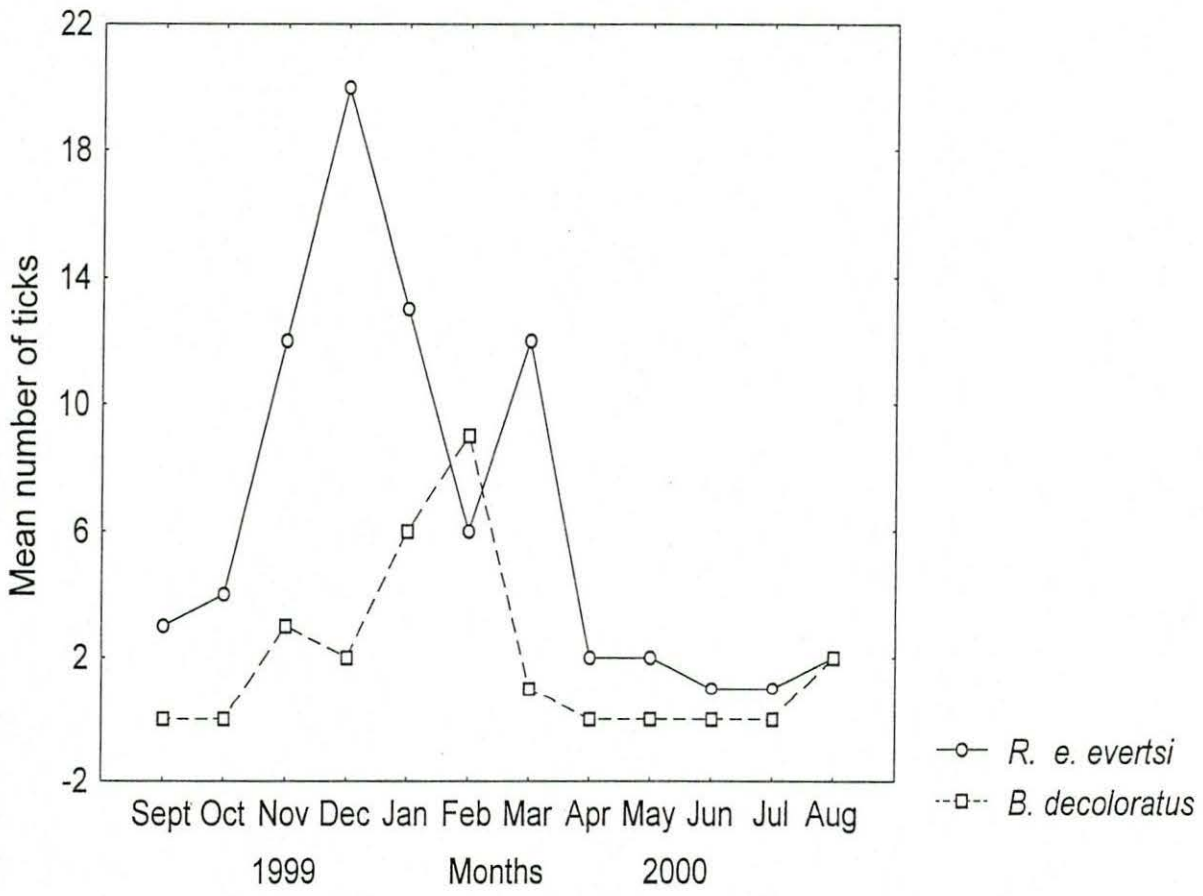

Figure 17: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on sheep in Harrismith

Rhpicephalus eversi evertsi was the dominant tick species to infect small livestock in the north eastern Free State. Two peaks were observed for adult ticks on sheep in Kestel, the first between November 1999 and February 2000 and the second between February 2000 and May 2000. This supports the observation that *R. e. eversti* are active during the warmer month and are capable of going through more than one generation per annum (Howell *et al.*, 1978). All stages were present on hosts throughout the year, although their abundance varied from one season to the other (Figure 18).

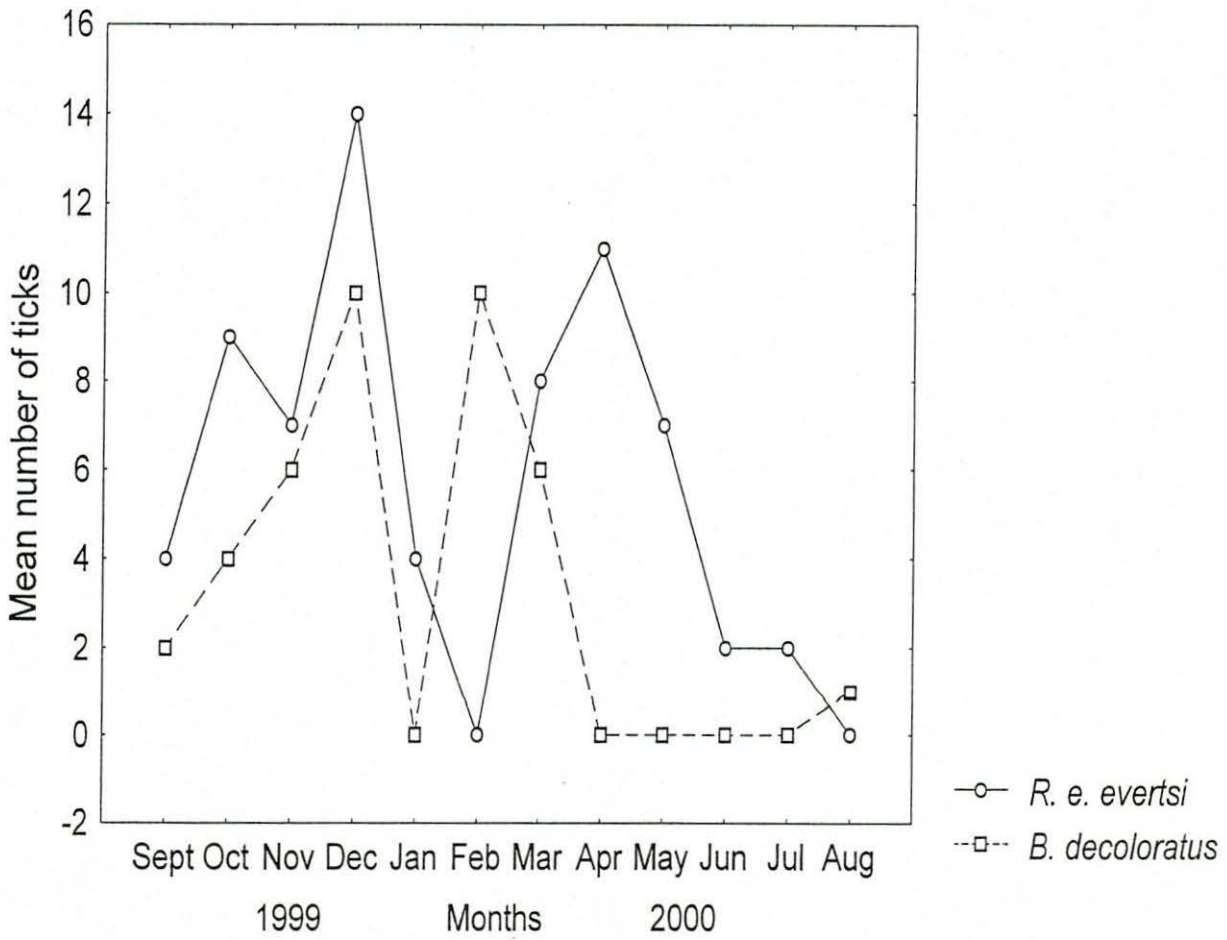

Figure 18: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on sheep in Kestel

Rhipicephalus evertsi evertsi was the abundant tick species on sheep in Qwa-Qwa grazing areas. There was a gradual rise in tick abundance at the commencement of the project, with a peak between December 1999 and April 2000, followed by a drop in tick numbers. With *Boophilus decoloratus* a peak was observed between December 1999 and March 2000, followed by a drop in the number of tick counts between April 2000 and July 2000 (Figure 19).

