A SERO-EPIDEMIOLOGICAL SURVEY OF PARASITES IN CATTLE IN THE NORTH EASTERN FREE STATE, SOUTH AFRICA

By

MOSES SIBUSISO MTSHALI

BSc, BSc Hons

Student number: 9511652

A thesis submitted in fulfilment for the degree of Master of Science in Parasitology

University of the North

Qwa-Qwa Campus

Faculty of Natural and Applied Sciences

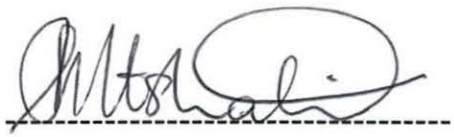
School of Life Sciences

Parasitology Research Program

2001

DECLARATION

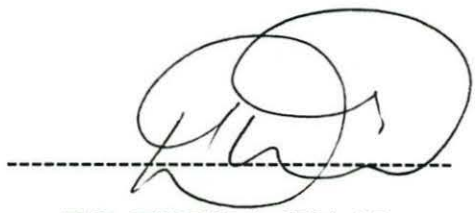
I, **Moses Sibusiso Mtshali**, hereby declare that this thesis is my original work and has not been presented in any other University



MOSES SIBUSISO MTSHALI

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

PROF. PETER AMUNGA MBATI



DR. THEO de WAAL

DEDICATION

This thesis is dedicated to my parents, Themba Hamilton Mtshali and Harriet Bonisiwe Mtshali and my late younger brother, Sizwe Mtshali

ACKNOWLEDGMENTS

I am deeply indebted to my supervisors, Prof. Peter Amunga Mbati and Dr. Theo de Waal for their guidance and encouragement during the entire period of this study. I sincerely thank Dr. Laura Lopez, Mr Olivier Matthee, Ms Andrea Spickett and Mr Jonas Komape of the Onderstepoort Veterinary Institute for training and guidance in sero-diagnostic techniques. I wish to thank Mr Motseki Hlatshwayo for his considerable assistance with tick identification and classification. I would also like to acknowledge Dr. Peter Njuho of the University of Natal, Pietermaritzburg for helping me with statistical analysis.

I also thank Mr Mothea Tsosane, Mr Thobelani Lose, the late Ms Zolelwa Mxhebe and Dr Sello Maboe for their assistance with data collecting. I would also like to express appreciation for the different type of assistance and support that I received from the following people of UNIQWA's Parasitology Research Program: Esther Maine, Kagiso Mogaswane, George Mosebi, Lehlohonolo Taoana, Theki Moloi, Adam Rathebe, Ana Tsotetsi, Limpho Taoana, Oriel Thekisoe, Flatha Masangane and Patrick Leeto. Farmers from the north eastern Free State who participated in this study are acknowledged for allowing us to use their animals in this survey. Lastly, I would like to thank my family, my two grandmothers, Ms Alice Sibisi and Ms Mildred Mtshali and above all God Almighty for the various type of assistance and support they gave me throughout this study, without them I couldn't have made it.

This research is based on a concept developed by Prof. Peter Mbati and received financial support from two grants made available to him:

(i) University of the North Qwa-Qwa Campus Senate Research Grant 4521

(ii) The National Research Foundation (GUN 2039188).

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ABSTRACT

Global economic losses due to parasitic infections in livestock are enormous. There is little or no information on parasites of veterinary importance afflicting livestock in the north eastern Free State, South Africa. A survey to determine the incidence of parasites in cattle (n=386) was conducted in the north-eastern Free State between August 1999 and July 2000. Giemsa-stained blood smears were negative for blood parasites. A total of 94% of the cattle were sero-positive for *Babesia bigemina* by Indirect Flourescent Antibody Test (IFAT) while 87% were sero-positive for *Anaplasma* by Enzymed-linked Immunosorbent Assay (ELISA). All the animals were sero-negative for *B. bovis* and this is probably because the tick vector (*Boophilus microplus*) which transmits the disease is not present in the Free State Province. There was no significant difference in the incidence of either anaplasmosis or babesiosis between the seasons. Two tick species belonging to the family Ixodidae were found on cattle, namely, *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi*. In the present study significant differences in seasonal burdens of *B. decoloratus* occurred, with the highest infestations recorded from February to June. The presence of *R. evertsi evertsi* throughout the year without any or with small fluctuations in winter months was observed, with a peak from February to May. The observation of negative blood smears but high incidence of positive serological results for *Anaplasma* and *Babesia* for the same group of cattle indicates that this area is endemic for these diseases but with a stable disease situation.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 PREAMBLE

With its great 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 has an infinite variety of habitats and an overwhelming variety of animal species, and an equally diverse parasitic fauna. Superimposed on this system are the parasites of domestic animals, brought into the country by successive waves of immigrants: first, the sheep-herding Khoi and later, in the early iron age, the Blacks with their cattle, goats, dogs and chickens. Western Europeans added horses, donkeys, pigs and various species of poultry (Penzhorn and Krecek, 1997).

It has been estimated that one hundred years from now the population of the earth will exceed ten billion people, about twice what it is today. The question that arises is whether or not the human species will be able to feed itself and if so, how? Parasites are a major cause of disease of man and domestic animals. More than half of the human population live in misery and pain and suffer vast economic losses due to parasites. A possible solution to these problems lies in the development of new agricultural technologies for expanding food production. One of the ways in which livestock production can be increased is by reduction of losses due to disease. Estimates suggest that only a 6% reduction in disease could provide food for an estimated 250 million people. The situation is most serious in developing countries where 75% of the world's population resides. According to the Food and Agriculture Organization, it is estimated that up to 70% of the world's livestock

resources exist in these regions, yet they account for only 30% of the world's meat output (Ambrosio and De Waal, 1990).

1.2 INFECTIOUS DISEASES OF CATTLE

Animal diseases in general and tick and tickborne diseases (TTBD)in particular are among the many factors which directly and indirectly hamper the growth of the livestock sector due to the many roles of livestock as a source of food and income through generating employment, delivery of energy (dung, biogas), fertiliser, weed control, use of marginal lands, investment and savings as well as transport (Sansoucy, 1995). Tick-borne diseases of major economic importance in Southern Africa which affect cattle are heartwater caused by *Cowdria ruminantium*, babesiosis caused by *Babesia bigemina* and *B. bovis*, anaplasmosis caused by *Anaplasma marginale*, and theileriosis caused by the *Theileria parva*-complex comprising of *T. parva parva*, *T. p. bovis* and *T. p. lawrencei*. Of lesser importance in cattle are the generally non-pathogenic mild theileriosis caused by *T. mutans*, *T. velifera* and *T. taurotragi*, spirochaetosis (borreliosis) caused by *Borrelia theileria*, benign babesiosis caused by *B. occultans*; and bovine ehrlichiosis caused by *Ehrlichia bovis* (Horak, *et al*, 1991).

1.2.1 The impact of ticks and tick-borne associated diseases

An important component of livestock improvement in the developing world, together with good nutrition, management and the availability of markets, is the control of animal diseases. The problem is particularly serious in Africa, Asia, the Caribbean, and Latin America. Losses caused by tick infestation are usually defined as tick worry, blood loss, damage to hides and skins of animals and introduction of

toxins (De Castro, 1997).

Regarding the indirect effects of ticks, four major tick-disease complexes are recognised affecting cattle on a world-wide basis (McCosker, 1979):

(i) *Boophilus* spp. - *Babesia* spp. (Babesiosis) - *Anaplasma marginale* (Anaplasmosis). This is probably the most important complex. It occurs in Latin America, Oceania and Asia, large areas of Africa and the Near East. The ticks cause damage per se as well as transmitting anaplasmosis and babesiosis which particularly affect imported and/or crossbred/high grade dairy and beef cattle;

(ii) *Hyalomma* spp. - *Theileria annulata* (Tropical theileriosis). These ticks and the disease are mainly distributed across northern Africa, southern Europa, the Near East and West Asia. In the latter, small holders, peri-urban dairies and imported cattle in general are the main production systems affected by this complex and it is likely to gain importance in northern Asia as the dairy sector develops in that region;

(iii) *Amblyomma* spp. - *Cowdria ruminantium*. This tick species, which transmits heartwater, occurs throughout sub-Saharan Africa. It is particularly important in sheep and goats. This complex is also present in Caribbean. In addition to heartwater, *Amblyomma* ticks also facilitate the development of dermatophilosis which is important in West Africa and the Caribbean. *Amblyomma cajennense* infestations are locally important in areas of Latin America such as Brazil, Cuba and Mexico. *Amblyomma* spp. also transmit *Theileria mutans*, a generally mild disease of cattle found throughout sub-Saharan Africa;

(iv) *Rhipicephalus* spp. - *Theileria parva* (East Coast fever, Corridor Disease, January Disease). It occurs in cattle throughout Eastern, Central and Southern

Africa.

In Africa, tick-borne diseases are important and they are considered as one of the greatest animal disease problem. It is accepted that theileriosis, dermatophilosis and heartwater are the major tick-borne or tick-associated diseases of grazing cattle. Babesiosis and anaplasmosis may be important in certain regions and may cause problems in grazing and zero-grazing situations. The complexity of determining the direct and indirect economic impact of tick-borne diseases and their control is reflected in the fact that only rough estimates are available for the cost of some of the components (De Castro, 1997).

The only world-wide account of the costs (control expenses + damage) of tick-borne diseases in cattle was given by McCosker (1979), who estimated these as ca. US$ 7 billion per year. This calculation was based on Australian figures of A$ 42 million in losses in 1973 in a cattle population of 8.5 million head (ca. A$ 5 per head), multiplied by the world cattle population at risk. An adjustment of these values to current levels using various indices results in a cost per head of cattle of US$ 13-18 at 1996 prices. This estimate was confirmed by using the estimates of McLeod (1995) to a cost per head in Australia of ca. US$ 14. About 80% of the world's cattle population of 1288 million (FAO/OIE/WHO, 1994) live in areas of tick-borne diseases risk. The global costs of tick-borne diseases would now be estimated between US$ 13.9 and 18.7 billion.

1.2.2 Tick-borne diseases diagnostic and control methods

Efficient sustainable tick-borne disease control can only be accomplished if the epidemiology is known and reliable diagnosis can be established.

1.2.2.1 Diagnostics

A number of diagnostic tests for tick-borne diseases, mostly based on the use of complement fixation, agglutination and flourescent antibodies, have been available for some time for anaplasmosis and babesiosis (Böse *et al.*, 1995; Wright, 1990), heartwater (Du Plessis and Malan, 1987) and theileriosis (Norval *et al.*, 1992b). These tests have enabled workers to perform field studies in the epidemiology of these diseases (Latif *et al.*, 1995). New tests based on enzyme-linked immunosorbent assay (ELISA) have been developed or are in different stages of development for anaplasmosis (Duzgun *et al.*, 1988), babesiosis (Jorgensen *et al.*, 1994), heartwater (Martinez *et al.*, 1993) and theileriosis (Musoke *et al.*, 1993).

The diagnosis of acute babesiosis in cattle caused by *B. bigemina* and *B.bovis* is relatively straightforward when clinical signs are evident and supported by microscopic examination of stained blood films. Mild and inapparent infections are more difficult to recognise since peripheral blood parasitemias fluctuate and frequently do not rise to levels detectable by microscopy. In these circumstances direct staining is unreliable even when improved thick blood film methods are used. Equally limited are the more complicated direct fluorescent staining techniques using fluorescein labeled antibody or non-conjugated acridine orange (Ross and Lohr, 1968). A complement fixation test for *B. argentina* and *B. bigemina* reliable for 7 and 4 months, respectively has been developed. However, since parasites

persisted in the blood for some months after the test ceased to be fully effective, negative results could not be taken as indicative of susceptibility or the non-carrier state (Ross and Lohr, 1968).

Successful use has been made of indirect immunofluorescence to study plasmodium antibodies in human sera. One of the most successful and widely used tests in protozoology is the indirect fluorescent antibody test (IFAT) which has been employed in diagnosis and in epidemiological studies of malaria, trypanosomosis and toxoplasmosis. The IFAT has also been extensively used for the detection of antibodies against cattle piroplasms (Morzaria *et al.*, 1977). The antigenic characteristics of the *Babesia* spp. are now investigated by this test (Gray and De Vos, 1981). This test has been used in several studies on bovine *Babesia* spp. (Ross and Löhr, 1968). The indirect fluorescent antibody test is considered to have a high degree of specificity (Du Plessis and Malan, 1987).

1.2.2.2 Immunisation

Effective anti-protozoan drugs are now at hand in case of tick-control failure (Peregrine, 1994) and as an essential back-up during tick-borne disease immunisation. Resistance to these drugs has not been reported (Peregrine, 1994).

Immunisation against both babesiosis and anaplasmosis is widely used throughout the world. A number of laboratories produce attenuated *B. bovis* and *B. bigemina* vaccines. In addition, *Anaplasma centrale*-based vaccines are successfully used to protect against *A. marginale* and combined *B. bigemina*, *B. bovis* and *A. marginale* vaccines are available and used widely in Australia, South Africa, Argentina, Brazil

and Uruguay. Although progress has been made on recombinant vaccines for anaplasmosis and babesiosis, no vaccine of this nature is yet available for field use (De Castro, 1997).

Difficulties still exist with regard to the development of an appropriate immunisation procedure against heartwater. The traditional Ball 3 strain is being used, particularly in sheep and goats, but its use in cattle is restricted and requires close monitoring. Recently, sheep have been successfully immunised with an inactivated *C. ruminantium* vaccine (Mahan, 1996). A schizont-based *Theileria annulata* vaccine has been available for some time for tropical theileriosis, and it is being widely used. Work is also being carried out towards a sporozoite-based vaccine and also sub-unit vaccines (Mahan, 1996).

The infection and treatment method developed for the immunisation of cattle against East Coast fever has successfully been used through the FAO-implemented 'Coordinated Multi-Donor Programme for Integrated Tick and Tick-borne Disease Control in Eastern, Central and Southern Africa' as well as by bilateral projects in Kenya and Zambia (Musisi *et al.*, 1992). A trivalent vaccine composed of three stocks (Kiambu 5, Muguga and Serengeti transformed) has been successfully used to immunise cattle in Malawi, Tanzania, Uganda and Zambia (Musisi *et al.*, 1992).

1.3 VECTORS OF TICK-BORNE DISEASES

Vector-borne diseases of livestock cause economic hardships throughout the tropical and subtropical regions of the world. Ticks are important vectors of infectious diseases of domestic livestock in southern Africa (Howell *et al.*, 1978).

They are sessile ectoparasites with life cycles usually consisting of a parasitic phase and a non-parasitic free living phase (Londt *et al.*, 1979). They are the most important external parasites of domestic animals 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).

They do harm both directly and indirectly. Directly ticks cause deterioration in the condition of their hosts resulting from loss of blood, tick-worry and tick toxicosis. Their bites are damaging in themselves and can result in the degrading of hides. Frequently, also, they form a route for invasion of the host's tissues by secondary organisms. Abscesses and deep-seated suppurating wounds may form which sometimes become maggot-infested and cause crumpled ear pinnae, sloughed teats, missing tail tips, lameness and foot-rot. Their indirect effects are even more serious in that ticks act as vectors of a wide range of pathogenic organisms which frequently cause the death of their hosts (Howell *et al.*, 1978).

Ticks are often referred to as insects but they are in fact much more closely related to spiders, scorpions and mites. They are all bloodsuckers for part of their lives and some species apparently feed on almost any kind of animal. Some, however, appear to prefer animals belonging to a particular group, for example cattle, sheep, goats and other herbivores. Others are extremely host specific and may only be found on one kind of animal. Elephants and rhinos, for example, have their own species of ticks, as do dassies, tortoises and snakes. Species of economic importance from the veterinary point of view belong mainly to the families Ixodidae (hard ticks) and Argasidae (soft ticks) (Howell *et al.*, 1978).

1.3.1 The life system of ticks

The life system of ticks is divided into three components: Free-living developmental phase, host finding, and the parasitic phase. Each component is interrelated with each other and can also be studied separately and then integrated into a life system (Sutherst *et al.*, 1978). These three components are common to all types of ticks, with the three-host and argasids tick repeatedly passing through each phase:

a) Developmental phase in vegetation, *i.e.*, engorged female ticks and their eggs, and fed larvae and nymphs of three-host ticks.
b) Host finding, which comprises the distribution, dispersal, longevity, behavior, and rate of host/tick contacts of unfed larvae, nymphs, and adults as appropriate to one, two or three host ticks.
c) The parasitic phase.

(a) Free-living developmental phase

During the free-living developmental phase, ticks are relatively immobile, which enables experimental as well as census data to be collected with relative ease. The major mortality factors known for ticks are extremes of temperature and desiccation (Sutherst *et al.*, 1978). Localized predation by spiders, ants, mice, and birds may also occur (Wilkinson, 1970). Parasites have been recorded from several ticks, but their parasitism has not prevented ticks from becoming important pests. Estimates of microclimatic temperature and moisture are needed to study developing, free living ticks. The fed larvae, nymphs, and females of the three-host ticks are more tolerant to dry conditions than are eggs. Neither eggs nor engorged immature ticks are able to compensate for water loss before molting. In contrast, the unfed ticks can

drink dew (Wilkinson and Wilson, 1959) or absorb moisture from atmosphere with a high relative humidity (Less, 1946).

(b) Host finding and unfed stages of the life cycle

During the host finding and unfed stages of the life cycle the proportion of a tick population that finds a host depends upon the behavior and longevity of unfed ticks and the intensity of host/tick contacts, which depend in turn upon the effective area covered by each host and the density of hosts. The first requirement is to estimate the efficiency of coverage of the habitat by each individual host. The problem is complicated by the modification of host coverage of the habitat by interference from other hosts (Sutherst *et al.*, 1978). The proportion of unfed ticks which will find a host in a continuously grazed area is determined by their median longevity and the rate at which they find a host. The success of active ticks in finding a host can be estimated in two ways. Firstly, the rate of removal or loss of unfed ticks from grazed pasture may be compared with the rate to loss due to mortality and dispersal in the ungrazed pasture. An alternative method is to graze an infested pasture with groups of host for short intervals and count the numbers of ticks they pick up. If the process is repeated once or twice, the host-finding rate can be estimated (Sutherst *et al.*, 1978).