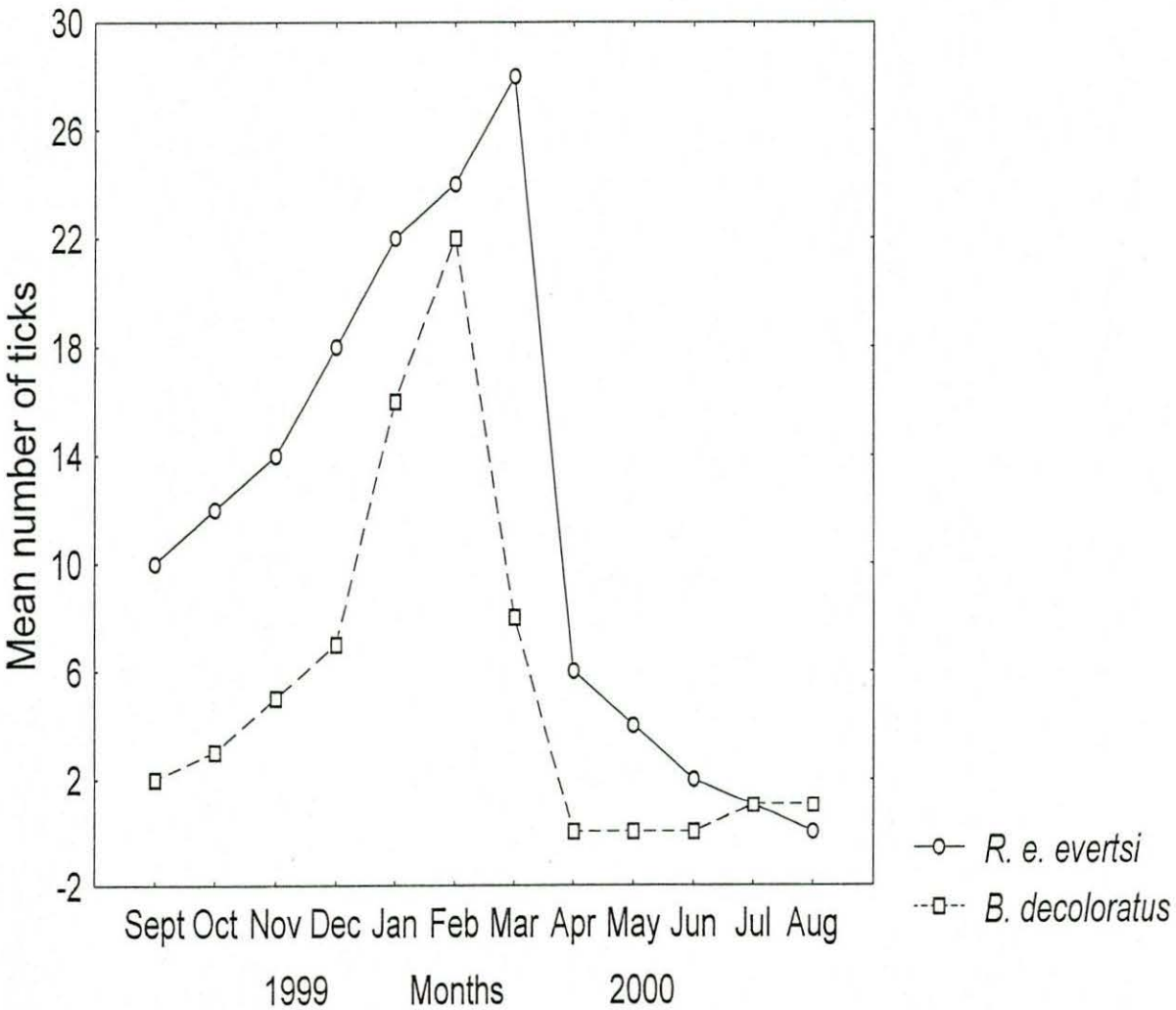

Figure 19: Monthly fluctuation in abundance of *R. e. evertsi* and *B. decoloratus* on sheep in Qwa-Qwa.

CHAPTER 5

DISCUSSION AND CONCLUSION

Ticks and tick-borne diseases are the most important external and internal parasites, respectively of livestock in South Africa and can easily constitute a limiting factor to successful stock farming unless appropriate measures are taken to control them (Howell *et al.*, 1978). Between 1960 and 1990 the world population increased by 75%, but in developing countries, the population increased by 97% (Wilson, 1995). It is the rapid urban growth and the growing demand for meat and milk that presents a particular challenge to the livestock sector. Although food production in Africa is increasing at a rate of approximately 33% per decade, it is unable to keep up with the population growth (Pinstrup-Anderson, 1993).

5.1 Serodiagnosis results for *Anaplasma* and *Theileria* species in sheep and goats across Harrismith, Kestell and Qwa-Qwa

In South Africa small livestock production is not only important in the supply of food but also in the economic growth (Meissner and Naude, 1982). Production losses due to ticks and tick-borne diseases pose important and significant challenges to improve animal health. On the basis of quantitative relationship between the tick infestation rate and the incidence of disease, regions are designated as either recently or permanently enzootic, epizootic or disease free. The incidence of infection, the severity of the disease syndromes and the economic importance of theileriosis in southern Africa (in sheep and goats) vary considerably from one geographical region to another. The incidence of anaplasmosis is determined by serological survey and hematocrit examination. Although infection rates can be high, anaplasmosis in South Africa does not always cause significant economic losses (Van Rensburg, 1981). The prevalence of clinical syndrome

depends on the maintenance of enzootic stability. The incidence and economic importance varies from one region to another depending on climatic conditions and movement of livestock. Unless new susceptible small livestock are introduced into an enzootic region, the problem of anaplasmosis, as with some other tick-borne diseases, is usually minor (Van Rensburg, 1981). Introduction of highly susceptible sheep and goats particularly those imported from temperate climates to improve local industry, results in the outbreak of several clinical diseases (Losos, 1986). Tick-borne diseases of sheep and goats have not been thoroughly investigated in the southern African region (Norval *et al.*, 1992b). Heartwater (which is caused by *C. ruminatium*), the most important of the group, was reported only in 1925 and malignant theileriosis of sheep (caused by *Theileria ovis*) in 1957 (Neitz, 1957). The other tick-borne diseases of sheep and goats that are of economic importance include anaplasmosis (caused by *Anaplasma ovis*) and babesiosis (caused by *Babesia ovis*) (Fourie *et al.*, 1988).

Among the studies done in South Africa concerning tick-borne diseases includes the one done in the Eastern Cape where 1 215 blood smears were taken from tick infested goats and sheep and examined for tick-borne parasites (Fourie *et al.*, 1988; Friedhoff, 1997). The total prevalences of *Anaplasma* and *Theileria* species were 3.85 and 2.6%, respectively. The *vectors* responsible for the transmission of these parasites are *B. decoloratus* and *R. e. evertsi*. In a review by Friedhoff (1997) on *Theileria* and *Anaplasma* species infecting sheep and goats, it was observed that *B. ovis* is transmitted by *R. bursa* and probably *R. e. evertsi*. Its distribution in south and east Asia and in South Africa is widely unknown. Malignant theileriosis of sheep and goats is an important disease in southern Africa (Friedhoff, 1997). *Anaplasma ovis* is transmitted by *R. bursa* (in Kenya), *R. e. evertsi* (in South

Africa) and *Dermacentor andersoni* in Europe. *Anaplasma ovis* is widely spread in the old world. Outbreaks occur only under extreme conditions. The identity of the tick-borne agents of sheep and goats and of their vectors is uncertain in many regions of the Old and New World (Friedhoff, 1997). Based on the information gained from previous serological studies, the immune status of animals in an area can be classified into (i) an endemically stable condition if between 81-100% of the sera is positive to a particular parasite; (ii) a situation approaching stability if between 61-81% of the sera is positive; (iii) an unstable situation if between 21-60% of the sera tested is positive (iv) a minimal disease situation if between 1-20% of the sera tested is positive and (v) a disease free situation of 0% sera is positive (Norval *et al.*, 1983a). The results of the present study revealed that 85% of the sheep and 100% goats were seropositive for *Anaplasma* species by ELISA and; 100% of both goats and sheep were seropositive for *Theileria* by IFAT. It is evident from the serological results of the present study that the immune status of sheep and goats in the north eastern region of the Free State can be classified into an endemically stable condition because between 81-100% of the sera tested positive to *Anaplasma* and *Theileria* whilst all the smears were negative for parasites. This means that goats and sheep from the north eastern Free State may have been exposed to tick infestation when they were still young and developed immunity against *Anaplasma* and *Theileria* species.

Serodiagnosis for the detection of circulating antibodies to *Theileria* species revealed that 100% of the goats sampled for the 12-months period in this study were seropositive even though all smears were negative for parasites. This observation suggests the presence of premunity in the population examined. In the three study sites clinical signs attributable to ovine theileriosis and ovine

anaplasmosis were not apparent in the animals studied. In comparing body condition to percentage seropositivity, it was observed that 99% and 89% of sheep and goats sampled with fair body condition were seropositive. There was no correlation between poor body condition and seropositivity ($p<0.05$). The results of the present study are similar to those obtained by Friedhoff (1997), in the Eastern Cape, where a high seroprevalence in goats was observed for *Theileria ovis*. Shompole *et al.,* (1989) observed *Anaplasma* organisms in erythrocytes from goats with anemia and weight loss in Kenya. In this study, serodiagnosis for the detection of circulating antibodies to *Theileria* species in sheep revealed a seroprevalence of 100%.

This study revealed that all blood smears collected from sheep and goats over the 12-months period in Harrismith, Kestel and Qwa-Qwa were negative for *Anaplasma* and *Theileria* parasites. Yet in contrast, serological tests results on sheep for the detection of circulating antibodies against *Anaplasma* and *Theileria* revealed that 85% and 100% of the samples were seropositive, respectively, whilst 100% of goats samples were seropositive for both *Anaplasma* and *Theileria* species. Throughout the 12-months period of sampling animals in all three study sites, no clinical cases of anaplasmosis and theileriosis were observed. The seroprevalence between sheep and goats in all three study sites was not significantly different ($p<0.05$).

The sensitivity of a serological test is its ability to correctly detect diseased or infected animals, a negative test on animals that have been infected being designated as false negative (Morzaria *et al.*, 1975). Applied to the study of *Theileria* species, the sensitivity of the IFAT is its ability to detect animals that

have been infected with *Theileria* species either artificially or naturally through the tick, irrespective of whether clinical disease is manifested or not (Du Plessis and Malan, 1987).

Although anaemia can be caused by factors other than tick-borne diseases, it remains one of the most important indicators of tick transmitted diseases. The PCV profile and average PCV of both sheep and goats is affected by the number of tick infestation on animal species. The mean PCV for sheep across all three study areas of the north eastern Free State was 28%. The normal PCV for sheep and goats is given as 27% (Fourie *et al.*, 1991). On comparing the normal PCV and the observed PCV, there was no difference between the two values.

The mean PCV value for goats over the 12-months period in the three study sites was 28%. A student t-test indicates that there was no difference when looking at the mean PCV of the three study sites. When performing the analysis of variance, it was observed that there was no significant difference between PCV values of males and females. The results of the present study are similar to those of a study done by Zaug (1987) in the Eastern Cape Province, which showed that the average PCV of sheep and goats was between 26-30%.

Parasitological examination of slides made from the blood smears of sheep and goats were negative for haemoparasites including *Anaplasma* and *Theileria*. The most probable reason may be because blood samples were collected from animals (mostly adults) showing no clinical signs of infection and therefore would have very low parasitaemia. The microscopic examination of blood smears from suspected cases of infection is usually performed at a time when the clinical signs

are most pronounced (Potgieter and Stoltsz, 1994). According to Shompole *et al.*, (1989), in a study conducted in Kenya, *Anaplasma* organisms were observed in erythrocytes from goats with anaemia and weight loss, using two *Anaplasma* DNA probes. Anaplasmosis often shows seasonal incidence, with outbreaks of the disease occurring more commonly during the warmer summer and autumn months. This increased incidence of the disease is related to the increased abundance and activity of ticks during this period (De Waal *et al.*, 1998a).

A possible explanation for the negative results obtained from the blood smears in this particular study could be that animals were exposed to tick infestation when they were young thus developing an immunity against the disease. On the other hand, mild and inapparent infections as in the case of *Anaplasma ovis and Theileria ovis*, are more difficult to recognise since blood parasitaemia is very low, fluctuate and frequently do not rise to levels detectable by microscopy (Ross and Lohr, 1968).

The occurrence of theileriosis coincides with the seasonal activity of the adult stages of its tick vector *Boophilus decoloratus* (Fourie *et al.,* 1988) and clinical signs are encountered more frequently during the summer season where the tick peak is high (Bezuidenhout, 1989). It is impossible to say that there are no haemoparasites such as *Anaplasma* and *Theileria* infecting sheep and goats in the north eastern Free State because serological tests confirm the presence of *Anaplasma* and *Theileria* parasites in this region. It is worthwhile for future studies in this area to use parasite-specific DNA probes to determine whether or not *Anaplasma* and *Theileria* parasites can be isolated from goats and sheep.

5.2 Tick species collected and identified from the three study sites

The two tick species found to infect sheep and goats over the 12-months period in this region were *Rhipicephalus evertsi evertsi* and *Boophilus decoloratus*, commonly referred to as the red-legged tick and the African blue tick, respectively. It is premature to conclude that these are the only two tick species infecting sheep and goats in the north-eastern Free State because full body searches were not conducted. According to Punyua (1992), it is rare to find more than six species commonly infesting hosts in one ecological zone. The ticks species identified in the present study are primarily parasites of wild and domestic ruminants (Howell *et al.*, 1978). The absence of suitable wild hosts in the area intensifies the dominant role played by cattle, sheep and goats in the population dynamics of these ticks. Because of the dense population of livestock in the area on the communal grazing and the expected fast rate of tick development on the ground due to high summer rains, the success rate of host finding is likely to be high. The absence of significant inter-and intraspecific differences may also be attributable to the small number of animals sampled in the entire study (Nkosi *et al.*, 1999).

5.2.1 Favourite sites of tick attachment in sheep and goats

Sheep and goats are the most preferred hosts of *R. e. evertsi* on which it completes its life cycle (Hoogstraal, 1956). *Boophilus decoloratus* is found mostly on cattle but also frequently parasitises goats, sheep, horses and donkeys as well as several wild ruminants including impala (*Apyceros melampus*), eland (*Taurotragus oryx*) and others. The most preferred predilection sites for adult goats infected by *R. e. evertsi* compared to the rest of the body is the ears. While lying down, goats tend to rest their heads on the ground or grass and the release of CO_2 through the nose acts as an attractant for the ticks (Fourie *et al.*, 1991). With *B. decoloratus*, the

favourite site of attachment for goats is the ear pinna; whereas in sheep most of the anal region, non-woolled areas of the head and non-woolled parts of the body underline and legs, may be infested (Howell *et al.*, 1978). The ticks favour the ear for goats and anal region for sheep as attachment sites since they offer easier access to blood because of their more superficial blood vessels. The ear pinna and anal region also offer great protection against predators and adverse environmental conditions (Howell *et al.*, 1978). But the present study revealed that ticks favourite attachment site was the anal region for both sheep and goats. One possible reason for such an observation may be because while the animals are lying down on the grass, nymphs which are attached to the grass will get into the animals body and move towards the soft areas where they will access blood easily or they could be attracted by ordours from the anal region.

Specificity in the site of attachment to hosts is common for many species of ticks (Nelson *et al.*, 1975). A variety of factors such as host type (Bloemer *et al.*, 1988), host age (Fourie *et al.*, 1991), habitat type (Wilkinson, 1985), attractants and time of the year (Evans, 1952), can affect the preferential feeding sites of the tick on the host. The results in the present study confirmed the concept of preferential attachment. From the previous Figures 8 and 9, we can deduce that a heavy tick infestation is prevalent during summer season in the anal region. This is attributable to the fact that for *R. e. evertsi*, in summer the females lay eggs five days after dropping from the host and produce up to 7 000 eggs which hatch in about a month (Howell *et al.*, 1978). The larval-nymphal feeding period in the ear lasts about 10-14 days and the nymphae subsequently take 3-4 weeks to develop into adults. Thus, red-legged ticks are most active in summer, though some species can be found all through the year. According to Howell *et al.*, (1978), the number

of immature red-legged ticks start to increase early in November, are at their peak from January to April and then slowly decrease again. This is similar to a study in Natal and Eastern Cape, where the number of immature red-legged ticks start to increase early in November, are at peak from January to April and slowly decrease again. Adult numbers are highest from January to the end of May (Howell *et al.*, 1978). A gradual increase in both the mean and the standard deviation signals the presence of a seasonal pattern of tick infection. When comparing the infestation rate between the three study sites in this study, it was observed that Qwa-Qwa had the highest infestation rate, followed by Kestel and the least infected area being Harrismith. This is possibly because Qwa-Qwa is dominated by resource-poor farmers.