(c) Parasitic phase

The parasitic phase of the life cycle is the main reason for studying ticks, because it is during this phase that damage is inflicted on domestic stock or infected with disease organisms. Some of the more important factors affecting the success of ticks during this phase are density of ticks, favourability of hosts, previous tick

experience, host species, age, nutrition, season, health, lactation, sex, *etc*. Two research activities are involved in studying the ecology of parasitic ticks. Firstly, the numbers of parasitic ticks must be monitored, and secondly, the favourability of the host must be assessed experimentally (Sutherst, *et al*., 1978).

1.3.2 Systematic position of ticks

Ticks are all bloodsuckers for part of their lives and some species feed on almost any kind of animal. Others are extremely host specific and may only be found on one kind of animal. Ticks belong to class Arachnida and are related to scorpions and spiders. Ticks and mites belong to the order Acarina and are closely related. This order includes three families of ticks, namely, Argasidae (soft ticks), Ixodidae (hard ticks) and Nuttaliellidae (Howell *et al*, 1978).

Classification of ticks is given as follows (Service, 1996):

Phylum : Arthropoda
Class : Arachnida
Order : Acarina
Sub-order : Ixodides
Families : Ixodidae
Argasidae
Nuttaliellidae

1.4 ANAPLASMOSIS

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

Order : Rickettssiales

Family : Anaplasmataceae

Genus : *Anaplasma*

Anaplasmosis is an important tick-transmitted disease of cattle caused by a rickettsia-like organism. It is more commonly known as gallsickness (De Waal, *et al*, 1998a). It occurs in the red blood cells of infected animals. The infectious organism invades and destroys red blood cells, causing anemia, weakness, and sometimes death. Infected cattle may recover but become carriers that serve as a reservoir of the disease (Iowa Cooperative Extension Service, 1992). The two causative organisms of anaplasmosis, namely *Anaplasma marginale* (Plate 1)and *A. centrale* (Plate 2) have been described in cattle in the Free State Province (Fourie *et al*., 1996).

1.4.1 Distribution

Anaplasmosis occurs throughout most of South Africa and Namibia (except in the very low rainfall areas where few ticks occur). In many areas it is permanently established (endemic). The distribution of anaplasmosis often corresponds to that of babesiosis, but it may also occur in areas free of babesiosis (De Waal *et al*, 1998a).

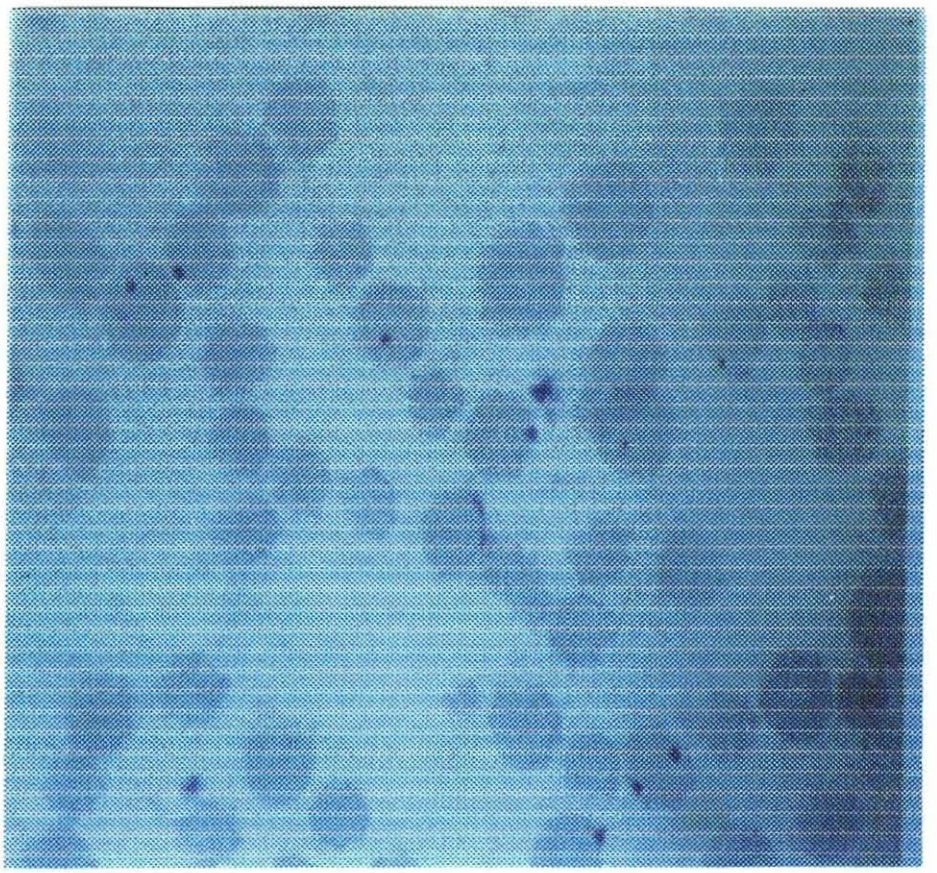


Plate 1: *Anaplasma marginale* in a thin blood smear

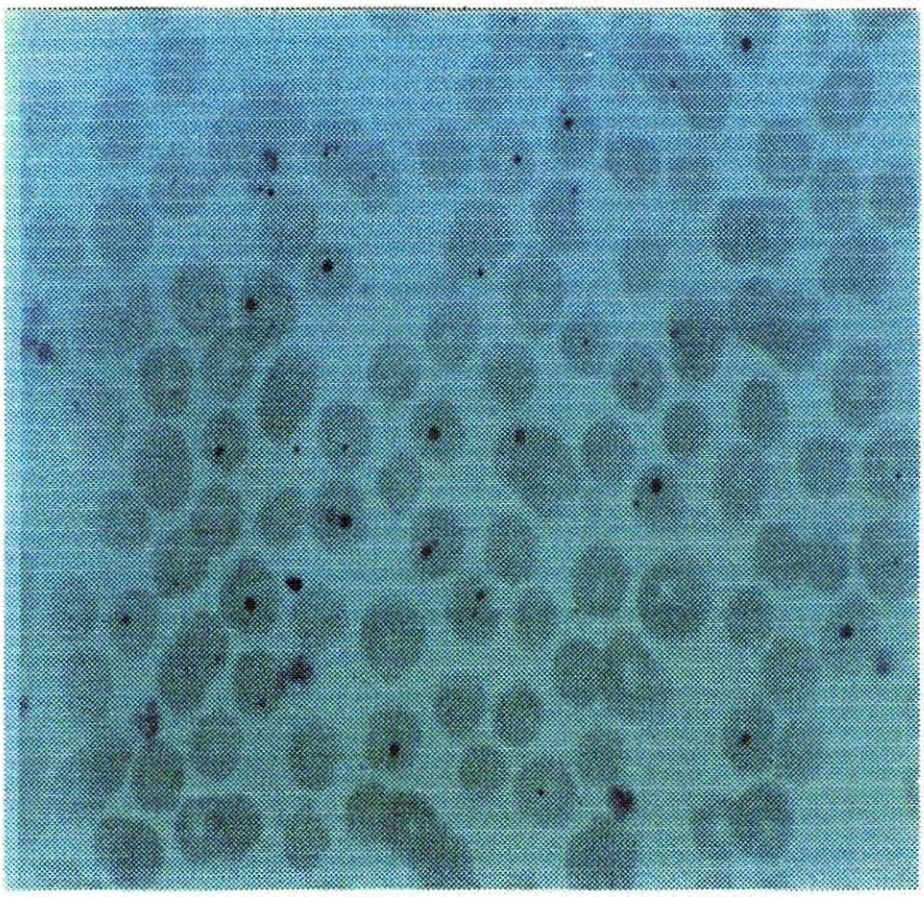


Plate 2: *Anaplasma centrale* in a thin blood smear

1.4.2 Symptoms

Anaplasmosis is most commonly diagnosed when a seriously ill or dead adult animal is discovered. This usually occurs in mid to late summer. Affected individuals are anemic, weak, off feed, and lose weight rapidly. Jaundice, or yellowing of the skin, is common but is only observed when the eyes, mouth, and other membranes are examined closely. Difficult respiration, fever of 105^0 F or higher, dehydration, and constipation are common (Iowa Cooperative Extension Service, 1992). Milk production of lactating cows declines dramatically. Affected animals are often excitable or belligerent. Death often occurs within 24 to 48 hours of the onset of symptoms and may be the first indication that anything is wrong in a herd. Abortion after recovery from clinical disease is common (Iowa Cooperative Extension Service, 1992).

In an infected herd, recovery from undiagnosed infection and development of a carrier state is actually more common than death. Many carriers were never known to have been sick. Infected carriers may be more susceptible to other conditions such as parasitism, severe winter weather stress, or malnutrition. In these cases, anaplasmosis may be a contributing factor but is not the immediate cause of death (Iowa Cooperative Extension Service, 1992).

Animals less than 1.5 or 2 years of age seldom show symptoms, although they may become infected and serve as a reservoir of infection for other herd members. They can regenerate red blood cells rapidly, replacing those destroyed by the infection. Younger animals may be off feed and moderately anemic, but usually recover rather quickly. Anaplasmosis is not a major cause of disease in feedlot cattle. It

occasionally affects confined dairy cows, but is much more common in adult beef cattle (Iowa Cooperative Extension Service, 1992).

1.4.3 Economic effects on cattle herds

Losses in breeding herds can be severe, although it is more common to have only a few individuals show symptoms. Mortality may be high however, especially if there is a massive exposure in a herd that has never been exposed to anaplasmosis. It is not unusual to find several infected individuals in a herd even though there has never been any evidence of disease. Losses depend on the degree of exposure, how the infection was introduced into the herd, and possible immunity from previous exposure to the infectious agent (Iowa Cooperative Extension Service, 1992).

Death losses of 20 or 30 percent may occur, although a mortality rate of 5 to 10 percent in newly infected herd is more common. In chronically infected herds, losses of 1 to 2 percent each year is common if control or eradication procedures are not instituted (Iowa Cooperative Extension Service, 1992).

1.4.4 Diagnosis

Post mortem examination of cattle that die of anaplasmosis reveals icterus (jaundice), anemia, pale tissues, an enlarged spleen, swollen liver, and an enlarged gall bladder. Hemoglobin in the urine is not found in cattle that die of anaplasmosis (as in cases with leptospirosis, bacillary hemoglobinuria, babesiosis and several other infectious diseases of cattle).

Animals that recover develop antibodies to the *Anaplasma* organism. This immunity

can be detected by a card agglutination or complement fixation test. Blood tests are used to confirm a diagnosis of anaplasmosis and to detect infected carriers. Antibody levels decline after infection is eliminated, so that the success of herd treatment and eradication programs can be monitored over time (Iowa Cooperative Extension Service, 1992).

1.4.5 Transmission

Anaplasmosis is spread from infected to susceptible cattle by ticks and biting insects or through careless use of instruments such as the dehorning, castrating, tatooing, or ear tagging equipment. Biting insects spread the disease by transmitting infected blood form infected to susceptible animals. Insects such as biting (tabanid) flies must feed on an infected animal and then a susceptible animal within a few minutes in order for transmission to occur (Iowa Cooperative Extension Service, 1992).

In South Africa it is transmitted by at least five tick species (De Waal *et al*, 1998a). After the tick feeds on an infected animal, the infectious organism localizes and multiples in the cells lining the gut of the tick. The organism can then be transmitted to succeeding generations of ticks. By this method, the infectious organism can survive outside of infected cattle for long periods of time. When future generations of infected ticks feed on susceptible cattle, the disease is transmitted. Tick control is an important aspect of control of anaplasmosis (Iowa Cooperative Extension Service, 1992).

1.4.6 Treatment

The anaplasmosis organism is susceptible to oxytetracycline (Terramycin) or chlortetracycline (Aureomycin) antibiotics. Cattle showing clinical signs usually respond to high dosages of oxytetracycline given by injection. In some cases, however, 60 or 70 percent of the red blood cells in cows with clinical disease are infected. These cattle may die in spite of treatment because the tetracycline only prevents spread of the organisms between blood and cells. They do not kill the organism once it is inside the cell. Early recognition and treatment is important (Iowa Cooperative Extension Service, 1992).

When clinical disease is diagnosed, immediate treatment of affected individuals with high dosages (10 mg oxytetracycline per pound of body weight or more for 3 to 5 days) is recommended. Blood transfusion of cattle with severe anemia may be helpful if practical under field conditions. Supportive treatment with fluids and electrolytes is helpful. Affected individuals should be isolated from other cattle (Iowa Cooperative Extension Service, 1992).

1.4.7 Prevention and Control

In southern Africa, where anaplasmosis is endemic in many of the important cattle farming areas, its eradication followed by the implementation and long-term maintenance of strict tick control programmes is not generally recommended. Eradication of anaplasmosis on the subcontinent and most other parts of Africa, is not practically possible for obvious reasons. Stringent tick control would render the cattle population susceptible to several other tick-borne diseases with potentially catastrophic consequences (Potgieter and Stoltsz, 1994).

It is generally recommended that a stable disease situation be persued in southern African region by allowing natural exposure of calves to tick-borne diseases, icluding anaplasmosis, during the period when they are naturally resistant or protected by passively-acquired maternal antibody. The existence of epidemiological factors which inhibit the exposure of calves to infected ticks within the relevant period can be compensated for by the administration of the *A. centrale* live-blood vaccine (Potgieter and Stoltsz, 1994).

1.5 BOVINE BABESIOSIS

The babesias are a group of protozoan parasites of red blood cells that are transmitted biologically by ticks. The economically most important species which affect bovines are *Babesia bigemina* (Plate 3) and *B. bovis* (Plate 4). They are important because of their wide geographic distribution. The disease causes fever, malaise and anemia. Hemoglobinuria is common with *B. bigemina*. Signs of circulatory shock and central nervous system are common with *B. bovis*. The animals that survive the infection remain as healthy carriers, developing a resistance to the infection (Barriga, 1994).

Babesiosis is caused by infection with species of tick-borne, intra-erythrocytic and generally host-specific protozoan parasites of the genus *Babesia* is classified as follows (De Vos *et al.*, 1994):