The critical annual rainfall level for *B. decoloratus* to survive is 375 mm (Theiler, 1969), which is below the recorded 800mm for the north eastern region of the Free State. In the present study, significant differences in seasonal burdens of this one host tick occurred, with high infestation recorded in December 1999 to April 2000. This is similar to studies done by Robertson (1981) in the Eastern Cape where *B. decoloratus* was abundant from February to May but scarce between May to July. Since north-eastern Free State region is dominated by colder climates, *B. decoloratus* is inactive in winter and early spring but evident from October to April. The engorged female usually lays about 2 500 eggs, which take 3-6 weeks to hatch, depending on environmental conditions. From larval attachment to complete engorgement the female takes three weeks. Thus under optimum conditions the blue tick can complete its life cycle in about two months (Howell *et al.*, 1978). There was a seasonal pattern in terms of monthly fluctuation in tick abundance across the three study sites. In the present study tick species were evident from

September and April and most probably indicated that the blue tick produces two generations during the year. A similar seasonal periodicity was observed by Baker and Ducasse (Dreyer *et al.*, 1998) in the southern region of the Free State. In other places such as the Northern Province, *B. decoloratus* is present throughout the year without any distinct seasonal peaks (Jooste, 1966), indicating a possible overlap between generations.

According to Rechav (1982), temperature is probably the main regulatory factor in the seasonal patterns of the blue tick. The slow development of eggs in the field during the cold winter and also the longer ovi-position period of the females (Robertson, 1981) will result in a low number of adult ticks in the early spring. A similar pattern was observed by Fourie *et al.*, (1996) in the southern region of the Free State Province where the duration of the pre-hatch period was influenced by seasonal fluctuations in numbers, resulting in a sharp drop in tick abundance during winter and early spring. Seasonal fluctuations in the three study areas of the north eastern Free State were very similar but tick abundance and thus tick infestation in Qwa-Qwa were significantly higher compared to the other two areas. One possible reason for this was the more number of animals with poorer body condition compared to those of the other two localities, possibly resulting in higher tick burdens but lowered tick resistance (De Castro and Newson, 1993; Hlatshwayo and Mbati, 1999). A possible reason for the poor body condition could have been the severe overgrazing of the veld in this specific locality.

The critical rainfall level of *R. e. evertsi* to survive is 250mm and it can maintain itself in grassy areas with any rainfall above that (Theiler, 1950). The presence of this two-host tick species throughout the year with small fluctuations in winter

months, was also observed by Punyua *et al.*, (1991) and Dreyer *et al.*, (1998). In the present study, the peak observed from February 2000 to May 2000 resembled the March-May peak in a study on sheep in the southern region of the Free State Province (Horak *et al.*, 1991). The average life cycle of *R. e. evertsi* is completed in 63 days under controlled temperature conditions (Rechav *et al.*, 1977), the life cycle undoubtedly must take longer under the climatic conditions of the north eastern Free State. Results obtained in a study by Hlatshwayo (2000) indicate that temperature and specifically the winter temperature, is probably the major factor regulating seasonal activity of the red-legged tick. The continuous presence of adult ticks throughout the year indicates that more than one life cycle can be completed annually, as was suggested by Matson and Norval (1977)

The findings of this study suggest that only two ixodid tick species infect sheep and goats in the north eastern Free State. The two ticks are *R. e. evertsi* and *B. decol*oratus ticks which are vectors of anaplasmosis and theileriosis. The infestation rate in Qwa-Qwa was significantly higher compared to the other two study sites. Harrismith is dominated by commercial farmers who would certainly be in a better position to apply acaricides treatment more regularly resulting in lower densities of ticks in their livestock. On the other hand Qwa-Qwa is dominated by resource-poor farmers who cannot afford the effective but expensive acaricides. With high infections occurring in summer, it is recommended that acaricides used for the tick control should be applied more intensively in this season.

REFERENCES

Andrew, R.A. and Norval, R.A.I. (1989). Carrier status of sheep, cattle and African buffalo recovered from heartwater. *Veterinary Parasitology*, **34**: 261-266.

Anon, R. (1990). Anaplasmosis. Monthly report of the Regional Veterinary Laboratories for the month of January, Department of Veterinary Services, Republic of South Africa.

Bachman, M. (1990). Tsetse/Nagana situation, Zululand. A provisional evaluation of nagana in Zululand. Report to the Director, Department of Animal Health. Peitermaritzburg: Department of Agricultural Economic and Marketing, Republic of South Africa.

Baker, M.K. and Ducasse, F.B.W. (1967). Tick infestation of livestock in Natal. The predilection sites and seasonal variation of cattle ticks. *Journal of South African Veterinary Medicine Association*. **38**: 447-453.

Barnard, C.J., Gilbert, F.S. and Mcgregor, P.K. (1993). *Asking questions in Biology*. Lomgman Scientific and Technical, Harlow.

Barriga, O.O. (1994). A review on vaccination against protozoa and arthropods veterinary importance. *Veterinary Parasitology*, **55**: 29-55.

Barry, D.M. and Van Niekerk, C.H. (1990). Anaplasmosis in improved Boer goats in South Africa artificially infected with *Anaplasma ovis*. *Small Ruminant Research,* **3**: 191-197.

Bevan, C.E.W. (1912). Anaplasmosis in sheep. *In*: Infectious disease of livestock with special reference to southern Africa. Vol. 1 (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.), pp 462-470. Oxford University Press, Cape Town.

Bezuidenhout, J.D., Prozesky, L., Du Plessis, J.L. and Van Amstel, S.R. (1994). Cowdriosis. *In*: Infectious Diseases of Livestock with special reference to southern Africa, Volume 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.), pp 351-357. Oxford University Press Cape Town.

Bezuidenhout, J.D. (1989). Cowdria vaccines. WRIGHT, I.G (ed). *Veterinary Protozoan and Haemoparasites Vaccines,* **4**: 35-40.

Bloemer, S.R., Zimmerman, R.H. and Fairbanks, K. (1988). Abundance, attachment sites and density estimators of lone star ticks (Acari: Ixodidae) infesting white-tailed deer. *Journal of Medical Entomology*, **25**: 295-300.

Botha, N. (1994). A modern approach to rural development. Farmer's Weekly, 4th March, pp 38-39.

Brown, C.C. and Skowronek, A.J. (1990). Histologic and immunochemical study of the pathogenesis of heartwater (*Cowdria ruminantium* infection) in goats and mice. *American Journal of Veterinary Research,* **51**: 1476-1480.

Burridge, M.J and Kimber, C.D. (1972). The Indirect Flourecent Assay Test for experimental of East Coats fever (*T. parva* infection of cattle). Evaluation of a cell culture schizont antigen. *Research Veterinary Science*, **13**: 451-455.

Camus, E. and Barre, N. (1987). Diagnosis of heartwater in live animals. Experiences with goats in Gaudeloupe. *Onderstepoort Journal of Veterinary Research*, **54**: 291-294.

Connor, R.J. (1994). African animal trypanosomiases. *In*: Infectious Diseases of Livestock with special reference to southern Africa, Volume 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.) pp 167-188. Oxford University Press, Cape Town.

Cowdry, E.V. (1925). Studies on the aetiology of heartwater. II. Observation of a rickettsia, *Rickettsia ruminantium* (n-sp.), in the tissues of infected animals. *Journal of Experimental Medicine*, **42**: 253-274.

Dargie, J.D. (1989). Helping farmers to improve their livestock. *In*: International Energy Year Book (IAEA), Vienna. pp B35-B55.

De Castro, T.J. (1997). Sustainable tick and tick-borne diseases improvement in developing countries. *Veterinary Parasitology,* **71**: 77-97.

De Castro, T.J. and Newson, R.M. (1993). Host resistance in cattle tick control. *Parasitology Today*, **9**: 13-17

De Vos, A.J., Potgieter, F.T., De Waal, D.T. and van Heerden, J. (1994). Babesiosis. *In*: Infectious Diseases of Livestock with special reference to southern Africa, Volume 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.), pp 295-298. Oxford University Press, Cape Town.

De Vos, A.J. (1992). Distribution, economic importance and control measures of *Babesia* and *Anaplasma*, *In*: Recent developments in the control of anaplasmosis, babesiosis and cowdriosis. (Dolan, T.T., ed.), pp 3-12.

De Vos, A.J. and Potgieter, F.T. (1983). The effect of tick control on the epidemiology of bovine babesiosis. *Onderstepoort Journal of Veterinary Research*, **50**: 3-5.