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

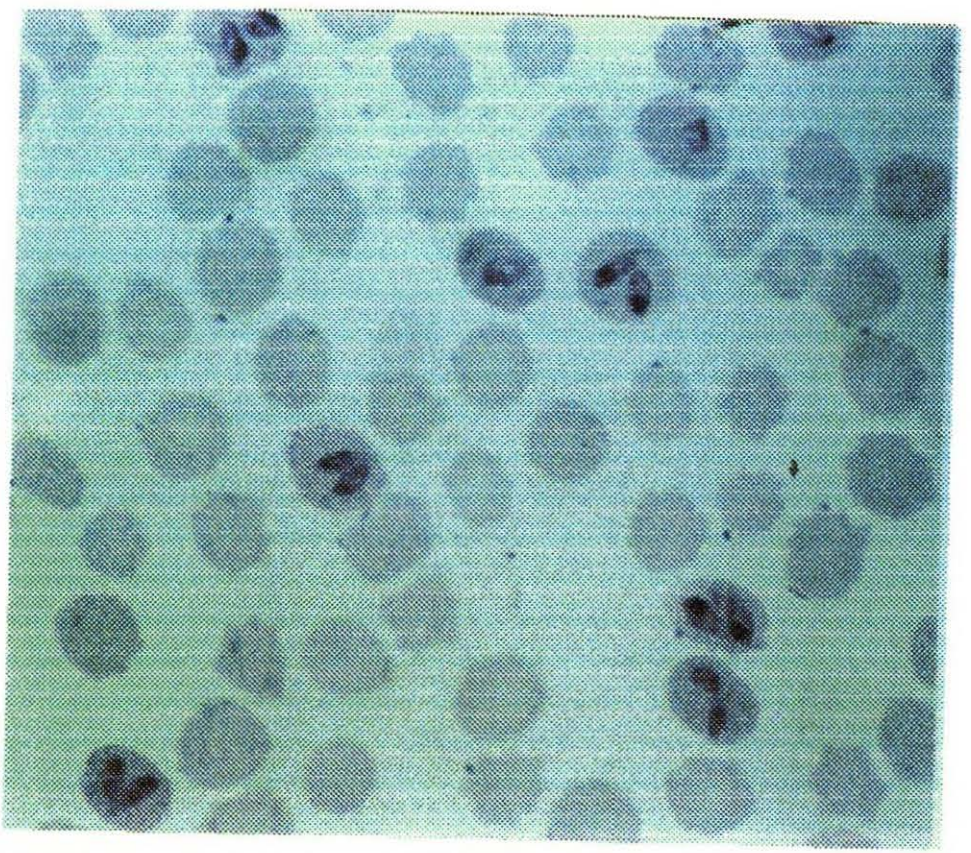


Plate 3: *Babesia bigemina* in a thin blood smear

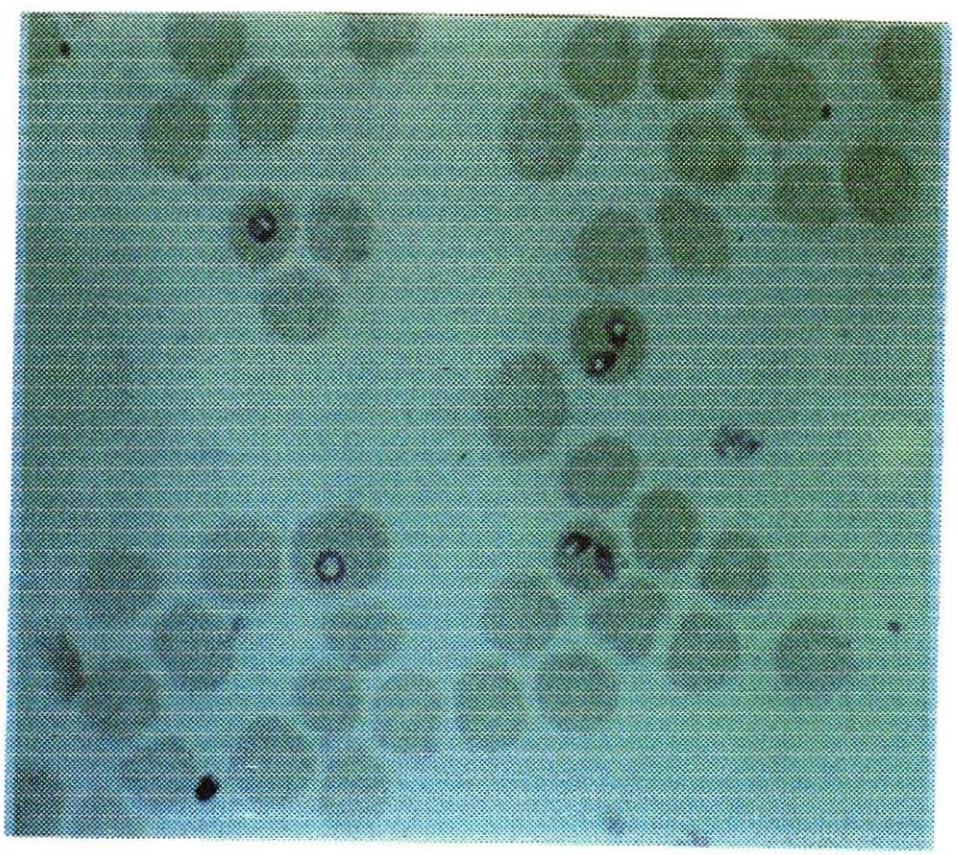


Plate 4: *Babesia bovis* in a thin blood smear

Family : Babesiidae

Genus : *Babesia*

1.5.1 Distribution and transmission

Babesiosis is widespread in South Africa, except in the very low rainfall areas where blue ticks do not exist. However, in many areas it is permanently established, that is, endemic. *Babesia bigemina* infection has the widest distribution, coinciding with the distribution of its two tick vectors, namely the one-host blue tick *Boophilus microplus* and *Boophilus decoloratus*. These ticks are absent in the Western and Northern Cape Provinces, western Free Sate and high lying parts of the Drakensberg and Lesotho. *Babesia bovis* infection has a more limited distribution because it is transmitted only by *B. microplus*, which occurs mainly in high rainfall areas. These include parts of the Eastern Cape Province, KwaZulu-Natal and the eastern parts of Mpumalanga (De Waal *et al*, 1998b).

Under natural conditions babesiosis is transmitted only by ticks (De Waal *et al*, 1998b).

1.5.2 Symptoms

Cattle usually begin to show signs of disease approximately 2-3 weeks after exposure to infected ticks. Fever is usually present for several days before other signs become obvious. This is followed by signs of poor appetite, depression, weakness and a reluctance to move. A light to dark red or brown discoloration of the urine is often present. Anaemia or even jaundice develop and are especially obvious in more advanced cases. Dry nose, rough hair coat, and diarrhoea are less

specific symptoms that may occur (De Waal *et al*, 1998b).

1.5.3 Diagnosis

In suspected cases of babesiosis a diagnosis of the disease can be confirmed by the microscopic examination of a blood smear prepared either from a live animal, take before the animal was treated with a redwater drug, or from one that had died shortly before (De Waal *et al*, 1998b).

1.5.4 Treatment

Babazene, Berenil, Veriben, Euflavine and Imizol are all effective redwater drugs that are available without prescription in South Africa (De Waal *et al*, 1998b).

1.5.5 Prevention and Control

Sustainable intensive tick control measures may under certain conditions succeed in preventing outbreaks of babesiosis, even in endemic areas. This, however, should be regarded as a temporary measure only. It is accompanied by risks of later outbreaks of the disease should the control measures be relaxed subsequently (De Waal *et al*, 1998b).

1.6 COWDRIOSIS

Cowdriosis is a tick-transmitted disease of cattle, sheep, goats and some wild ruminants. It is caused by *Cowdria ruminantium*. This organism is found in the cytoplasm of endothelial cells and some white blood cells of infected animals (De Waal *et al.*, 1998c). The disease causes fever and an accumulation of fluid around the heart, in the thoracic cavity, lungs and brain. In most cases it leads to death. In

South Africa the causal organism, *C. ruminantium* is transmitted by *Amblyomma hebraeum* (bont tick). The disease is transmitted only by bont ticks in the nymphal and adult stages (De Waal *et al.*, 1998c).

1.6.1 Distribution and transmission

Amblyomma hebraeum prefers warm and humid lowveld and bushveld areas. It is found in the bushveld areas of eastern Botswana, northern Gauteng, the Northern and Northwest Province, Mpumalanga, parts of Swaziland, and KwaZulu-Natal, as well as in the coastal area of the Eastern Cape Province as far as Mossel Bay in the Western Cape Province.

The disease develops after an infected tick has fed on a susceptible animal. Bont ticks become infected with the heartwater organism when they feed on an infected animal. Usually less than 10% of bont ticks found in heartwater areas are infected (De Waal *et al.*, 1998c).

Apart from cattle and small stock, various large and small game animals, hares and some ground-dwelling birds (*e.g.* guinea-fowl) can also become subclinically infected with heartwater. Although they may show no clinical signs of the disease, they may have the organism circulating in their blood and therefore be able to infect ticks that feed on them (De Waal *et al.*, 1998c).

1.6.2 Symptoms

The incubation period in naturally-infected cattle ranges from 9 to 29 days and that of naturally-infected sheep and goats from 7 to 35 days.

Affected animals have a fever ranging between 40-42^{0}C, are listless, lose their appetite and lag behind the rest of the herd. Animals often develop a high-stepping gait that is more pronounced in the front limbs. Breathing becomes gradually more laboured and animals may stand with their heads held low, make constant chewing movements and push against objects (De Waal *et al.*, 1998c).

Animals suffering from heartwater are sensitive to intensive light and twitch their eyelids rapidly when exposed to it. The eyes roll from side to side. The body temperature rapidly drops to below normal shortly before death (De Waal *et al.*, 1998c).

1.6.3 Diagnosis

A tentative diagnosis of heartwater can usually be made on the basis of the symptoms and the post-mortem-lesions, but it is often difficult to distinguish from other conditions that manifest similar signs. In suspected cases of heartwater the confirmation of such diagnosis requires the demonstration of *C. ruminantium* organisms in the cytoplasm of endothelial cells of blood vessels in stained smears of the brain (De Waal *et al.*, 1998c).

1.6.4 Treatment

Various short or long-acting formulations of injectable tetracycline antibiotics are recommended for the treatment of heartwater. A dosage of 10 mg/kg body weight of oxytetracycline is given intravenously (De Waal *et al.*, 1998c).

1.6.5 Prevention and Control

Heartwater can be controlled by immunization of calves, lambs or kids, treatment of sick animals infected by ticks, and the strategic control of the number of bont ticks to which livestock are exposed (De Waal *et al.*, 1998c).

1.7 THEILERIOSIS

Theileria is a genus comprising tick-transmitted parasitic protozoa classified as follows (Lawrence *et al.*, 1994):

Phylum : Apicomplexa
Class : Sporozoa
Sub-class : Piropalsmia
Order : Piroplasmida
Family : Theileriidae
Genus : *Theileria*

Theileria is a protozoan genus closely related to *Babesia*. The main theilerias of bovines are *T. parva*, which causes East Coast fever (ECF) in the eastern half of Africa south of Sudan and north of South Africa, and *T. annulata*, which causes Mediterranean Coast Fever around the Mediterranean, Middle East, India, Russia and other parts of Asia (Irvin, 1987). Theilerias are also transmitted by ticks. The vector inoculates sporozoites that invade and multiply in the lymphocytes to form schizonts. The schizonts generate numerous merozoites that leave the lymphocyte to invade and multiply in the erythrocytes. These forms in the red blood cells are still called 'piroplasms'. Lymphocyte parasitism causes blastogenesis, proliferation and destruction of the cells. Erythrocyte parasitism is intense and destroys the cells

in *T. annulata* but not in *T. parva* infections. Both theilerias cause fever, non-specific proliferation of lymphocytes, and debilitation. *Theileria annulata* also causes anemia (Barriga, 1994).

1.7.1 Distribution and transmission

The situation regarding theileriosis in South Africa appears to be stable at present. The mildly pathogenic *Theileria* species (*T. mutans* and *T. taurotragi*) and the apothogenic species (*T. velifera* and *T. ovis*) are common in South Africa, but apparently of little economic consequence in most cases. Buffalo-associated theileriosis (Corridor disease) caused by *T. p. lawrencei* is still present, but fortunately restricted to well defined areas bordering on certain game reserves where buffalo occur (Stoltsz, 1989).

Theileria parva bovis has not been isolated in South Africa. However, both confirmed vectors of this parasite, namely *R. appendiculatus* and *R. zambeziensis*, are known to occur over a large area of the country (Stoltsz, 1989). Considering that *T. p. bovis* is widely distributed in Zimbabwe and that strong evidence exists for its presence in Botswana, it seems likely that the parasite was introduced into South Africa by the cattle trade, both legally and illegally, that existed between these countries for many years (Stoltsz, 1989).

A further reason for the apparent absence of solid evidence for the existence of *T.p. bovis* in South Africa is probably the fact that non-pathogenic strains of the parasite occur, with the mortality rate during outbreaks in endemic areas as low as 1.5%. It would thus appear that the presence of the parasite could easily be obscured by the

presence of other relatively more pathogenic tick-borne diseases like babesiosis and anaplasmosis (Stoltsz, 1989).

Although several tick species have been shown experimentally to transmit *T. p. parva*, it is generally accepted that *Rhipicephalus appendiculatus* is the main vector. Plate 5 shows *T. p. parva* in a blood smear. After the introduction of ECF into South Africa in 1902 the disease rapidly spread within the low-lying areas of the country and by 1912 had invaded all the areas within which it could establish and maintain itself.

1.7.2 Symptoms

East Coast fever in the classical form is characterized by pyrexia, enlargement of the superficial lymph nodes, severe pulmonary oedema and wasting. It usually terminates in death. The disease usually progress over a period of about 15 days, but may terminate after five days or be prolonged to 25 days. The fever remains high, although in a small proportion of cases there may be a temporary remission for one or two days. Appetite and rumination become increasingly depressed and there is severe loss of body condition, increasing weakness and ataxia, and frequent recumbency. Constipation is succeeded by diarrhoea and there may be blood and mucus in the faeces. Anaemia and icterus have been reported but are inconsistent. Nodular skin lesions have been described in a small number of experimental cases. Pregnant cows may abort (Lawrence *et al.*, 1994).

1.7.3 Diagnosis

Diagnosis of classical East Coast fever is based on the characteristic clinical signs

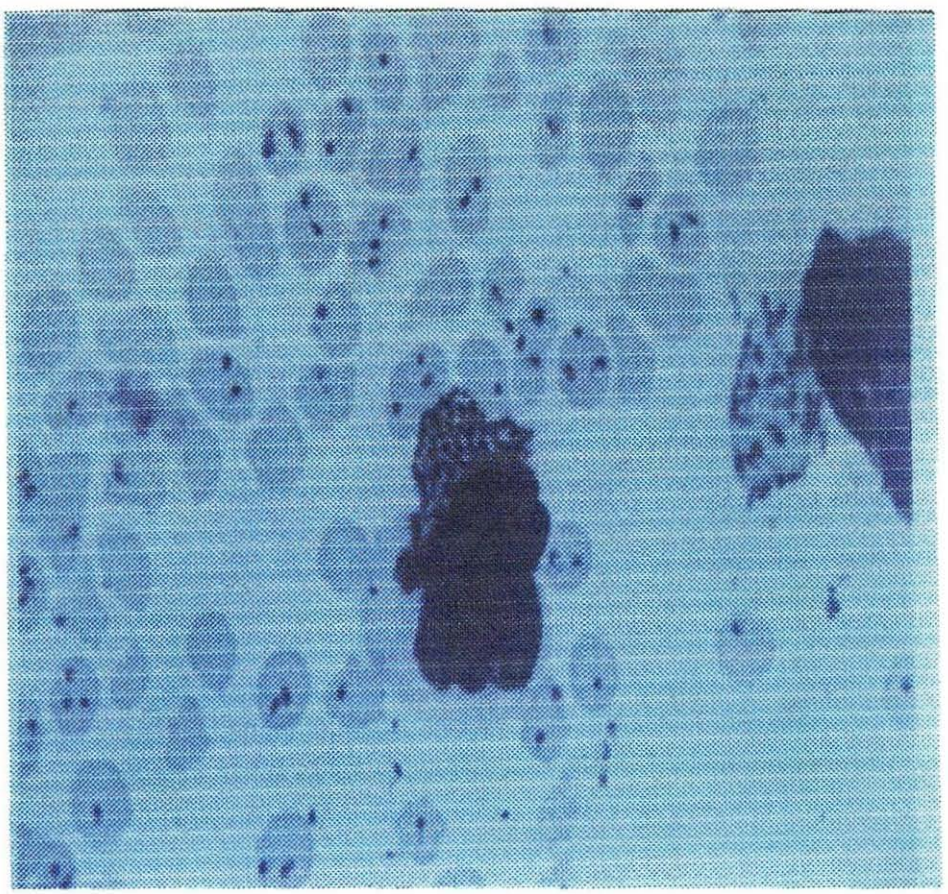


Plate 5: *Theileria parva* in a thin blood smear

and lesions, and may be confirmed by demonstration of schizonts and, in the later stages of the disease, piroplasms. Theilerial parasites are generally numerous. In the live animals they can be demonstrated in smears prepared from peripheral blood and fine-needle aspirates of superficial lymph nodes. Parotid, prescapular or precrucal lymph nodes may be sampled. In the dead animals, smears prepared from the cut surface of the spleen and any enlarged lymph nodes should be examined (Lawrence *et al.*, 1994).

Retrospective diagnosis of East Coast fever can be made by a demonstration of a rising titre to *T. parva parva* schizonts using indirect immunofluorescence. Antibodies persist on average for a period of 30 weeks with a range of 12 to 73 weeks (Lawrence *et al.*, 1994).

1.7.4 Treatment

Tetracyclines have been widely used in the treatment of clinical disease, sometimes to good effect, but they have not proved to be completely reliable therapeutic agents. A second drug, halofuginone, a quinazoline derived from febrifugine, is effective when administered orally as the hydrobromide or preferably the lactate salt at a rate of 1.2 mg/kg, repeated after 48 hours. It is active against the schizonts but not the piroplasms. Both drugs have the disadvantage of being relatively expensive and this reduces their application in the field, but they can be used as a back-up to prevent mortality if other forms of control prove ineffective (Lawrence *et al.*, 1994).

Effective methods of immunization are achieved by controlled infection of cattle using sporozoites derived from ticks or schizont-infected cells. The infective

material is virulent and the method of administration must be carefully regulated to avoid a fatal outcome (Lawrence *et al.*, 1994).

1.7.5 Prevention and Control

Initial attempts to control the disease were aimed at the eradication of vector ticks, particularly *R. appendiculatus*. The implementation of short-term dipping programmes, combined with fencing and quarantine of infected areas, eliminated the disease from many areas, but failed to eradicate it completely (Stoltsz, 1989).

Control measures were than intensified and aimed at the early diagnosis of the disease, strict control of cattle numbers, close supervision of short-interval dipping (5:5:4-day cycle) and the adoption of a permit system for the movement of cattle. This intensified campaign led to a steady decline in the number of outbreaks, but also revealed the need to eliminate isolated outbreaks of the disease by application of a slaughter policy. This latter approach eventually led to the total eradication of the disease in South Africa. The last recorded outbreaks of ECF in Gauteng occurred in 1944 and in the Eastern Cape and KwaZulu-Natal in 1954 and 1955 respectively. Continued close veterinary surveillance, based largely on the examination of spleen smears from dead cattle, has failed to show any recurrence of this disease in the former endemic areas (Stoltsz, 1989).

Considering the potential East Coast fever threat to the cattle population in this country, either through reintroduction of *T. p. parva* or the transformation of *T. p. lawrencei*, strict control measures will be maintained. Indications are that *T. p. lawrencei* infections will not be easily sterilized and probably not with the existing

Plate 6: *Trypanosoma vivax* in a thin blood smear

anti-theilerial drugs. Having shown that recovered intact cattle can infect ticks, the use of chemotherapeutic drugs in infected cattle during Corridor disease outbreaks is prohibited (Stoltsz, 1989).

1.8 TRYPANOSOMOSIS

The African trypanosomes are blood parasites of vertebrates, including humans, and are transmitted biologically by tsetse flies (*Glossina spp*), though some species freed themselves from their specific vector dependency and migrated to other continents (*Trypanosoma evansi*, *Trypanosoma vivax*, and *Trypanosoma equiperdum*) (Wilson *et al.*, 1963).

Trypanosomes are protozoan parasites of the genus *Trypanosoma*, order Kinetoplastida, and have organelles, a kinetoplast and a flagellum. Typically, trypanosomes are digenetic parasites and thus require two hosts to complete their life cycle: they multiply in the blood, tissues or body fluids of a vertebrate host and, with the exception of *T. equiperdum* which is venereally transmitted, are ingested by a haematophagus invertebrate vector (Connor, 1994).

Human African trypanosomiasis (sleeping sickness) is caused by *T. rhodesiense* and *T. gambiense*, currently considered as subspecies of *T. brucei*. More than 50 million people are at risk of infection and 20-30 thousand acquire it every year. Livestock trypanosomiasis of bovines (nagana) is caused mainly by *T. congolense*, *T. vivax* (Plate 6) and *T. brucei* (Barriga, 1994). Nagana negates the full use of about 10.4 million km^2 of African land that could support 125 million head of cattle of high productivity (Wilson *et al.*, 1963).