De Vos, A.J. (1981). *Rhipicephalus appendicularis*. Cause and vector of diseases in Africa. *Journal of the South African Veterinary Association*, **52**: 315-322.

De Vos, A.J. (1979). Epidemiology and control of bovine babesiosis in South Africa. *Journal of the South African Veterinary Association*, **50**:357-362.

De Waal, D.T. (1999). Making Blood Smears. Onderstepoort Veterinary Institute (OVI), Pretoria, pp 1–4.

De Waal, D.T., Stoltsz, W.H. and Combrink, M.P. (1998a). Anaplasmosis: Tick-transmitted gallsickness of cattle. Onderstepoort Veterinary Institute, Pretoria, pp 1-6.

De Waal, D.T. and Stoltsz, W.H. (1998b). Heartwater. Onderstepoort Veterinary Institute. pp 1-6.

De Waal, D.T. and Stoltsz, W.H. (1998c). Cowdriosis: Heartwater of cattle, sheep and goats. Report of Onderstepoort Veterinary Institute, Pretoria, pp 23-28.

De Waal, D.T. (1998d). Theileriosis: East Coast fever of cattle. Report of Onderstepoort Veterinary Institute, Pretoria, pp 23-34.

Di Domizo, G. (1919). *Dell' Anaplasma marginale*. *In*: Infectious diseases of livestock with special reference to southern Africa, Vol 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, C.R., eds.), pp 743-472. Oxford University Press, Cape Town.

Dipeolu, O.O., Mongi, A.O., Punyua, D.K., Latif, A.A., Amoo, O.A. and Odhiambo, T.R. (1992). Current concepts and approach of livestock ticks in Africa. *Discoveries and Innovations*, **4**: 35-44.

Dixon, R.W. (1899). Heartwater experiments. *Agricultural Journal of the Cape of Good Hope,* **14**: 754-760.

Dreyer, K., Fourie, L.J. and Kok, D.J. (1998). Tick diversity, abundance and seasonal dynamics in a resource-poor community. *Onderstepoort Journal of Veterinary Research*, **65**: 291-296.

Du Plessis, J.L., De Waal, D.T. and Schotsz, W.H. (1994). A survey of the incidence and importance of the tick-borne diseases; Heartwater, redwater and anaplasmosis in the heartwater-endemic regions of South Africa. *Onderstepoort Journal of Veterinary Research*, **6**: 295-301.

Du Plessis, J.L. and Malan, L. (1987). The application of the IFAT in the research of heartwater. *Onderstepoort Journal of Veterinary Research*, **54**: 319-325.

Edington, A. (1900). Heartwater. *Agricultural Journal of the Cape of Good Hope,* **12**: 748-754.

Ellis, J. (1994). Climate variability and complex ecosystem dynamics: implications for pastoral development. *In*: Living with uncertainty: new directions in pastoral development in Africa (Scoones, I, ed.), pp 37-46. Intermediate Technology Publications Ltd, London.

Evans. G.O. (1952). Distribution of Ixodes: *Rianus* (L.) on body of cattle and sheep. *The Bulletin of Entomological Research.* Volume 26 (4).

Ewang, P.N. (1995). Address the basics. Farmer's Weekly, 12th May, pp 4-5.

Food and Agricultural Organisation. (1996). Food Requirements and Population Growth. WF5 96/TECH/10. Provisional Version of Technical Paper for the World Food Summit. pp 60.

Flach, D., Woodford, J.D., Morzaria, S.P., Dolan, T.T. and Hambwana, K. (1990). Identification of *Babesia bovis* and *Cowdria ruminantium* on the island of Unguja, Zanzibar. *The Veterinary Record,* **126**: 57-59.

Fourie, L.J., Kok, D.J. and Heyne, H. (1996). Adult ixodid ticks on two cattle breeds in the south-western Free State, and their seasonal dynamics. *Onderstepoort Journal of Veterinary Research*, **63**: 19-23.

Fourie, L.J., Horak, I.G. and Van Zyl, J.M. (1991). Sites of attachment and intraspecific infestation densities of the brown paralysis tick (*Rhipicephalus punctatus*) on Angora goats. *Experimental and Applied Entomology*, **12**: 243-249.

Fourie, L.J. and Horak, I.G. (1990). Parasites of cattle in the south-western Orange Free State. *Journal of South African Veterinary Association*, **12**: 27-28.

Fourie, L.J., Horak, I.G. and Marais, L. (1988). Seasonal abundance of adult ixodid ticks on Merino sheep in the south western Orange Free State. *Journal of South African Veterinary Association,* **59**: 191-194.

Fourie, L.J. and Horak, I.G. (1987). Tick individual paralysis of the springbok. *South African Journal of Wildlife Research,* **17**: 131-133.

Friedhoff, K.F. (1997). Transmission of *Babesia*. *In*: Ristic, M., (ed.) Babesiosis of domestic animals and man. Boca Raton, Florida: CRC Press, Inc.

Gothe, R. and Bezuidenhout, J.D. (1989). Studies on the ability of different strains or populations of female *Rhipicephalus evertsi evertsi* (Acarina: Ixodidae) to produce paralysis in sheep. *Ondertsepoort Journal of Veterinary Research*, **53**: 19-24.

Gruss, B. (1981). A practical approach to the control of Heartwater in the Angora goats and certain sheep breeds in the Eastern Cape Coastal Region. Tick Research Unit. Rhodes University, South Africa.

Harith, A.E., Kolk, A.H.J., Kager, P.A., Leeuwenberg, J., Mugai, R., Kiungu, S. and Laarman, J.J. (1986) A simple and economical Direct Agglutination Test for serodiagnosis studies of visceral leishmaniais. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**: 583-586.

Hermans, P., Dwinger, R.H., Buening, G.M. and Herrero, M.V. (1994). Seasonal incidence and haemoparasites infection rates of Ixodid ticks (Acari: Ixodidae) detached from cattle in Costa Rica. *Review of Tropical Biology*, **42**: 623-632.

Hlatshwayo, M. (2000). Studies on tick species (Acari: Ixodidae) infesting cattle in the eastern Free State Province, South Africa: Epidemiology, biology and control. MSc Thesis, University of the North, Qwa-Qwa Campus.

Hlatshwayo, M. and Mbati, P.A. (1999). Seasonal abundance of Ixodid ticks in the eastern Free Sate Province of South Africa. *Uniqwa Research Chronicle*, **1**: 243-245.

Hoogstraal, H. (1956). African Ixoidea. I. Ticks of the Sudan (with reference to Equitoria Province and eigth preliminary reviews of the genera *Boophilus*, *Margaropus* and *Hyalomma*). Research Report NM 005.050.29.07. Bureau of Medical Survey, Department of Navy, Washington D.C.

Horak, I.G., Williams, E.J. and Van Schalkwyk, P.C. (1991). Parasites of domestic and wild animals in South Africa. XXV. Ixodid ticks on sheep in the north-eastern Orange Free State and in the Eastern Cape Province. *Onderstepoort Journal of Veterinary Research,* **58**: 115-123.

Horak, I.G. (1982). Parasites of domestic and wild animals in South Africa. XV. The seasonal prevalence of ectoparasites on impala and cattle in the northern Transvaal. *Ondertepoort Journal of Veterinary Research*, **49**: 85-93.

Hove, T., Musis, F.L., Kanhai, G.K., Latif, A., Masaka, S., Munatwsa, F.C., Pegram, R.C., Kamwendo, S.P., Quiroga, J.C., Mwangodwe, R. and Dolan, T.T. (1996). Challenge of *T. parva* (Bolemi) immunised cattle with selected East African *Theileria* stocks. *Tropical Animal Health Production*, **27**: 202-210.

Howell, D.J., Petney, T.N. and Horak, I.G. (1989). Host status of striped mouse *Rhapdomys pumilio* in relation to the tick vectors of heartwater in South Africa. *Onderstepoort Veterinary Journal of Research*, **56**: 289-291.

Howell, C.J., Walker, B.J. and Walker., E.M. (1978). Ticks, mites and insects infesting domestic animals in South Africa. Part 1. Description and biology. *Science Bulletin*. No. 393.

Jooste, K.F. (1966). Seasonal incidence of the immature egg of the brown ear tick. *Rhodesian Agricultural Journal*, **63** (1): 16-18.

Joyner, L.P., Donnely, J., Payne, R. and Brocklesby, D.W. (1972). The Indirect Flourescent Antibody Test for the differentiation of infections with *Babesia divergens* or *Babesia major. Research in Veterinary Science*, **13**: 515-518.

Kaufmann, J. (1996). Parasitic infections of domestic animals. A diagnostic Manual, Birkhauser Verlag, Schweiz, pp 188-189.

Khera, S.S. (1962). Rickettsial infections in animals. A review. *Indian Journal of Veterinary Science*, **32**: 282-301.

Kok, D.J. and Fourie, L.J. (1995). The role of *Hyalomma* ticks in foot infestations and temporary lameness of sheep in a semi-arid region of South Africa. *Onderstepoort Journal of Veterinary Research*, **62**: 201-206.

Krecek, R.C., Cornelius, S.T. and McCrindle, C.M.E. (1995). Socio-econmic aspects of animal diseases in southern Africa: Research priorities in Veterinary Science. *Journal of South African Veterinary Association,* **66**: 115-120.

Krige, D.S. (1995). Demographic profile of the Free State. Department of Urban and Regional Planning, University of the Orange Free State, Bloemfontein.

Kritzinger, A.S. and Pieterse, J.M. (1987). Qwa-Qwa: Introductory Economic and social memorandum. Institute for Development Research, Development Bank of southern Africa, Sandton, South Africa.

Latif, A.A. (1993). Sustainable control methods for ticks and tick-borne diseases in southern Africa. *Veterinary Research Laboratory*, Harare, Zimbabwe. Proceedings of the workshop held at Kadoma Ranch Hotel, Zimbabwe 20-23 July 1992.

Latif, B.M., Al-Shenawi, F.A. and Al-Alousi, T.I. (1979). The Indirect Fluorescent Antibody Test for diagnosis of kalazar infection in Iraq. *Annual Tropical Medicine and Parasitology*, **73**: 35-45.

Lawrence, J.A., De Vos, A.J. and Irvin, A.D. (1994). Theileriosis. *In*: Infectious Disease of Livestock with special reference to southern Africa, Volume 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.). pp 307-330. Oxford University Press, Cape Town.

Lawrence, J.A. (1979). The differential diagnosis of the bovine theileriosis of South Africa. *Journal of South African Veterinary Association*, **50**: 311-313.

Lestoquard, T. (1924). Deuxieme note sur les Piroplasmoses du mouton en Algerie. L'anaplasmose: *A. ovis. Bulletin dela Society of pathology Exotic*, **17**: 784-787.

Levine, N.D., Corliss, J.O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B.M., Leedale, G.F., Loeblich III, A.R., Lom, J., Lynn, D., Merinfeld, J. and Wallace, F.G. (1985). A newly revised classification of the Protozoa. *Journal of Protozoology*, **27**: 37-58.

Losos, G.J. (1986). Infectious tropical diseases of domestic animals. Harlow, U.K: Longman Scientific and Technical.

Lounsbury, C.P. (1904). The transmission of African coast fever. *Agricultural Journal*, Cape Town, **24**: 428-432.

Lounsbury, C.O. (1899). The bont tick, *Amblyomma hebraeum*. Its life history and habits. *Agricultural Journal*, Cape Town, **15**: 728-743.

Lu, W.S., Lu, W.X., Zhang, Q.C., Dou, H.F., Yu, F., Yin. H., Ji, Z.D., Hu, T.Y., Sun, B.G., Han, J.S., Gao, D.F. and Bao, Y.J. (1987). Study of anaplasmosis in goats. Identificatioon of the pathogen and observation of the clinical signs. *Chinese Journal of Veterinary Science and Technology*, **3**: 5-8.

Maas, J. and Buening, G.M. (1981). Characterization of *Anaplasma marginale* infection in splenectomised domestic goats. *American Journal of Veterinary Research,* **42**: 142-145.

Marchette, N. (1982). Origin and evolution of the ehrlichieae. *In*: Marchette, N. and Stitter D., (eds). Ecological Relationships and evolution of the rickettsiae. Volume 2. Balon Bouge.

Matheron, G., Barre, N., Camus, E. and Goque, Y. (1987). Genetic resistance of Gaudeloupe native goats to heartwater. *Onderstepoort Journal of Veterinary Science,* **54**: 337-340.

Matson, B.A. and Norval, R.A.I. (1977). The seasonnal occurrence of adult ixodid ticks on cattle on a Rhodesian farm. *Rhodesian Veterinary Journal*, **8**: 2-6.

Meissner, H.H. and Naude, R.T. (1982). Aksie: Beesvleisproduksie tot die jaar 2003/Action: Beef production until the year 2003. *South African Journal of Animal Science*, **12**: 187-195.

Moffett, R.O. (1997). Grasses of the eastern Free State: Their description and uses. University of the North Qwa-Qwa Campus, Phuthaditjhaba. pp 13-14.

Molloy, J.B., Bowles, P.M., Teston,. P.J., Bruyeresr, A.G., Bowden, J.M., Bock, R.E., Jorgesen, B. and Daglies, N.J. (1998). Development of an ELISA for detection of antibodies to *Babesia bigemia* in cattle. *Parasitology Research*, **84**: 651-656.

Morzaria, S.P., Brocklesby, D.W. and Harradine, D.L. (1975). Evaluation of the IFAT for B*abesia major* and *Theileria mutans* in Britain. *The Veterinary Record*, 454-87.

Moshkovski, S.D. (1974). Comments by readers. *Science,* **106**:62.

Mukhebi, A.W., Perry, B.D. and Kruska, R. (1992). Estimated economics of theileriosis control in Africa. *Preventive Veterinary Medicine*, **12**: 73-85.

Ndungu, L.W., Aguire, C., Rurangirwa, F.R., McElwain, T.F., Mcguire, T.C., Knowle, D.P., Palmer, P.P. and Guy, H. (1995). Detection of *A. ovis* infection in goats by major surface protein competitive inhibition assay. *Journal of Clinical Microbiology,* 675-679.

Neitz, W.O. (1968). *Ehrlichia ovina* infection. *Bullentin del'Office Internaat des Epizooties*, **70**: 337-340.

Neitz, J. (1967). The epidemiological pattern of viral, protophytal and protozoal zoonosis in relation to game preservation in South Africa. *Journal of South African Veterinary Association*, **38**: 129-141.

Neitz, J. (1957). Theileriosis, Gondonoses and Cytauxzoonoses: A review. *Onderstepoort Journal of Veterinary Research*, **27**: 275-430.

Neitz, W.O. (1956). A consolidation of our knowledge of transmission of tick-borne diseases. *Onderstepoort Journal of Veterinary Research,* **70**: 347-350.

Nelson, W.A., Keirans, J.E., Bell, J.F. and Clifford, C.M. (1975). Host ecto-parasites relationships. *Journal of Medical Entomology*, **12**: 143-166.

Nkosi, W.T., Heideman, N.J.L., Mtshali, M.S. and Mogaswane, H.R. (1999). Tick species and their pattern of infection in angora goats for resource-poor farmers in the north-eastern Free State, South Africa. *UNIQWA Research Chronicle*, Vol 1 (2), pp 123-139.

Norval, R.A.I., Perry, B.D. and Young, A.S. (1992a). The epidemiology of theileriosis in Africa. Academy Press, London. pp 481.

Norval, R.A.I., Barret, J.C., Perry, B.D. and Mukhebi, A.W. (1992b). Economics, epidemiology and ecology: A multidisciplinary approach to the planning and appraisal of tick and tick-borne diseases control in southern Africa. *In*: Tick vector biology: Medical and veterinary aspects (Fivaz, B., Petney, T. and Horak, I.G. (eds.), pp 35-54. Spriger-Verlag, Berlin.

Norval, R.A.I., Andrew, H.R. and Yunker, C.C. (1990). Infection rates with *Cowdria ruminantium* of nymph and adults of the bont tick *Amblyomma hebraeum* collected in the field in Zimbabwe. *Veterinary Parasitology*, **36**: 277-283.

Norval, R.A.I., Fivaz, B.H., Lawrence, J.J and Daillecort. T. (1983a). Agricultural extension by training and visit- its suitability for application in South Africa. *Tropical Animal Health Production,* **13**: 87-94.

Norval, R.A.I. (1983b). Arguments against dipping. *Zimbabwe Veterinary Journal*, **14**: 19-25.

Norval, R.A.I. (1982). The ticks of Zimbabwe. IV. The genus *Hyalomma*. *Zimbabwe Veterinary Journal*, **13**: 2-10.