African trypanosomes are typically chronic diseases. The human infection is invariably lethal if not treated. The infection of bovines is generally fatal but some animals are able to control the parasitemia and survive with a moderate parasitic load. This ability, known as 'trypanotolerance', is inherited and largely dependent on immunological mechanisms. In South Africa cases suggestive of trypanosomosis have been reported in the KwaZulu-Natal region. The symptoms include fever, wasting and lethargy (Barriga, 1994).

1.8.1 Distribution and transmission

The majority of animal diseases caused by trypanosomes occur in the tropics. In Africa, several species of tsetse-transmitted trypanosomes cause African trypanosomiasis in domestic animals, which in southern Africa are collectively known as 'nagana', a word derived from the Zulu 'nakane' meaning tsetse fly disease. An important form of trypanosomosis known as dourine, caused by *T. equiperdum*, also occurs in southern Africa, but has no arthropod vector, but it is transmitted during coitus (Bachmann, 1990).

The epidemiology of African animal trypanosomoses is almost entirely dependent on tsetse flies. African trypanosomosis is primarily caused in cattle by *T. congolense* and *T. vivax*. In endemic areas the disease causes huge economic losses, but it can also occur in areas remote from tsetse infestations, as a result of the movement of previously infected animals in which, when stressed, show clinical signs of trypanosomosis. Additionally, small numbers of infected tsetse flies, carried along distances by vehicles, can transmit trpanosomes in areas otherwise free of trypanosomosis. Recognition of the wide range of clinical signs associated with the

disease is thus important, both within and outside endemic areas (Connor, 1994).

1.8.2 Symptoms

Cattle living in tsetse -infested areas frequently have mixed trypanosomal infections that cause more severe disease than infections with a single species of trypanosome. Generally, in eastern and southern Africa, *T. congolense* is more pathogenic to cattle than *T. vivax* and produces serious disease, whereas *T. brucei* is widely regarded as being of minor clinical significance. The course of disease due to infection with salivarian trypanosomes is variable and there are no clinical signs specific to bovine trypanosomosis. The manifestations of disease depend upon the degree of damage to specific organs and the degree of anaemia. Trypanosomosis in cattle which may be acute, subacute or chronic disease may be fatal after illness lasting two to six weeks, but chronic disease lasting many months or even years is more common (Connor, 1994).

Chancres are rarely seen in cattle with naturally-acquired infections. The first signs of disease are due to the fever which accompanies the onset of parasitaemia. As parasitaemia falls, so does fever; the course of the infection is characterized by fluctuating parasitaemia and parallel fluctuations in body temperature. Intermittent parasitaemia and malaise are followed by increasingly severe clinical signs. Acute trypanosomosis causes sudden reduction of milk yields and also abortion. Oxen, bulls and young stock with acute infections are reported to be suddenly 'off-colour'. They are generally in good bodily condition but are dejected, their ears droop and they may walk stiffly. Pulse and respiratory rates are raised in febrile animals and there may be piloerection. Conjunctival mucosae may be congested early in the

acute phase, but later, as anaemia develops, pallor of the mucous membranes is evident. Acutely affected animals quickly lose weight and body condition, although they continue to eat. They become weak, dejected, and lethargic, often standing alone away from the rest of the herd, not seeking shade. After a short illness some acutely affected cattle become recumbent for a few days before death occurs (Connor, 1994).

In subacute cases, animals are intermittently 'off-colour', becoming weak and dejected, with drooping ears and flaccid tail. Within a few weeks they lose weight and condition, the coat becomes dull and marked jugular pulse develops. Thin, rough-coated, anaemic, lethargic cattle with generalized lymph node enlargement are said to have a 'fly-struck' appearance. Death may occur between four and six months after the onset of disease, but many animals make a gradual recovery, which is assisted by a good plane of nutrition (Connor, 1994).

Chronic bovine trypanosomosis is by far the most common form of the disease in endemic areas. It may arise either as a result of partial recovery from acute or subacute disease, or from an initial subclinical or mild infection. Severely affected cattle may be extremely emaciated, having lost a large proportion of their muscle mass; they are often just 'skin and bone'. Their general condition is very poor, that is, the hair is sparse and the coat is rough, dull and staring. The skin is dry and scaly, and in long-standing infections the hair of the tail switch may be completely absent. The combined effect of anaemia, circulatory disturbance and myocardial damage frequently produces acute cardiac decompensation which leads to sudden death from congestive heart failure (Connor, 1994).

1.8.3 Diagnosis

Although in many tsetse-infested areas diagnostic facilities are not available, clinical signs of trypanosomosis are well recognized. Farmers and veterinary personnel commonly resort to treatment of sick animals, and use the response of therapy for retrospective diagnosis. In such areas, a history of the presence of tsetse flies and the use of trypanocidal drugs, when considered with presenting clinical signs, are sufficient to make a tentative diagnosis. The presence of concurrent disease may mask trypanosomosis and complicate the clinical picture. Thus, the only way to confirm a diagnosis in clinically affected animals is to demonstrate and identify the parasites of the body fluids (Connor, 1994).

The body fluid most commonly examined is blood, either capillary blood from the tip of the tail or venous blood from an ear vein or from the jugular vein. Lymph, aspirated from a punctured superficial lymph node (usually the prescapular), provides useful supplementary diagnostic material (Connor, 1994).

The indirect fluorescent antibody test (IFAT), has been advocated as a tool for herd diagnosis, and the enzyme-linked immunosorbent assay (ELISA) is also considered to be a valuable diagnostic method. The micro-ELISA compares favourably with the IFAT and has been found to give results which correlate with the local history of trypanocide usage. Monoclonal antibodies have been developed that distinguish *T. brucei*, *T. congolense* and *T. vivax*. When these antibodies are used in an antigen-trapping ELISA, diagnosis is sensitive and specific, enabling the detection of many latent infections (Connor, 1994).

1.8.4 Treatment

The satisfactory treatment of trypanosomosis requires more than a correctly administered trypanocidal drug, and the speed of recovery is largely determined by the plane of nutrition, the amount of exercise during convalescence and the duartion of the disease. Chronic trypanosomosis often fails to respond to therapy; the ferrokinetic disturbances and accompanying dyshaemopoiesis appear to be irreversible, and affected animals may remain thin and anaemic despite trypanocidal treatment (Connor, 1994).

1.8.5 Prevention and Control

Several strategies for the control of African animal trypanosomosis are evident from the epidemiology of the disease. Control methods may be directed against the vector or the parasite, and may be directed towards the livestock and modified management. The efforts of the Tsetse and Trpanosomosis Control Branch of Zimbabwe's Department of Veterinary Services have achieved notable success in controlling the disease over the past 50 years. Different strategies have been used, and within each strategy different methods have been applied as they have become available (Connor, 1994).

CHAPTER 2

GENERAL OBJECTIVES OF THE STUDY

2.1 RATIONALE

Ticks are important biological vectors of many diseases of animals including viral, bacterial, fungal, protozoal and rickettsial agents (Philip, 1963). Bovine babesiosis and anaplasmosis form part of this complex of diseases sharing the feature of being predominantly transmitted by ticks. Worldwide, babesiosis and anaplasmosis are considered to be amongst the most important tick-borne diseases of cattle (Morzaria, 1986). Up to 500 million cattle are at risk to contract these diseases (De Vos, 1992). According to Ezra Sigwela, the former Eastern Cape MEC for the Department of Agriculture and Environmental Affairs, approximately 53 000 cattle (valued at R53 million) died in the first six months of 1996 in the Eastern Cape, due to tick-borne diseases (Die Burger, 1996). Babesiosis and anaplasmosis are severe economical constraints on successful animal production and the development of livestock industries (Verhulst *et al*, 1983). The economic impact can be expressed in terms of mortality, loss of production (including liveweight gain, milk production, draught potential and manure), the cost of control and, in some cases, restrictions placed on the movement of animals (Norval *et al*, 1992).

There is little or only outdated information on parasites of veterinary importance afflicting livestock in the north eastern Free State (Mbati, 1999). The following tick species has been documented to occur in the north eastern Free State: *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi* (Hlatshwayo *et al.*, 1999). It is therefore critical that we investigate the presence of the following possible

infections due to *B. decoloratus*: bovine babesiosis (*Babesia bigemina*), anaplasmosis (*Anaplasma marginale*) and spirochaetosis (*Borrelia theileria*) in cattle.

Data collected from this research work will be important in devising strategies for controlling these diseases in the north eastern Free State region of South Africa.

2.2 GENERAL OBJECTIVE

2.2.1 To record current livestock parasites of economic importance in the north eastern Free State region of South Africa.

2.3 SPECIFIC OBJECTIVES

2.3.1 To diagnose blood parasites of major economic importance in cattle in the north eastern Free State by the use of Giemsa-stained thin and thick peripheral blood smears.

2.3.2 To diagnose anaplasmosis and bovine babesiosis by enzyme linked immuno-sorbent assay (ELISA) and indirect fluorescent antibody test (IFAT) respectively in the north eastern Free State.

2.3.3 To determine tick species which infect cattle in the north eastern Free State.

2.3.4 To determine the intensity, distribution and seasonal dynamics of infections of these parasites in this region.

CHAPTER 3

MATERIALS AND METHODS

3.1 DESCRIPTION OF THE STUDY AREA

3.1.1 Experimental locations

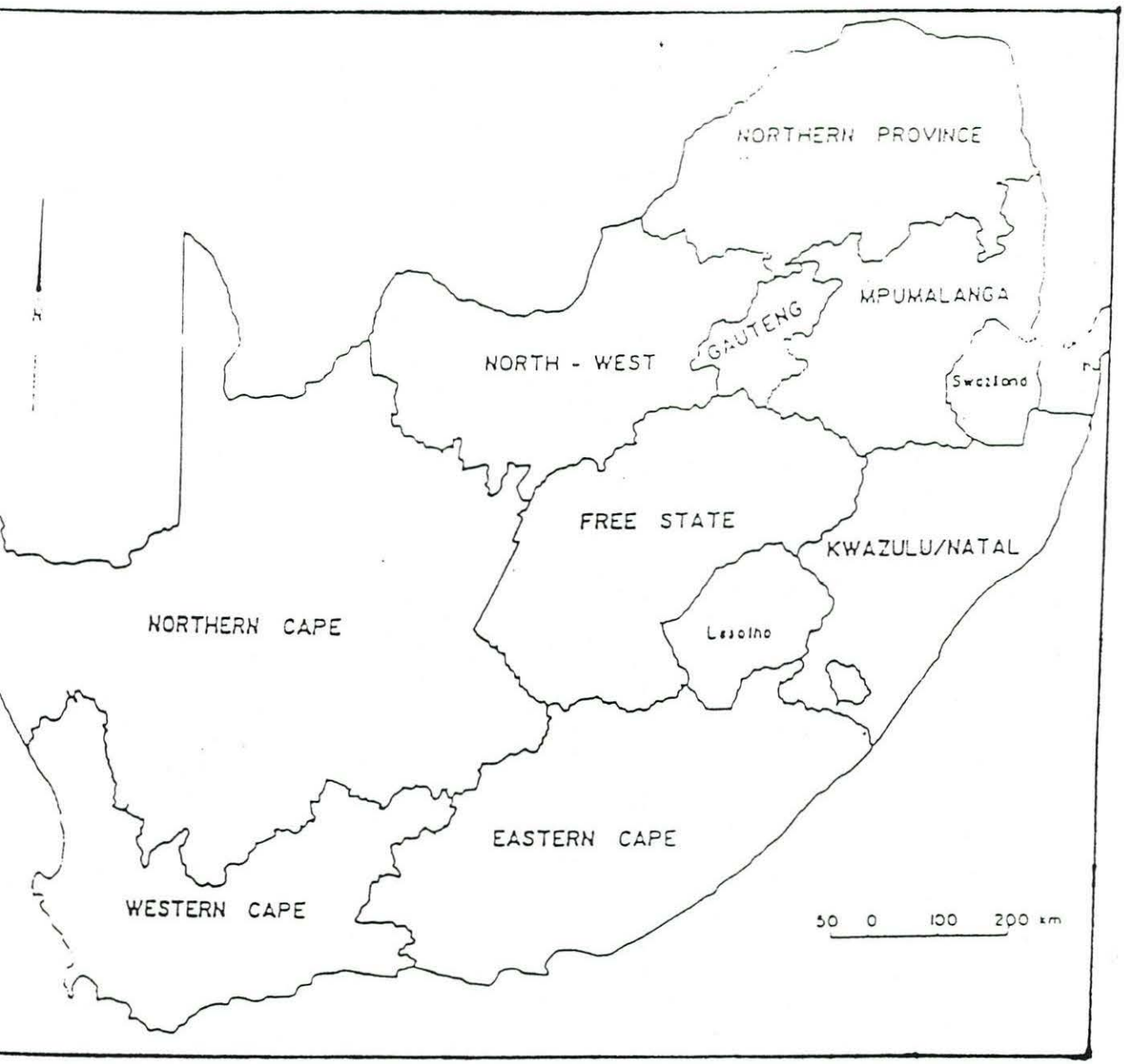
The north eastern Free State was divided into three study sites that included Harrismith ($29^0$5'E, $28^0$18'S), Kestell ($28^0$38'E, $28^0$20'S) and Qwa-Qwa ($28^0$50'E, $28^0$35'S) which are three established study sites of UNIQWA's Parasitology Research Program. They are located at 40, 28 and 5 km respectively away from the Qwa-Qwa Campus of the University of the North. The geographical position of the Free State Province in South Africa is indicated in Figure 1. The location of Harrismith, Kestell and Qwa-Qwa in the Eastern Free State Province is shown in Figure 2.

3.1.2 Topography and altitude

The eastern Free State is a predominantly hilly and mountainous area situated between latitudes 28^0 and 30^0S , 28^0 and 30^0 E. It is bounded to the south by Lesotho and to the east by KwaZulu-Natal. It is situated at a height of about 1500 to more than 3000m above sea level (Kritzinger and Pieterse, 1987).

3.1.3 Geology

The Eastern Free State region with its beautiful landscape, consist of the rocks of the Karoo super-group, consisting of the Beaufort and Stormberg groups, with the soil types belonging to the Clovely-Avalon, Katspruit, Kroonstad-Katspruit and the Sterkspruit formations (Vrey and Smith, 1980). Numerous dolerite dykes and



gure 1. **Location of the Free State Province in a national South African context (Adapted from Krige, 1995.)**

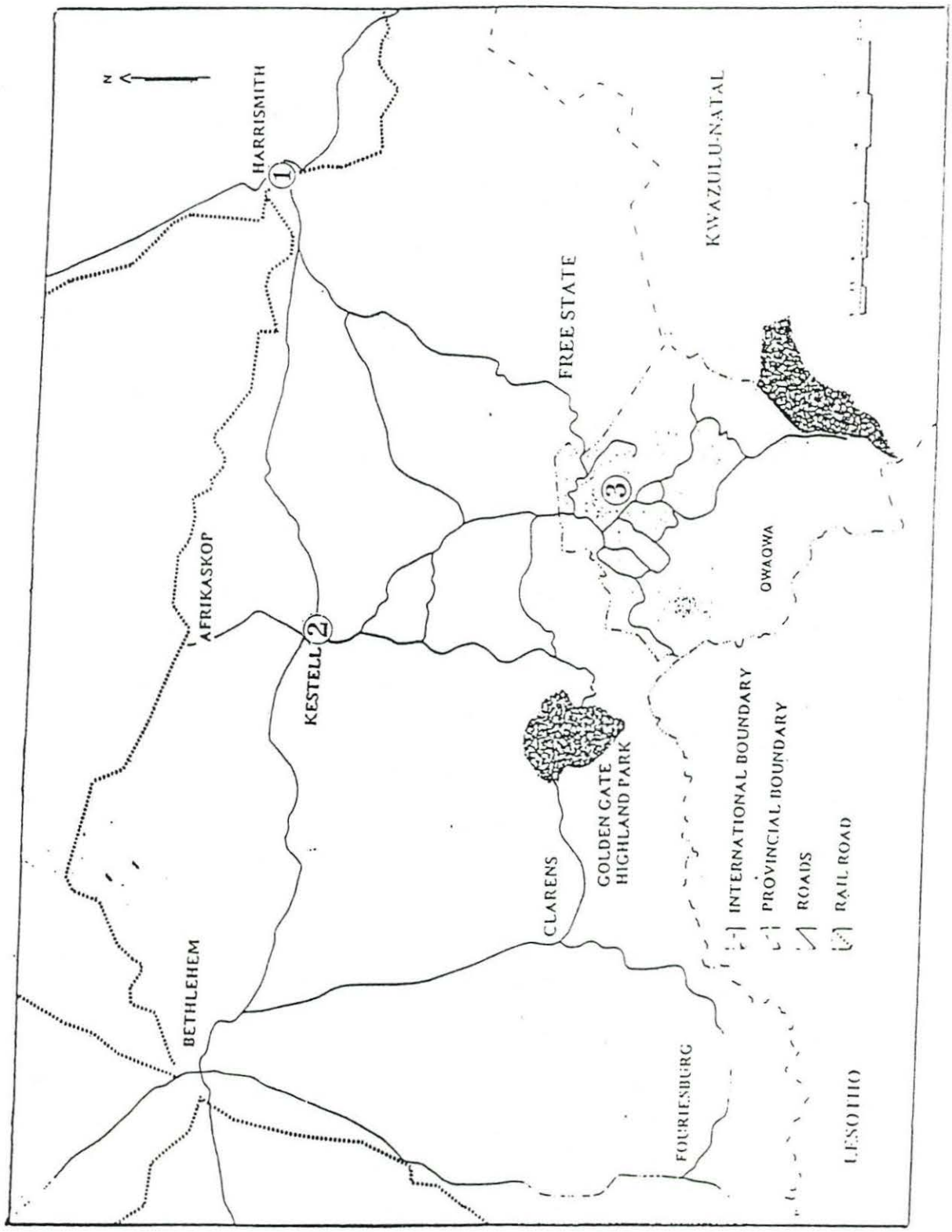


Figure 2: Location of the study sites, Harrismith, Kestell and Qwa-Qwa, indicated by 1, 2, 3 respectively

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).

3.1.4 Climate

Climate variablity 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 crucial to understand the influence of climate in African ecosystems (Ellis, 1994).

3.1.4.1 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 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 700mm per annum, increasing gradually to approximately 1340mm per annum in the Qwa-Qwa mountains as one progresses in a southerly direction. Hail storms are not uncommon, occurring three to five times per annum. Snowfalls occur in the high-lying south with the heaviest falls occurring towards the end of June (Kritzinger and Pieterse, 1987).

3.1.4.2 Atmospheric temperatures

The temperature in the eastern Free State region may be described as being cool to moderately warm. The average temperature during mid-winter is 7.4^0 C and during mid-summer is 17.9^0C. The average daily minimum temperature during winter is 4.6^0C and the average daily maximum temperature during summer is 29.9^0C. Cold spells, *i.e.* temperature decreases in excess of 5^0C can occur 30 times a year, lasting 2 to 3 days at a time. Hot spells, *i.e.* temperature in excess of 35^0C also occur for 4 to 5 days per year. During September and October temperatures are regarded to be unstable, but during April and May to be quite stable (Kritzinger and Pieterse, 1987).

3.1.5 Vegetation

The area belongs to the grassland biome, with five vegetation types, namely: moist cool highveld, moist cold highveld, wet cold highveld, afro-montane and alti-montane grassvelds (Moffet, 1997). The vegetation can also be decribed as highland sourveld which changes into Themeda-Festuca veld at heights in excess of approximately 2 200m. In the almost pure sourveld, red grass (*Themeda triandra*), red seed grass (*Tristachya hispida*) and spear grass (*Heteropogon contortus*) predominate and these are easily reduced, through over-grazing, to tough grass (*Eragrostis plana*) and wiregrass (*Elionurus argenteus*) (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 Eric-Helichrysum (Kritzinger and

Pieterse, 1987).

3.2 EXPERIMENTAL ANIMALS

Cattle (Plate 7) in the experimental sites were identified in terms of their breeds. These breeds included Bonsmara, Brahman, Friesland, Hereford, Jersey, Nguni and Simenthaler in the three research sites. Most of the experimental animals in Qwa-Qwa were of mixed breed. A total of 10-16 animals were randomly selected and examined monthly at each of the three study sites. The study was conducted over a continuous period of 12 months from August 1999 to July 2000. Cattle were kept under small-scale farming systems. During the day the cattle graze on the communal pastures. The usual grazing time is 7-9 hours per day, determined by the time of the year. At night, cattle are herded, milked and kept in kraals with a manure floor.

3.3 PARASITOLOGICAL DIAGNOSIS

3.3.1 Handling and management procedures

Animals were individually restrained in a mobile facility consisting of a collecting kraal, a race, crush and neck clamp. Standard glass microscopic slides were used for the preparation of thin and thick blood smears. These slides were cleaned with cotton gauze or absorbent material. During transport to the field, slides were kept in dust-proof slides boxes or sealed in plastic packets.

3.3.2 Preparation of thin film blood smears

Blood smears were prepared essentially as described in the OVI Sero-diagnostic Laboratory Manual (OVI, 1999). Peripheral blood for smears was taken from the edge of the tail by pricking the skin with sharp scissors. Before pricking the skin,

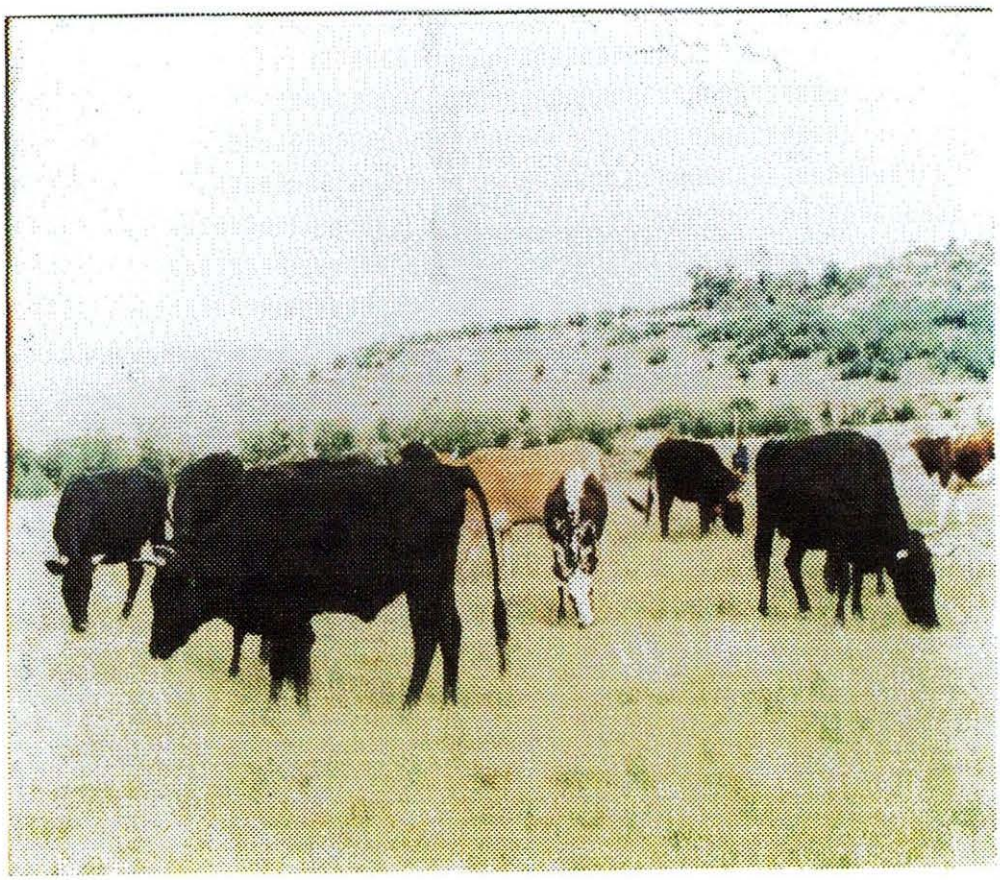


Plate 7: Cattle in the field at Hatswanyane farm, Qwa-Qwa

the hair was clipped and the area wiped with a dry cloth to remove dust or debris. The tail tip was folded back so that the tip was easily given a sharp jab with scissors. A drop of blood was obtained by squeezing the tail between the thumb and forefinger. After the scissor-prick had been made in the skin, a drop of blood that was formed was picked up with a corner of a microscopic slide (spreader slide). Another clean microscopic slide was held firmly on a flat surface so that the drop of blood was transferred from the corner of the spreader slide to the end of the second slide, about 1 cm from its edge. The drop of blood was spread by touching the edge of the spreader to the microscopic slide at an angle of 30^0 slightly in front of the droplet and then gently drawing the spreader backwards until it touched the droplet. The spreader slide was moved forward quickly and smoothly away from the drop of blood towards the far end of the microscopic slide, drawing the blood with it whilst being held against the smear slide. This leaves a characteristic-shaped smear which tails off towards its end. Slides were left to air-dry and were wrapped individually in clean tissue.

3.3.3 Preparation of thick film blood smears

A drop of blood was put on the microscopic slide and smeared in a circular direction. Thick smears differ from 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.

3.3.4 Parasitological screening

Microscopic slides were fixed using analytical grade absolute methanol (Shalom, Laboratories). Slides were stained for 30-35 minutes using a 10% Giemsa solution

(90 ml buffer solution and 10 ml Giemsa). To prepare the Giemsa buffer, 104.41g of $Na_2HPO_4.2H_2O$ was mixed with 19.2g of $NaH_2PO_4.2H_2O$ in 10L distilled water at pH 7.4. After thirty minutes, the slides were removed and rinsed under running tap water until all stain was removed. This prevents precipitates from adhering to the smears. After washing, the slides were allowed to dry in a slide rack. Then a drop of 518C immersion oil (Zeiss Germany) was put on both thin and thick smears and examined using a 100 X oil immersion objective lens of the Nikon SE light microscope (Nikon).

3.4 SEROLOGICAL DIAGNOSIS

Blood samples were collected aseptically from the jugular vein or under the tail using vacutainers without anticoagulant and 18G X 1.5" needles. Blood samples were centrifuged for 30 minutes at 2000 rpm using a Medifriger centrifuge (J.P. Selecta). Sera was harvested into cryovials (Plate 8) and kept at -35^{0}C until used for serological analysis.

3.4.1 Indirect Fluorescent Antibody test (IFAT) for *Babesia spp.*

The indirect test make use of a known antigen to determine the presence of antibody in the serum. The antigen/antibody complex is visualized by staining the complex with a secondary antibody conjugated with a fluorochrome. The secondary antibodies for the conjugate is prepared in an unrelated animal species. An indirect flourescent antibody test described by Morzaria *et al* (1977) was used to detect *B. bigemina* and *B. bovis* antibodies.

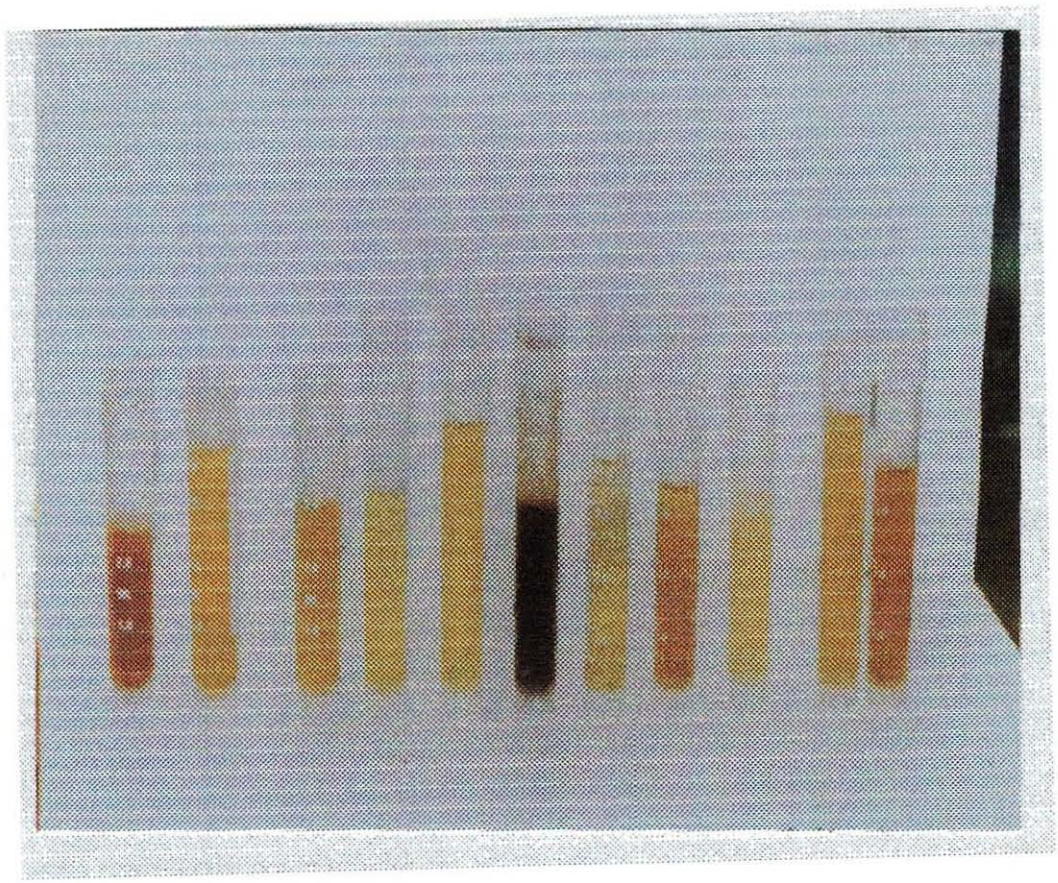


Plate 8: Harvested sera of cattle in cryovials

3.4.1.1 Test procedure

Antigen smears, test and control sera were obtained from Onderstepoort Veterinary Institute. They were taken out from storage at -20^{0}C and placed in the incubator at 37^{0}C for 10 min. Two-fold serial dilutions of control and test sera were made, *i.e.* 1/80, 1/160 and 1/320. Serum samples were marked numerically and annotated in the laboratory serological result book (Appendix I). The antigen slides were taken out of their protective covering and fixed in cold acetone at -20^{0}C for 1 min. They were then marked, *e.g. B. bovis* = b1, b2 ...; *B. bigemina* = B1, B2 ... A drop of the diluted serum was placed on the antigen slide wells. Slides were incubated in a humid chamber at 37^{0}C for 1 hour. After incubation serum dilutions were rinsed from slides by dipping the slides into a container with ±200 ml Phosphate Buffered Saline (PBS). After rinsing each slide was placed into another container with 1 L PBS and washed using a magnetic stirrer set at very low revolutions for 10 min. They were then washed in distilled water for 5 minutes and dilutions of the conjugate were made, *i.e.* 1/80, 1/160 and 1/320. Excessive distilled water was shaken off from the slides and were completely covered with the conjugate dilution. Slides were incubated in a humid chamber at 37^{0}C for 1 hour. After incubation they were rinsed in fresh PBS and washed in PBS for 10 min on a magnetic stirrer as before. Slides were left to air dry. One drop of 50% glycerine/PBS was placed on each slide, and were covered with a 24X50 mm coverslip. Slides were examined with a Nikon ECLIPSE E600 microscope with a Y-FL Epi-fluorescence attachment at a single wavelength of 405nm in a darkroom using a 100X fluorescence oil objective.

3.4.2 Competition Inhibition (CI) Enzyme Linked Immuno-Sorbent Assay (ELISA) for *Anaplasma*

The competition inhibition ELISA (CI-ELISA) format overcomes problems associated with antigen purity since the specificity of the CI-ELISA depends solely on the monoclonal antibody (MoAb) (Knowles *et al.*, 1995). Competition assays imply that two reactants are trying to bind to a third. Proper competition assays involve the simultaneous addition of the two competitors. Inhibition or blocking assays are similar, except that one of the reagents being examined for 'competing' ability of a system is added and incubated before the second 'competitor' is added. A lisate made from a recombinant *E.coli* that express an immunogenic major surface protein 5 (MSP 5) of *A. marginale* is used as the antigen. The test sera and 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 binds to the monoclonal and after adding 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 indicates high concentration of mouse MoAb bound to the antigen, indicating absence of *Anaplasma* antibodies in the test sera. The monoclonal antibody was obtained from the Onderstepoort Veterinary Institute.

A competition inhibition enzyme-linked immunosorbent assay (CI-ELISA) described by Visser *et al.* (1992) was employed to detect *A. marginale* antibodies.

3.4.2.1 Test procedure

Antigen was diluted with PBS and 100μl of the diluted antigen was transferred into ELISA plates (Plate 9). The plates were incubated overnight at 4^{0}C. The plates were rinsed once with PBS, and were filled with 225 μl PBS containing 5% elite milk powder (Appendix II). The ELISA plates were incubated at 37^{0}C for 1-2 hours in an incubator. The blocking buffer was expelled and 100 μl of a 1:10 serum dilution was dispensed into each well in duplicate. The plate set-up of the negative and positive control sera is shown in Appendix III. The plates were incubated at 37^{0}C for 30 min. The monoclonal antibody was diluted (1:200) and 25 μl of the dilution was dispensed into each well with the 100 μl test sera after the 30 min incubation. The plates were incubated at 37^{0}C for 60 min. The plates were then rinsed once with washing buffer, and washed twice for 5 min on the plate shaker. Anti-mouse antibodies were diluted (1:3000) and 100μl of the dilution was dispensed into each well. The plates were incubated at 37^{0}C for 30 min. After incubation the ELISA plates were rinsed once with the washing buffer, and washed twice for 5 min on the plate shaker as before. Avidin/Biotin solution was diluted, and 100 μl of the dilution was dispensed into each well. The plates were incubated at 37^{0}C for 30 min. They were rinsed and washed twice for 5 min on the plate shaker. The substrate (4-nitrophenyl phosphatase) was diluted and 100 μl of the diluent was dispensed into each well. The plates were incubated at 37^{0}C for 30 min. The resulting color was read with Bio-Tek Model ELX800 Universal ELISA Microplate Reader at 405 nm (single wavelength). Data and time was recorded on the ELISA serological result book (Appendix III) and were analyzed using Microsoft Excel computer program (Appendix IV) of a Mecer-desktop computer.