Norval, R.A.I. (1981). The ticks of Zimbabwe. III. *Rhipicephalus evertsi evertsi*. *Zimbabwe Veterinary Journal*, **12**: 31-35.

Norval, R.A.I. (1979). The limiting effect of host availability for the immature stages on population growth in economically important Ixodid ticks. *Journal of Parasitology*, **62**: 285-287.

Norval, R.A.I. (1977). Tick problems in relation to land utilization in Rhodesia. *Rhodesian Veterinary Journal*, **8**: 33-38.

Oberem, P.T. and Bezuidenhout, J.R. (1989). Heartwater in hosts other than domestic ruminants. *Onderstepoort Journal of Veterinary Research,* **54**: 271-275.

Ondestepoort Veterinary Research, Pertoria. (1999). Laboratory Manual-Serodiagnosis for Indirect Fluorescent Antibody Test (IFAT).

Persson. A. (1995). Study on the presence of *Theileria lestoquardi* infection in sheep in Sudan based on the IFAT. A minor fieldwork. Swedish University of Agricultural Science International Rural Development. pp 1-8.

Perry, B.D., Pegram, R.C., Musisi, F.L. and Mwanaumo, B. (1986). Ecology and phenology of ticks in Zambia: Seasonal dynamics on cattle. *Experimental and Applied Acarology*, **2**: 25-45.

Petney, T.N., Horak, I.G and Rechav, V. (1987). The ecology of African vectors of heartwater, with particular references to *Amblyomma hebraeum* and *Amblyomma variegatum*. *Onderstepoort Journal of Veterinary Research,* **51**: 381-395.

Penzhorn, K. and Krecek, R.C. (1997). Veterinary parasitology in South Africa: Some highlights of the past 100 years. *Veterinary Parasitology,* **71**: 69-76.

Pinstrup-Anderson, P. (1993). Future perspectives on food supply in developing countries. *Outlook Agriculture*, **22**: 225-232.

Potgieter, F.T. and Stoltsz, W.H. (1994). Bovine anaplasmosis. *In:* Infectious disease of livestock with special reference to southern Africa, Vol. 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., (eds.)), pp 371-375.

Potgieter, F.T. (1979). Epizootiology and control of anaplasmosis in South Africa. *Journal of South African Veterinary Association*, **50**: 367-432.

Price, C.P. and Newman, D.J. (1991). Principles and practise of immunoassays. M. Stockton Press, pp 1-8.

Provost, A. and Bezuidenhout, J.D. (1989). The historical background and global importance of heartwater. *Onderstepoort Journal of Veterinary Research*, **54**: 165-169.

Punyua, D.K. (1992). A review of the development and survival of ticks in tropical Africa. *Insect Science and its Application*, **13**: 538-539.

Punyua, D.K., Latif, A.A., Nokoe, S. and Capstick, P.B. (1991). Tick (Acari: Ixodidae) infestation of zebu cattle in western Kenya: Seasonal dynamics of

the four species of ticks on traditionally managed cattle. *Journal of Medical Entomology*, **28** (5): 630-636.

Rechav, Y. (1982). Dynamics of tick populations (Acari: Ixodidae) in the Eastern Cape Province of South Africa. *Journal of Medical Entomology*, **19** (6): 679-700.

Rechav, Y., Knight, M.M. and Norval, R.A.I. (1977). Life of the tick *R. e. evertsi* under laboratory conditions. *Journal of Parsitology*, **63**:575-579.

Ristic, M. and Kreier, J.P. (1984). Family Anaplasmataceae. *In*: Krieg, N.R. and Holt, J.G. (eds.). Bergey's Manual of Systematic Bacteriology. Vol 1. Baltimore and London: Williams and Wilkins.

Robertson, W.D. (1981). A four year study of the seasonal fluctuation in the occurrence of a blue tick *Boophilus decoloratus* (Koch) in the coastal regions of the Eastern Cape. *In*: Tick biology and control (Whitehead, G.B. and Gibson., J.D., eds.), pp 199-204. Proceedings of an International Conference held at the Tick Research Unit, Rhodes University, Grahamstown . 27-29 January.

Ross, J.P.J. and Lohr, K.F. (1968). Serological diagnosis of *Babesia bigemia* infection in cattle by the IFAT. *Research Veterinary Science*, **9**: 557-562.

Saunderson, M.D. (1995). Sustainable agriculture and rural development in South Africa. *Outlook Agriculture,* **24**: 163-165.

Schultz, H. (1939). A rickettsiosis new to South Africa. *Onderstepoort Journal of Science and Animal Industry,* **13**: 287-289.

Sewell, R.I. and Brandillesby, G. (1990). A handbook on animal diseases in the tropics. Baillere Tindall, London.

Shompole, S., Waghela, S.D., Rurangirwe, F.R. and McGuire, T.C. (1989). Cloned DNA probes identify *Anapalsma ovis* in goats and reveal a high prevalence of infection. *Journal of Clinical Microbiology,* **27**: 2730-2735.

Sinha, G.K. and Pathale, R.C. (1966). Anaplasmosis in sheep and goats. *Indian Veterinary Journal*, **43**: 490-496.

Spickket, T. and Heyne, H. (1988). A survey of Karoo paralysis in South Africa. *Onderstepoort Journal of Veterinary Research,* **55**: 89-92.

Stoltsz, W.H. (1994). Ovine and caprine anaplasmosis. *In*: Infectious Diseases of Livestock with reference to southern Africa, Volume 1. (Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C., eds.). pp 431-434. Oxford University Press, Cape Town.

Theiler, G. (1969). Factors influencing the existence and distribution of ticks. *In*: The Biology and control of ticks in Southern Africa, pp 17-36. Proceedings of a symposium held at Rhodes University, Grahamstown.

Theiler, G. (1950). Zoological survey of the Union of South Africa. Tick survey: Part V. Distribution of *R. e. evertsi*, the red tick. *Ondesretepoort Journal of Veterinary Science and Animal Industry*, **24**: 33-37.

Theiler, A. (1904). *Piroplasm mutans* in species of South African cattle. *Journal of Comparative Pathology and Therapeutics,* **19**: 292-300.

Uilenberg, G. (1983). Heartwater (*Cowdria ruminantium* infection): Current status. *Advances in Veterinary Science and Comparative Medicine*, **27**: 427-480.

Uilenberg, G. (1982). Disease problems associated with the introduction of European cattle in the tropics. Proceedings of the 12th World Congress on Disease of Cattle, 7-10 September 1982, Amsterdam, the Netherlands.

Uilenberg, G. (1964). *Haematoxonus veliferus,* n.g., sp., parasite incertae sedis u sang de boring a Madagascar. *Revenue d'Elevage et de medecine Veterinaire des Pays Tropicaux*, **17**: 655-662.

Van Niekerk, A. and Louw, B.P. (1990). Kondisiebepaling van Vleisbeeste/Condition Scoring of beef cattle. Department of Agricultural Development, Pretoria.

Van Rensburg, S.J.J. (1981). The importance and need for tick control: what it means to the industry. *In*: Tick Biology and Control (Whitehead, G.B. and Gibson, D. (eds.)), pp119-122. Proceedings of an International Conference, Tick Research Unit, Rhodes University, Grahamstown, South Africa. 27-29 January.

Vrey, W.J.H. and Smith, D.J.D. (1980). The development of Qwa-Qwa Institute of Social Development and Economics Research, University of Orange Free State, pp 1-5.

Visser, E.S., Riccardo, E.A. and De Waal, D.T. (1991). An *Anaplasma centrale* DNA probe that differentiates between *Anaplasma ovis* and *Anaplasma marginale* DNA. *Veterinary Microbiology*, **28**: 213-235.

Voller, A., Bidwell, D.E. and Barlett, A. (1976). Enzyme Immunoassays in Diagnostic Medicine Theory and Practice. *Bulletin of World Health Organisation*, **53**: 55-65.

Wagner, S.F. (1992). Introduction to statistics. Harper Collins Publisher, pp 35-56.

Walker, J.B., Mehlitz, D. and Jones, G.E. (1978). Notes on the ticks of Botswana. German Agency for Technical Cooperation, West Germany.

Walker, J.B. (1961). Some observations on the classification and biology of ticks belonging to the genus *Rhipicephalus* with special reference to the immature stage. *East African Medical Journal,* **38**: 232-246.

Walpole, R.E. (1983). Elementary statistics concepts. 2nd Edition. Macmillan Publishing Co., INC, New York.