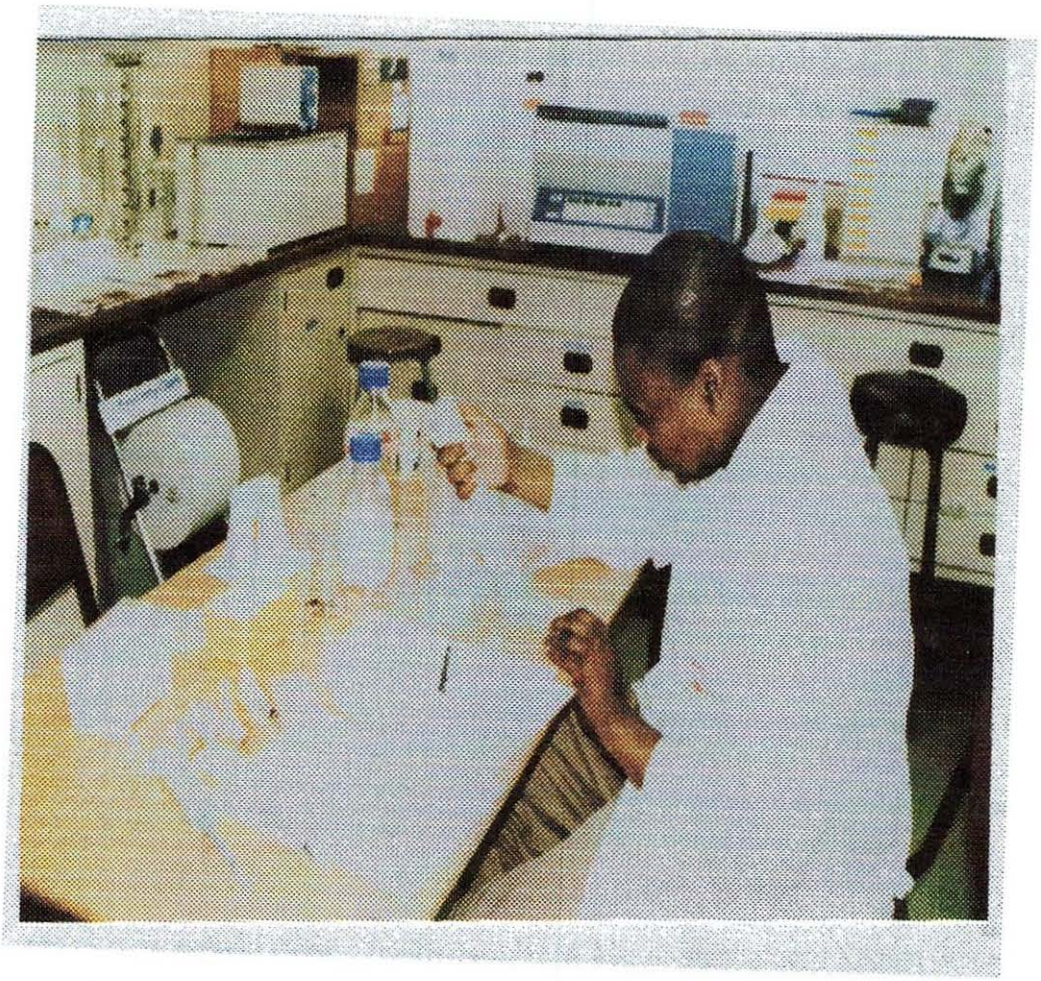


Plate 9: Diluted antigen is transferred into an ELISA plate

3.4.2.2 Interpretation of the test

The mean value and standard deviation (SD) 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 SD, *i.e.* [x (negative samples) - 3 X SD (of the x) = cut-off point]. Values higher than the cut-off point were positive and lower were negative.

3.5 DETERMINATION OF PACKED CELL VOLUME (PCV) VALUES IN CATTLE IN THE NORTH EASTERN FREE STATE.

Peripheral blood for packed cell volume was taken from the edge of the tail by pricking the skin with sharp scissors. Before pricking the skin, the hair was clipped and the area wiped with a dry cloth to remove dust or debris. The tail tip was folded back so that the tip was easily given a sharp jab with scissors. After the scissor-prick had been made in the skin, a drop of blood was formed and was collected into micro-haematocrit tubes. The blood was centrifuged in a micro-hematocrit centrifuge (Gemmy Industrial Corp..) for 10 minutes. A Micro-haematocrit Reader Scale was used to determine the PCV readings of the studied animals. The measurements were recorded in the data sheet (Appendix V).

3.6 COLLECTION AND IDENTIFICATION OF TICK SPECIES

Ticks were collected on a monthly basis between August 1999 to July 2000 from the same groups of cattle in Harrismith, Kestell and Qwa-Qwa from which blood samples had been obtained for diagnosis of haemoparasites. Only adult male and female ticks in all stages of engorgement were collected from the right-hand sides of the experimental animals. The method of counting ticks on one body side only

is generally used to estimate the overall level of tick infestation on animals (Hermans *et al.*, 1994; Dreyer *et al.*, 1998b).

Animals were individually restrained in a mobile handling facility consisting of a collecting kraal, a race, crush and neck clamp. The right sides of animals were divided into 13 different regions and ticks from each region were collected separately (Dreyer *et al.*, 1998b). These regions did not necessarily correspond to recognized anatomical regions, but were as indicated in Figure 3.

The position of attached adult ticks was firstly 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 the bovines. The ticks were removed by entomological forceps from each of the defined body areas and placed in labeled containers filled with 70% alcohol.

The collected ticks were counted, identified and recorded to species level using a standard stereo-microscope (Kyowa, Tokyo). Identification was done according to methods of Hoogstraal (1956) and Walker (1961). The ticks collected during the half-body counts were multiplied by two to give an indication of total tick burdens on the animals.

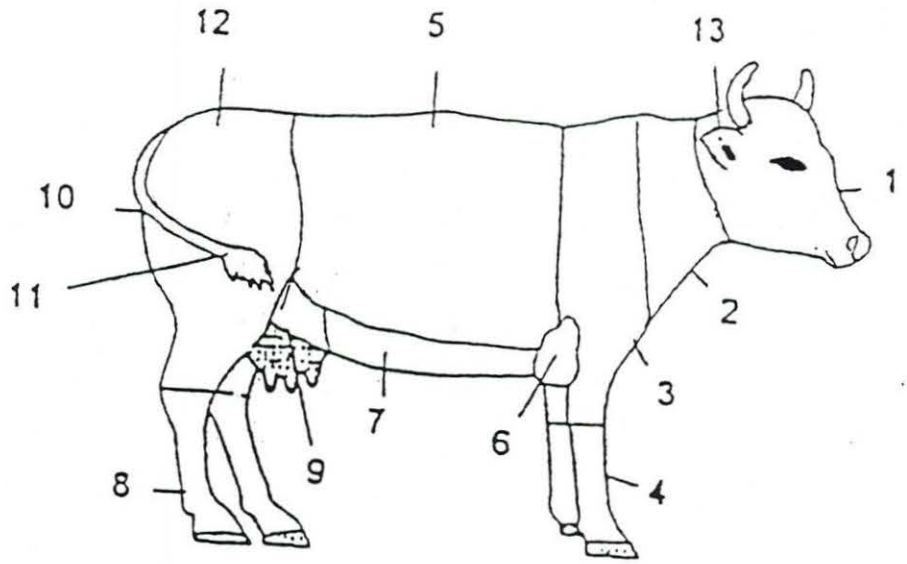


Figure 3: The regions on the bovine body from which ticks were collected

Key:

1:	Head	6:	Axilla	11:	Tail
2:	Neck	7:	Abdomen	12:	Buttocks
3:	Shoulder	8:	Hind leg	13:	Ear
4:	Front leg	9:	Inguineum		
5:	Flank/side	10:	Perineum		

3.7 DATA PRESENTATION AND ANALYSIS

The data sets were analyzed statistically using the appropriate analysis of variance techniques. All statistical data analyses were done on a Mecer-desktop computer. The software program used was Statistical Analysis System (SAS) for all the serological data.

Another software program used was Statistica 1998 Edition for Windows (StatSoft, Inc.). A One-way analysis of variance (ANOVA) test (Barnard *et al.*, 1993) was used to determine significant differences in burdens of *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi* during winter (May-July), spring (August-October), summer (November-January) and autumn (February-April). This was followed by a multiple range procedure, namely, the Least Significant Difference (LSD) test, to indicate the seasons causing the variance (Zar, 1974). A One-way ANOVA-test followed by a LSD test was used to determine if any significant variability existed in seasonality of tick species among the three different localities (Barnard *et al.*, 1993).

CHAPTER 4

RESULTS

4.1 PARASITOLOGICAL SCREENING OF HAEMOPARASITES INFECTING CATTLE IN THE NORTH EASTERN FREE STATE

No blood parasite was detected from the Giemsa-stained thick and thin blood smears prepared from a total of 386 cattle during the entire sampling period from August 1999 to July 2000 in Harrismith, Kestell and Qwa-Qwa as shown in Table 1. The majority of cattle in the area are of mixed-breed origin with predominantly Friessian-crosses. All the sampled animals appeared healthy and did not show any clinical signs of disease. Generally, tick control with commercial acaricides is not practised regularly by farmers in the study area but traditional methods such as the use of engine-oil and Jeyes fluid are frequently used, especially in Qwa-Qwa (Hlatshwayo, 2000). The problems and diseases observed due to husbandry and management systems as well as those due to poor grazing included alocepia, rumen alkalosis, halter absesses, rope sores and injuries (Hlatshwayo, 2000).

Table 1: Results of parasitological screening of cattle (n=386) by Giemsa-stained thick and thin smears for *Anaplasma*, *Babesia*, *Theileria* and *Trypanosoma* in Harrismith, Kestell and Qwa-Qwa

Month	Number of animals screened in Harrismith	Number of animals screened in Kestell	Number of animals screened in Qwa-Qwa	Parasitological results
August (1999)	11	12	10	-ve
September	10	11	10	-ve
October	12	10	10	-ve
November	12	10	10	-ve
December	10	10	10	-ve
January(2000)	11	10	10	-ve
February	10	10	10	-ve
March	13	10	10	-ve
April	16	13	10	-ve
May	12	11	10	-ve
June	12	10	10	-ve
July	10	10	10	-ve
Total	139	127	120	386

Key:

-ve means negative for *Anaplasma*, *Babesia*, *Theleria* and *Trypanosoma*

4.2 SEROLOGICAL DIAGNOSIS OF *ANAPLASMA* AND *BABESIA* IN CATTLE IN THE NORTH EASTERN FREE STATE

Animals were examined monthly in Harrismith over a continuous period of 12 months from August 1999 to July 2000. The average seroprevalence (Chi-square test, Statistical value=79.317, df=1, prob=0.001) of cattle (n=139) for *A. marginale* was 88% by ELISA while 92% of the animals were sero-positive (Chi-square test, Statistical value=98.482, df=1, prob=0.001) for *B. bigemina* by IFAT. The tested sera was sero-negative (100%) for *B. bovis* in Harrismith as shown in Table 2.

Table 2: The prevalence of *Anaplasma* and *Babesia* in cattle (n=139) in Harrismith as determined by ELISA and IFAT respectively

Anaplasma marginale	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
N	17	12	17	12
P	122	88	139	100
Babesia bigemina				
N	11	8	11	8
P	128	92	139	100
Babesia bovis				
N	139	100	139	100
P	0	0	0	0

Key:

N means the number of screened animals that were negative

P means the number of screened animals that were positive

Animals were examined monthly in Kestell over a continuous period of 12 months from August 1999 to July 2000. The average seroprevalence (Chi-square test, Statistical value=59.598, df=1, prob=0.001) of cattle (n=127) for *A. marginale* was 84% by ELISA while 91% of the animals were sero-positive (Chi-square test, Statistical value=86.811, df=1, prob=0.001) for *B. bigemina* by IFAT. The tested sera was sero-negative (100%) for *B. bovis* in Kestell as shown in Table 3.

Table 3: The prevalence of *Anaplasma* and *Babesia* in cattle (n=127) in Kestell as determined by ELISA and IFAT respectively

Anaplasma marginale	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
N	20	16	20	16
P	107	84	127	100
Babesia bigemina				
N	11	9	11	9
P	116	91	127	100
Babesia bovis				
N	127	100	127	100
P	0	0	0	0

Key:

N means the number of screened animals that were negative

P means the number of screened animals that were positive

Animals were examined monthly in Qwa-Qwa over a continuous period of 12 months from August 1999 to July 2000. The average seroprevalence (Chi-square test, Statistical value=73.633, df=1, prob=0.001) of cattle (n=120) for *A. marginale* was 89% by ELISA. All the animals (n=120) were sero-positive (100%) for *B. bigemina* by IFAT. The tested sera was sero-negative (100%) for *B. bovis* in Qwa-Qwa as shown in Table 4.

Table 4: The prevalence of *Anaplasma* and *Babesia* in cattle (n=120) in Qwa-Qwa as determined by ELISA and IFAT respectively

Anaplasma marginale	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
N	13	11	13	11
P	107	89	120	100
Babesia bigemina				
N	0	0	0	0
P	120	100	120	100
Babesia bovis				
N	120	100	120	100
P	0	0	0	0

Key:

N means the number of screened animals that were negative

P means the number of screened animals that were positive

Animals were examined monthly at each of the three study sites over a continuous period of 12 months from August 1999 to July 2000. The average seroprevalence (Chi-square test, Statistical value=211.907, df=1, prob=0.001) of cattle (n=386) for *A. marginale* was 87% by ELISA while 94% of the animals were sero-positive (Chi-square test, Statistical value=303.016, df=1, prob=0.001) for *B. bigemina* by IFAT. The tested sera was sero-negative (100%) for *B. bovis* in all the three study sites as shown in Table 5.

Table 5: The prevalence of *Anaplasma* and *Babesia* in cattle (n=386) in the three study sites, Harrismith, Kestell and Qwa-Qwa as determined by ELISA and IFAT respectively

Anaplasma marginale	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
N	50	13	50	13
P	336	87	386	100
Babesia bigemina				
N	22	6	22	6
P	364	94	386	100
Babesia bovis				
N	386	100	386	100
P	0	0	0	0

Key:

N means the number of screened animals that were negative

P means the number of screened animals that were positive

4.2.1 Means of optical density (OD) values of tested sera of cattle in the north eastern Free State as determined by ELISA

The mean monthly value and standard deviation (SD) of OD values of tested sera from cattle (n=139) in Harrismith were determined. The mean monthly OD values in cattle (n=139) in Harrismith ranged between 0.211- 0.307 as is shown in Table 6.

Table 6: Mean monthly OD values of tested sera from cattle (n=139) in Harrismith as determined by ELISA

Month	N	Mean	SD
August (1999)	11	0.267	0.080
September	10	0.223	0.058
October	12	0.232	0.073
November	12	0.304	0.161
December	10	0.248	0.127
January (2000)	11	0.273	0.117
February	10	0.211	0.046
March	13	0.216	0.090
April	16	0.227	0.080
May	12	0.307	0.101
June	12	0.288	0.099
July	10	0.226	0.105

Key:

N means the number of sampled animals

SD means standard deviation from the mean

The mean monthly value and standard deviation (SD) of OD values of tested sera from cattle (n=127) in Kestell were determined. The mean monthly OD values in cattle (n=127) in Kestell ranged between 0.201- 0.342 as is shown in Table 7.

Table 7: Mean monthly OD values of tested sera from cattle (n=127) in Kestell as determined by ELISA

Month	N	Mean	SD
August (1999)	12	0.269	0.085
September	11	0.282	0.098
October	10	0.268	0.029
November	10	0.201	0.022
December	10	0.342	0.113
January (2000)	10	0.276	0.084
February	10	0.288	0.115
March	10	0.212	0.049
April	13	0.263	0.089
May	11	0.307	0.091
June	10	0.213	0.056
July	10	0.235	0.081

Key:

N means the number of sampled animals

SD means standard deviation from the mean

The mean monthly value and standard deviation (SD) of OD values of tested sera from cattle (n=120) in Qwa-Qwa were determined. The mean monthly OD values in cattle (n=120) in Qwa-Qwa ranged between 0.198- 0.315 as is shown in Table 8.

Table 8: Mean monthly OD values of tested sera from cattle (n=120) in Qwa-Qwa as determined by ELISA

Month	N	Mean	SD
August (1999)	10	0.198	0.070
September	10	0.198	0.088
October	10	0.241	0.081
November	10	0.254	0.077
December	10	0.315	0.111
January (2000)	10	0.277	0.094
February	10	0.254	0.090
March	10	0.282	0.085
April	10	0.250	0.049
May	10	0.253	0.069
June	10	0.244	0.061
July	10	0.287	0.109

Key:

N means the number of sampled animals

SD means standard deviation from the mean

The mean monthly value and standard deviation (SD) of OD values of the tested sera from cattle (n=386) in Harrismith, Kestell and Qwa-Qwa were determined. The mean monthly OD values of the tested sera of cattle in all the three sites ranged between 0.236 - 0.302 as is shown in Table 9.

Table 9: Mean monthly OD values of tested sera from cattle (n=386) in Harrismith, Kestell and Qwa-Qwa as determined by ELISA

Month	N	Mean	SD
August (1999)	33	0.247	0.083
September	31	0.236	0.089
October	32	0.246	0.066
November	32	0.256	0.114
December	30	0.302	0.121
January (2000)	31	0.275	0.096
February	30	0.251	0.091
March	33	0.235	0.082
April	39	0.245	0.077
May	33	0.290	0.090
June	32	0.251	0.081
July	30	0.249	0.010

Key:

N means the number of sampled animals

SD means standard deviation from the mean

The mean value and standard deviation (SD) of OD values of tested sera from cattle (n=386) were determined. The mean OD values of the tested sera of cattle (n=386) in the three study sites, Harrismith, Kestel and Qwa-Qwa was 0.252, 0.264 and 0.254 respectively as is shown in Table 10. There was no significant difference (p=0.823, at 5% significance level) in the OD values of the tested sera of cattle in the three study sites.

Table 10: Mean optical density (OD) values of tested sera of cattle (n=386) in the three study sites, Harrismith, Kestell and Qwa-Qwa as determined by ELISA

Site	N	Mean	SD
Harrismith	139	0.252	0.101
Kestell	127	0.264	0.088
Qwa-Qwa	120	0.254	0.086

Key:

N means the number of sampled animals

SD means standard deviation from the mean

4.2.2 Means of Packed Cell Volume (PCV) values in cattle in the north eastern Free State

Peripheral blood for PCV values was also taken on a monthly basis between August 1999 to July 2000 from the same groups of cattle (n=139) in Harrismith. The mean monthly PCV valuesof cattle (n=139) in Harrismith ranged between 29-32% as is shown in Table 11.

Table 11: Mean monthly PCV values of cattle (n=139) in Harrismith

Month	N	Mean	SD
August (1999)	11	30	2.71
September	10	29	2.95
October	12	32	4.06
November	12	32	3.61
December	10	30	2.91
January (2000)	11	31	3.41
February	10	31	3.14
March	13	32	4.65
April	16	31	3.30
May	12	31	2.94
June	12	29	3.85
July	10	31	3.75

Key:

N means the number of sampled animals

SD means standard deviation from the mean

Peripheral blood for PCV values was also taken on a monthly basis between August 1999 to July 2000 from the same groups of cattle (n=127) in Kestell. The mean monthly PCV values of cattle (n=127) in Kestell ranged between 27-32% as is shown in Table 12.

Table 12: Mean monthly PCV values of cattle (n=127) in Kestell

Month	N	Mean	SD
August (1999)	12	29	2.54
September	11	31	7.88
October	10	27	1.35
November	10	29	3.24
December	10	30	4.95
January (2000)	10	31	7.72
February	10	31	3.47
March	10	31	3.10
April	13	29	3.62
May	11	32	4.80
June	10	29	2.50
July	10	29	2.84

Key:

N means the number of sampled animals

SD means standard deviation from the mean

Peripheral blood for PCV values was also taken on a monthly basis between August 1999 to July 2000 from the same groups of cattle (n=120) in Qwa-Qwa. The mean monthly PCV values of cattle (n=120) in Qwa-Qwa ranged between 18-32% as is shown in Table 13. PCV value of the month of July in Qwa-Qwa was low with the average of 18%.

Table 13: Mean monthly PCV values of cattle (n=120) in Qwa-Qwa

Month	N	Mean	SD
August (1999)	10	30	11.17
September	10	26	6.07
October	10	25	5.09
November	10	32	6.34
December	10	25	10.21
January (2000)	10	24	6.15
February	10	30	5.15
March	10	27	5.44
April	10	23	.5.07
May	10	23	4.81
June	10	20	5.80
July	10	18	2.59

Key:

N means the number of sampled animals

SD means standard deviation from the mean

Peripheral blood for PCV values was also taken on a monthly basis between August 1999 to July 2000 from the same groups of cattle (n=386) in Harrismith, Kestell and Qwa-Qwa. The mean monthly PCV values in all the three sites ranged between 26-31% as is shown in Table 14.

Table 14: Mean monthly PCV values of cattle (n=386) in Harrismith, Kestell and Qwa-Qwa

Month	N	Mean	SD
August (1999)	33	29	6.33
September	31	29	6.29
October	32	28	4.94
November	32	31	4.58
December	30	28	6.86
January (2000)	31	29	6.78
February	30	30	3.88
March	33	30	5.05
April	39	28	4.92
May	33	29	5.53
June	32	26	6.11
July	30	26	6.90

Key:

N means the number of sampled animals

SD means standard deviation from the mean

Peripheral blood for determination of PCV values was also taken on a monthly basis between August 1999 to July 2000 from the same groups of cattle in Harrismith, Kestell and Qwa-Qwa previously used for diagnosis of haemoparasites. The mean PCV of cattle in Harrismith, Kestel and Qwa-Qwa was 31%, 30% and 25% respectively as is shown in Table 15. Although the PCV value of animals in Qwa-Qwa was 25 %, there was no significant difference (p=0.731, at 5% significance level) in the mean PCV readings of cattle in the three study sites.

Table 15: Mean PCV values of cattle (n=386)in the three study sites, Harrismith, Kestell and Qwa-Qwa

Site	N	Mean	SD
Harrismith	139	31	4.46
Kestell	127	30	7.44
Qwa-Qwa	120	25	3.53

Key:

N means the number of sampled animals

SD means standard deviation from the mean

4.3 TICK SPECIES INFESTING CATTLE IN THE NORTH EASTERN FREE STATE

A total of 2 965 adult ticks belonging to two species were collected from cattle (n=386) over a continuous period of 12 months from August 1999 to July 2000 in Harrismith, Kestell and Qwa-Qwa as summarized in Table 16. High tick infestations were observed in animals of Qwa-Qwa. *Boophilus decoloratus* was generally a dominant species when comparing all the three study sites, whereas *Rhipicephalus evertsi eversti* was only dominant in Qwa-Qwa.

Table 16: Tick species of the studied animals (n=386) in Harrismith, Kestell and Qwa-Qwa

Site	N	*Boophilus decoloratus*	*Rhipicephalus evertsi evertsi*
Harrismith	139	467	246
Kestell	127	480	209
Qwa-Qwa	120	674	889
Total	386	1621	1344

Key:

N means the number of sampled animals

Ticks were collected on a monthly basis between August 1999 to July 2000 in Harrismith. Significant differences (p=0.641, at 5% significance level) in seasonal burdens of *B. decoloratus* occured, with the highest infestations recorded from February to June as is shown in Figure 4. Peaks were observed in May-June and December-February. *Rhipicephalus evertsi evertsi* was present throughout the year with only very small fluctuations in winter months (Figure 4). A peak of *R. evertsi evertsi* was observed from February to May in Harrismith.

Highest infestations of *B. decoloratus* were recorded from March to June (Figure 5). Peaks were observed in this graph in May-June and December-January. *Rhipicephalus evertsi evertsi* was present throughout the year with some small fluctuations in winter months, but with a peak in October (Figure 5). Increased numbers of *R. evertsi evertsi* were present on the animals from February to May, with a peak occuring in May, dropping off to low levels in June and July in Kestell.

Seasonal burdens of B. decoloratus occured, with the highest infestations recorded from March to June as is shown in Figure 6. Peaks were observed in this study in May-June and December-February. A peak of *R. evertsi evertsi* was observed from February to May in Qwa-Qwa.

Seasonal burdens and variations of the two tick species were compared for the three localities, Harrismith, Kestell and Qwa-Qwa as is shown in Figures 7 and 8. Seasonal variations among the three localities for the two tick species were small and mostly insignificant, and data from the three localities were thus combined for discussion of seasonal dynamics.

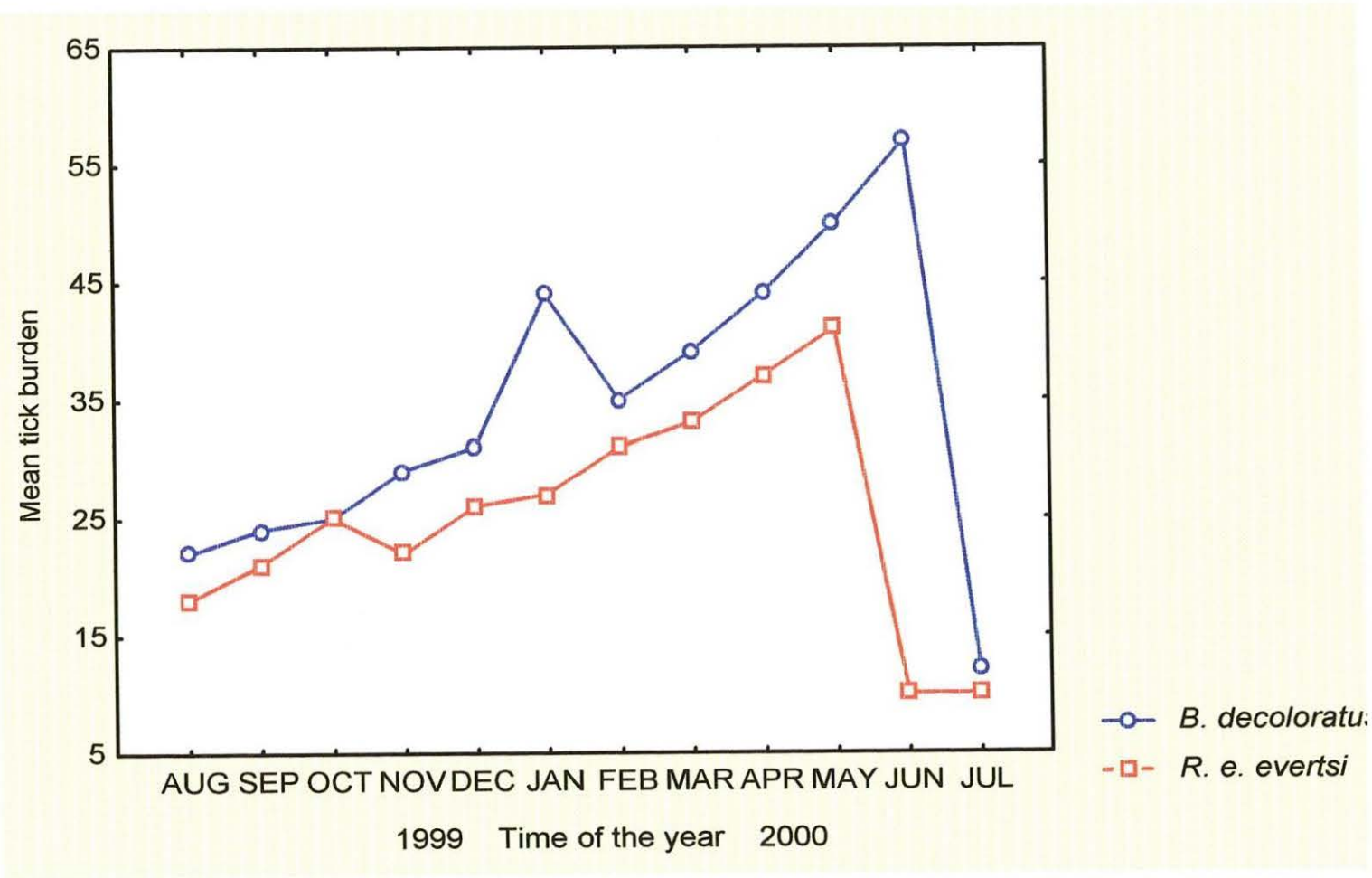


Figure 4: **Mean monthly *B.* decoloratus and *R. evertsi evertsi* burdens in cattle sampled in Harrismith during a twelve month study period (August 1999-July 2000)**

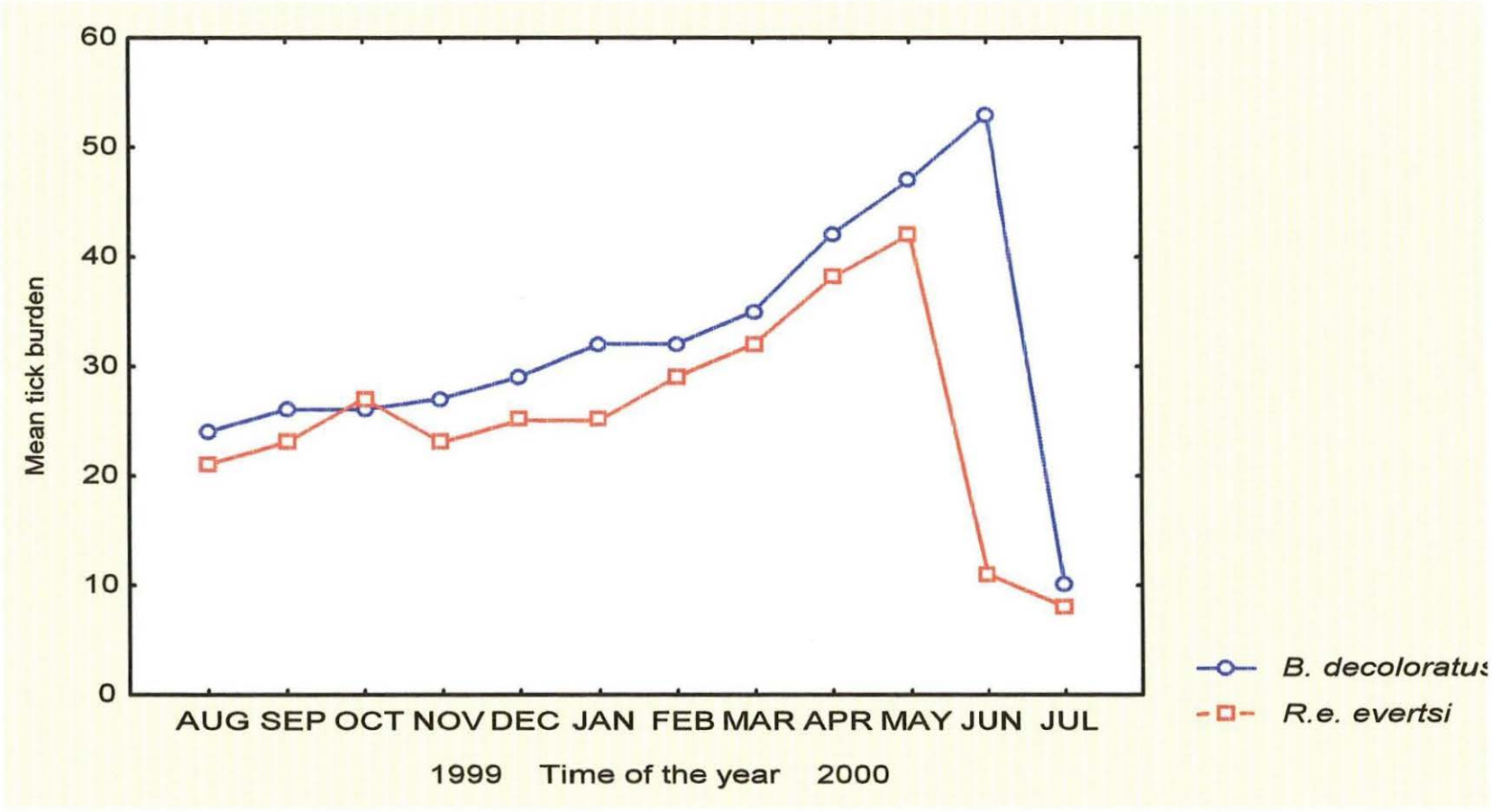


Figure 5: **Mean monthly *B.* decoloratus and *R. evertsi evertsi* burdens in cattle sampled in Kestell during a twelve month study period (August 1999-July 2000)**

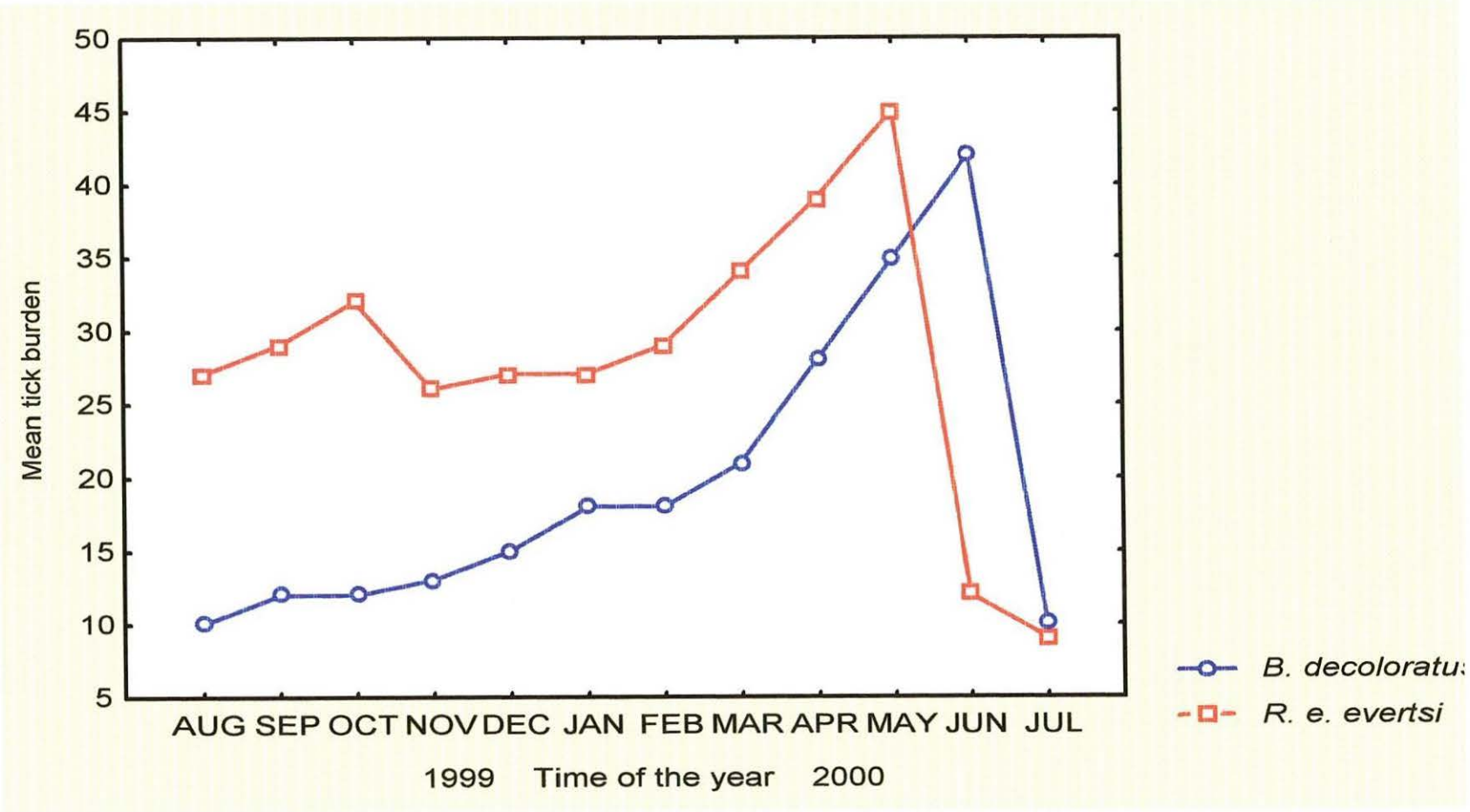


Figure 6: Mean monthly *B.* decoloratus and *R. evertsi evertsi* burdens in cattle sampled in Qwa-Qwa during a twelve month study period (August 1999-July 2000)