Whitehead, G.B and Gibson, J.D. (1981). Tick Biology and Control. Tick research Unit, Rhodes University, Grahamstown. South Africa, pp 7-53.

Wilkinson, P.R. (1985). Differences in paralyzing ability and sites attachment of cattle of rocky mountain wood ticks (Acarina: Ixodidae). From three regions of Western Canada. *Journal of Medical Entomology*, **22**: 28-31.

Wilson, R.T. (1995). Summary of the joint FAO/ILRI round table on livestock development strategies for low income countries. *In*: Supply of livestock products to rapidly expanding urban populations (Kim, W.Y. and Ha, J.K., (eds.)), pp7-13. Proceedings of an WAAP/KSAS International Symposium held in Seoul, Korea, FAO. 16-20 May.

Young, A.S. and Mchinja, S.J. (1977). Observations on *Haematoxenus separatus* in the erythrocytes of Kenyan sheep. *Research in Veterinary Science*, **23**: 387-388.

Zar, J.H. (1974). Biostatistical analysis. Prentice-Hall, INC., Englewood Cliffs, New Jersey.

Zaug J.L. (1987). Ovine anaplasmosis. *In*: utero transmission as it relates to stages of gestation. *American Journal of Veterinary Research,* **48**: 100-103

APPENDIX 1

1. Competition inhibition ELISA

Materials

Chemicals

Sodium Carbonate	$NaHCO_3$
Magnesium Chloride	$MgCl_26H_2O$
PBS Dulbeco	
Conjugate-Vecta Stain ABC KIT	(Vector Laboratories)
p-Nitrophenol Phosphate	Merck 1.06850

Plastic ware

Nunc C96 Pollysorp 96 well plates

Measuring cylinders	100 ml, 50 ml, 20 ml and 10 ml
Ehrlenmeyer flask	100 ml
Pipette	10ml
Micro pipette	0-200 µl

Apparatus

ELISA reader capable of reading at 405 and 620 nm (405 nm single reading is sufficient)

Printer

Shaking platform

ELISA plate shaker

Chemical balance

Micro pipette	Single	5 –200 µl

	Multi	25 – 250 µl
		20 – 1000 µl
Incubator		37°C

REAGENTS

PBS-dulbecco

NaCl	8.00 g
$Na_2HPO_4.2H_2O$	1.53 g
KCl	0.20 g
KH_2PO_4	0.20 g
$CaCl.2H_2O$	1.35 g
$MgCl.6H_2O$	0.24 g
D-H_2O up to	1000.00 ml

A - BLOCKING BUFFER

PBS	100.00 ml
Elite skimmed milk powder	5.00 g

B - SERUM DILUTION BUFFER

PBS/1%Elite/0.1% Tween 20

PBS	100.00 ml
Elite skimmed milk powder	1.00 g
Tween 20	0.10 ml

C - SUBSTRATE BUFFER

$NaHCO_3$	100 mM	0.84 g
$MgCl_26H_2O$	10 mM	0.203 g

D-H_2O	100.00 ml
pH	9.5

D - WASHING BUFFER

PBS	1000.00 ml
Tween 20 (0.1%)	1.00 ml

VECTA STAIN ABC

Portion A (anti-mouse antibodies)

Dilute 1:3000 with dilution buffer

AVIDIN/BIOTIN SOLUTION

Two solutions (portion B and C of Vecta ABC) are packed separately and need to be mixed.

For one plate, mix

Avidin	7.50 µl
Biotin	7.50 µl
Washing buffer	11.00 ml

Incubate for 30 minutes at 37°C after mixing.

SUSBTRATE

p-Hitrophenol phosphatase	0.01 g

Substrate buffer (Solution C)

2. Peparations of blood smears)

10% Giemsa Stain

10 ml Giemsa Buffer mixed with 10 ml Giemsa stain

Giemsa Buffer

$Na_2HPO_4.2H_2O$	104.41 g
$NaH_2PO_4.PO4.2H_2O$	19.2 g
D-H_2O	1000.00 ml

518C immersion Oil (Zeiss Germany)

3. Ticks preservation

Collecting bottles

Alcohol	70ml
D-H_2O	10 ml

APPENDIX 2

APPENDIX 2-1: The analysis of variance for PCV and OD values for goats in Harrismith.

Site for goats	Months	PCV LSMean	OD value LSMean
Harrismith	1	0.2759	1.3930
Harrismith	2	0.2709	0.6071
Harrismith	3	0.2698	0.3757
Harrismith	4	0.2745	0.8201
Harrismith	5	0.2680	0.8326
Harrismith	6	0.2654	0.6815
Harrismith	7	0.2895	0.6647
Harrismith	8	0.2720	1.2399
Harrismith	9	0.2699	0.3510
Harrismith	10	0.2622	0.3505
Harrismith	11	0.2757	0.3490
Harrismith	12	0.2704	0.2311

APPENDIX 2-2: The analysis of variance for PCV and OD values for goats in Kestell.

Site for goats	Months	PCV LS Mean	OD value LS Mmean
Kestell	1	0.2627	0.8960
Kestell	2	0.2712	0.6766
Kestell	3	0.2704	0.4812
Kestell	4	0.2712	0.3722
Kestell	5	0.2667	0.3360
Kestell	6	0.2672	0.2294
Kestell	7	0.2747	0.7282
Kestell	8	0.2687	0.5210
Kestell	9	0.2702	0.6147
Kestell	10	0.2737	0.1568
Kestell	11	0.2687	0.4950
Kestell	12	0.2727	0.1720

APPENDIX 2-3: The analysis of variance for PCV and OD values for goats in Qwa-Qwa.

Site for goats	Months	PCV LS Mean	OD value LS Mean
Qwa-Qwa	1	0.2812	0.4288
Qwa-Qwa	2	0.2807	0.5000
Qwa-Qwa	3	0.2787	0.5436
Qwa-Qwa	4	0.2787	0.3486
Qwa-Qwa	5	0.2712	0.4644
Qwa-Qwa	6	0.2662	0.7320
Qwa-Qwa	7	0.2759	1.3303
Qwa-Qwa	8	0.2537	0.8209
Qwa-Qwa	9	0.2668	1.1698
Qwa-Qwa	10	0.2687	1.07003
Qwa-Qwa	11	0.2687	0.7524
Qwa-Qwa	12	0.2631	0.2075

APPENDIX 2-4: The analysis of variance for PCV and OD values for sheep in Harrismith.

Site for sheep	Months	PCV LSMean	Odvalue LS Mean
Harrismith	1	0.2646	0.6738
Harrismith	2	0.2647	0.2722
Harrismith	3	0.2712	0.9782
Harrismith	4	0.2689	0.5732
Harrismith	5	0.2617	0.7125
Harrismith	6	0.2745	0.6262
Harrismith	7	01.2785	0.8233
Harrismith	8	0.264	0.733
Harrismith	9	0.2763	0.9372
Harrismith	10	0.2634	0.832
Harrismith	11	0.2645	0.7363
Harrismith	12	0.2645	0.7363

APPENDIX 2-5: The analysis of variance for PCV and OD values for sheep in Kestell

Site for Sheep	Month	PCV LS Mean	OD value LS Mean
Kestell	1	0.2765	0.5872
Kestell	2	0.2760	0.1862
Kestell	3	0.2870	0.2026
Kestell	4	0.2792	0.4888
Kestell	5	0.2653	0.3353
Kestell	6	0.2739	0.2209
Kestell	7	0.2874	0.1802
Kestell	8	0.2683	0.1733
Kestell	9	0.2772	0.4141
Kestell	10	0.2858	0.4664
Kestell	11	0.2862	1.5319
Kestell	12	0.2954	0.8424

APPENDIX 2-6: The analysis of variance for PCV and OD values for sheep in Qwa-Qwa.

Site for sheep	Months	PCV LS Mean	OD value LS Mean
Qwa-Qwa	1	0.2742	0.6912
Qwa-Qwa	2	0.2780	0.5904
Qwa-Qwa	3	0.2748	1.9882
Qwa-Qwa	4	0.2700	1.8844
Qwa-Qwa	5	0.2671	1.3969
Qwa-Qwa	6	0.2894	0.8712
Qwa-Qwa	7	0.2653	0.5305
Qwa-Qwa	8	0.2888	0.9274
Qwa-Qwa	9	0.2720	0.7073
Qwa-Qwa	10	0.2836	1.6712
Qwa-Qwa	11	0.3003	2.2996
Qwa-Qwa	12	0.2939	1.6789