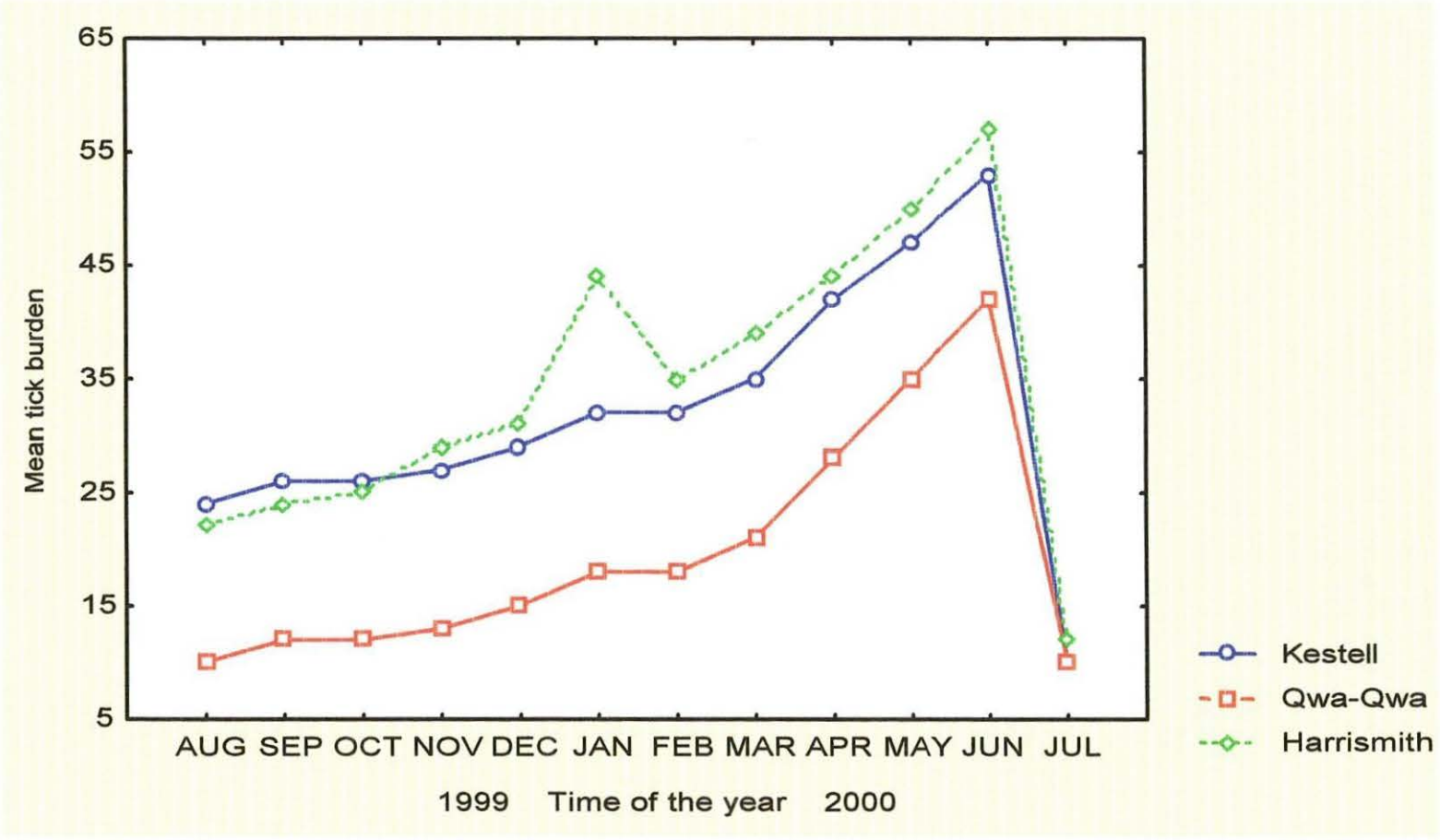


Figure 7: **Mean monthly *B.* decoloratus burdens in cattle sampled in Harrismith, Kestell and Qwa-Qwa during a twelve month study period (August 1999-July 2000)**

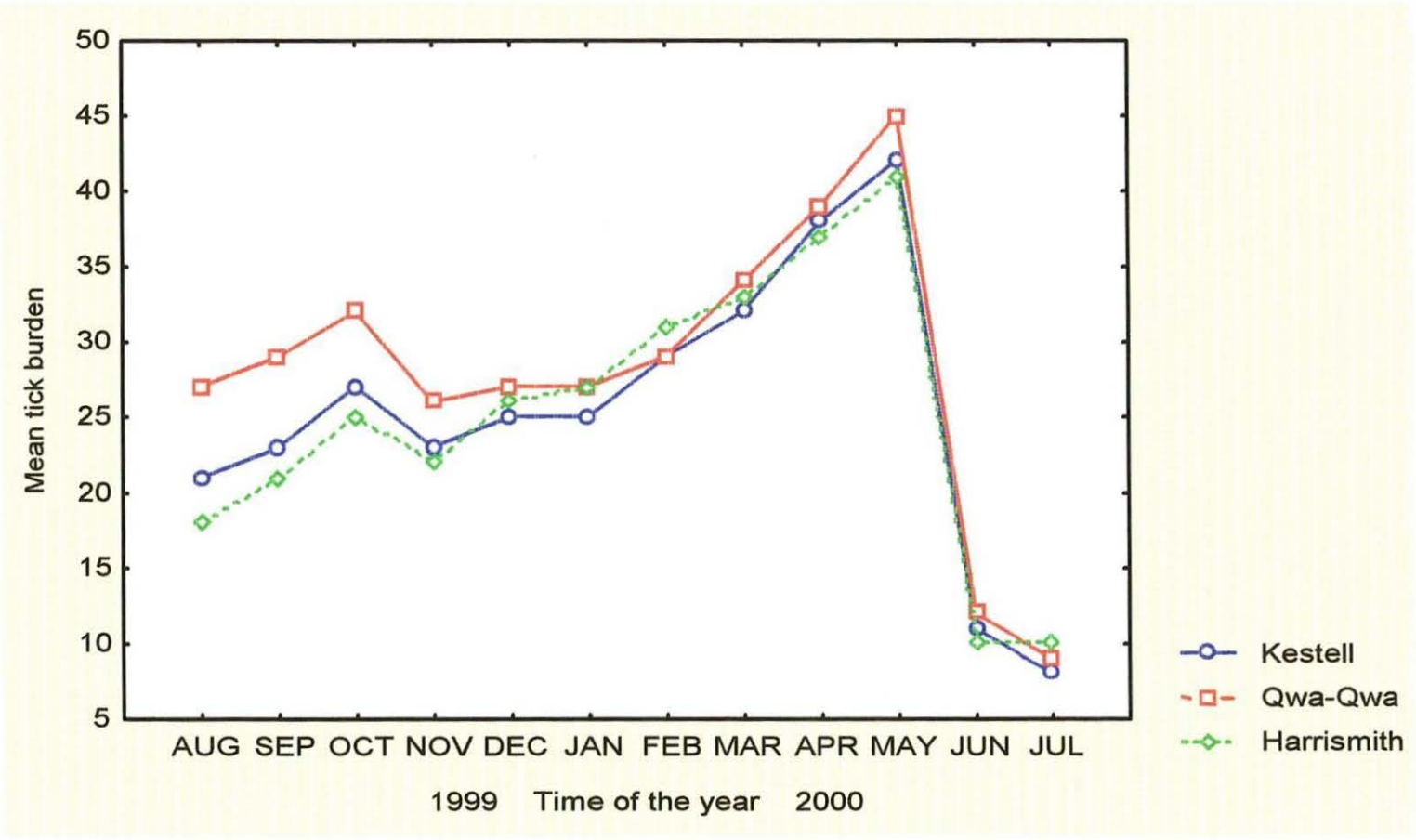


Figure 8: **Mean monthly *R. evertsi evertsi* burdens in cattle sampled in Harrismith, Kestell and Qwa-Qwa during a twelve month study period (August 1999-July 2000)**

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 SEROLOGICAL AND PARASITOLOGICAL DIAGNOSIS OF PARASITES IN CATTLE

It has been shown that based on the information gained from a serological study, the immune status of cattle in an area can be classified into an endemically stable (81-100% positive sera) situation, a situation approaching stability (61-80%), an unstable (21-60%) situation, a minimal disease situation (01-20%), and a disease-free situation (0% positive sera) (Norval *et al.*, 1983). Where the inoculation rate of *Babesia* and *Anaplasma* is adequate to ensure that all calves are infected while they are protected by innate and/or colostral immunity, clinical disease is minimal and endemic stability is achieved. The disruption of an existing situation of endemic stability is usually associated with drought conditions (De Vos, 1979), or increased tick control (Norval *et al.*, 1983). Endemic instability, on the other hand, describes the situation in a herd where some animals fail to become infected within nine months of birth. When such susceptible individuals encounter infected ticks, clinical disease develops (Dreyer *et al.*, 1998a).

A total of 94% of the studied animals (n=386) were sero-positive indicating a significantly large number of animals exposed to babesiosis (Chi-square test, Statistical value=303.016, df=1, prob=0.001) for *B. bigemina* by IFAT. When comparing the three study sites, all the animals in Qwa-Qwa (n=120) were sero-positive (100%) for *B. bigemina*, 92% of the animals in Harrismith (n=139) were sero-positive (Chi-square test, Statistical value=98.482, df=1, prob=0.001) and 91%

of the animals in Kestell (n=127) were sero-positive (Statistical value=86.811, df=1, prob=0.001) for *B. bigemina* by IFAT.

Based on the findings that 94% of cattle were sero-positive *B. bigemina*, it is evident that cattle in the north eastern Free State fit into the group of infection rates that is indicative of an endemically stable situation. No clinical cases of redwater due to this organism were observed during the 12 month study period. Although there were differences in seroprevalence between the cattle in Harrismith, Kestell and Qwa-Qwa areas, these differences were small and not statistically significant. A similar study by Dreyer *et al.* (1998a) investigating tick-borne diseases of cattle in Botshabelo and Thaba Nchu indicated a situation approaching endemic stability with 62.42% of the studied animals exposed to *B. bigemina* antibodies.

All the tested sera was sero-negative for *B. bovis* in all the three study sites. As far as it is known, *B. bovis* is only transmitted by *Boophilus microplus* (De Vos, 1979; De Vos and Jorgensen, 1992) which does not occur in the Free State Province (Howell *et al.*, 1983). Dreyer *et al.* (1998a) found an average seroprevalence of 19.47% against *B. bovis* for cattle sampled in the Botshabelo-Thaba Nchu area. These low seroprevalence indices indicated a situation of minimal disease, and the risk of clinical disease outbreak due to this organism was thus small. The occurrence of *B. bovis* antibodies in cattle of Botshabelo-Thaba Nchu area as described by Dreyer *et al.* (1998a), is difficult to explain, because no *B. microplus* ticks were found in this region (Dreyer, 1997). Further, many studies have concluded that *B. microplus* is the sole transmitter of *B. bovis* in southern Africa (De Vos, 1979; De Vos and Every, 1981; Howell *et al.*, 1981; Norval *et al.*, 1983).

Enzootic stability with regard to bovine babesiosis is a condition where there is frequent transmission of parasites and infection of all animals occurs during the period that young animals are protected by passively acquired and non-specific factors particularly within the first 6 to 9 months of life. Acquired immunity develops without the host becoming obviously sick and local animals are therefore generally immune to the specific *Babesia* organism involved and suffer minimally from the disease. A stable situation for one *Babesia* sp does not imply a similar situation for the other species (De Vos, 1979).

Enzootic instability on the other hand defines the situation in which some animals in the herd fail to become infected for a considerable period after birth. This could be due to a host-parasite imbalance resulting from infrequent transmission. Disease is then seen when susceptible animals in a herd encounter infected ticks. The creation of unstable situations in South Africa is to a large extent dependent on two factors: unfavourable climatic conditions and the injudicious control of ticks. One or both of these factors may influence the stability of either of the *Babesia* spp in any given area (De Vos, 1979).

The average seroprevalence (Chi-square test, Statistical value=211.907, df=1, prob=0.001) of cattle (n=386) for *A. marginale* was 87% in the three study sites. A seroprevalence above 81% is indicative of a situation of endemic stability. When comparing the three study sites the animals of Qwa-Qwa (n=120) had the highest sero-positive (Chi-square test, Statistical value=73.633, df=1, prob=0.001) percentage of 89%. It was followed by Harrismith where 88% of the animals (n=139) being sero-positive (Chi-square test, Statistical value=79.317, df=1,

prob=0.001) for *A. marginale* by ELISA. In Kestell 84% of the animals (n=127) were sero-positive (Chi-square test, Statistical value=59.598, df=1, prob=0.001) for *A. marginale* by ELISA.

Enzootic areas of anaplasmosis in South Africa appear to have high vector activity and for the maintenance of a stable situation the obvious source of infection would be infected cattle. It is still believed that adequate vector challenge will ensure early exposure of calves and result in the development of protective immunity (Potgieter, 1979).

Natural epizootic areas occur where none of the young calves are exposed to early infection because of the effect on vector survival resulting from geographical and climatic restrictions. Drastic fluctuations of these restrictions may result in the temporary settlement or elimination of vectors in these areas, with obvious consequences. Human-induced epizootics may result within enzootic areas, if excessive tick control is practised. If vector ticks are eradicated, a farmer would eventually breed susceptible stock. Under such conditions a laspe in the dipping programme, the possible introduction of carrier animals, the neglect by neighbours to dip their cattle, the presence of certain wild ruminants and migrating cattle could lead to serious disease outbreaks (Potgieter, 1979).

There was no significant difference (p=0.006, at 1% significance level) in the incidence of either anaplasmosis or babesiosis between the seasons. Further there was no significant (p=0.008, at 1% significance level) association on the occurrence of *Anaplasma* and *Babesia* infections when using the Likelihood Ratio of Chi-

Square indicating that the two infections are independent.

The observation of negative blood smears but high prevalence of positive serological results for *Anaplasma* and *Babesia* for the same group of animals indicates the north eastern Free State is endemic for these diseases but with a stable disease situation. It is now known that indigenous breeds can become very resistant to ticks (Bonsma, 1981) and acquire immunity to tick-borne diseases if exposed at an early age (Ross and Lohr, 1968).

5.2 ELISA OPTICAL DENSITY (OD) VALUES OF TESTED SERA AND PCV READINGS OF CATTLE IN THE NORTH EASTERN FREE STATE

The mean monthly ELISA OD values of the tested sera of cattle (n=386) in Harrismith, Kestell and Qwa-Qwa ranged between 0.211- 0.307, 0.201- 0.342 and 0.198 - 0.315 respectively. The mean monthly ELISA OD values of the tested sera of cattle in all the three sites ranged between 0.236 and 0.302. The mean ELISA OD values of the tested sera as a measure of exposure to *Anaplasma* in cattle in the three study sites, was 0.252, 0.264 and 0.254 in Harrismith, Kestel and Qwa-Qwa respectively. There was no significant difference (p=0.823, at 5% significance level) in the ELISA OD values of the tested sera of cattle in the three study sites.

The monthly PCV range values of cattle in Harrismith, Kestell and Qwa-Qwa ranged between 29 - 32%, 27 - 32% and 18 - 32% respectively. The monthly PCV range value of cattle (n=386) in all the three sites ranged from 26 - 31%. The overall mean PCV of cattle (n=386) in Harrismith, Kestel and Qwa-Qwa was 31%, 30%

and 25% respectively. There was no significant difference (p=0.731, at 5% significance level) in the mean PCV readings of cattle in the three study sites. The normal PCV range of 'healthy' animals is 24 - 40%. This study had shown that the studied animals have PCV ranges that fall within the normal PCV range of 'healthy' animals. This means that the studied animals are healthy carriers of anaplasmosis and babesiosis.

Although anaemia can be caused by factors other than tick-borne diseases, it remains one of the most reliable indicators of tick transmitted diseases. The PCV profile and average PCV of cattle is affected by the number of ticks infecting animals and the parasitological prevalence of *Anaplasma* and *Babesia*. This observation can be used as an additional indicator for tick-borne diseases even when *Anaplasma* and *Babesia* cannot be detected by parasitological diagnostic tests.

The General Linear Models Procedure showed no significant effect (p=0.922, at 5% significance level) of site, month, breed, *B. bigemina*, *A. marginale* on optical density (OD) values. Also there was no significant effect (p=0.0160, at 5% significane level) of site, month, breed, *B. bigemina*, *A. marginale* on PCV when using the General Linear Models Procedure. Finally, there was no significant association (p=0.7129, at 5% significance level) between PCV and OD values when using Correlation Analysis (Pearson Correlation Coefficients).

5.3 TICK SPECIES INFECTING CATTLE IN THE NORTH EASTERN FREE STATE

A total of 2 965 adult ticks belonging to two species were collected over a continuous period of 12 months from August 1999 to July 2000 in Harrismith, Kestell and Qwa-Qwa. *Boophilus decoloratus* (blue tick) was generally a dominant species. This is not surprising as north-eastern Free State lies at the centre of its distribution. The tick is inactive in winter and spring. In the present study significant differences in seasonal burdens of this one-host tick occurred, with the highest infestations recorded from February to June. This is similar to results obtained by Robertson (1981) in the Eastern Cape, and Dreyer *et al.*, (1998b), where *B. decoloratus* was abundant from May to June but sparse from July to January. In a study done by Rechav (1982) in the Eastern Cape, no definite pattern of seasonal occurrence was observed but the lowest numbers on hosts were also seen in early spring, with peaks in summer and autumn (February to June). The various peaks in adult infestation resulting in population 'waves', indicate the completion of several generations in one year (Punyua *et al.*, 1991). Peaks were observed in the present study, namely, in May-June and December-January. This indicates that the blue tick produced three generations during the year. A similar seasonal periodicity with three generations was observed by Dreyer *et al.*, (1998b) in Botshabelo and Thaba Nchu areas of the Free State Province.

Boophilus decoloratus is also responsible for the transmission of two tick-borne diseases, namely anaplasmosis and babesiosis. This tick is usually most evident from about 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

(Howell *et al.*, 1978). The observation in our study is similar to that of Howell *et al.*, (1978) in that we experienced a little rise in tick numbers during September and a peak in June when the blue ticks were mostly evident.

The positive correlation (r^2=0.364, $p<0.05$) between burdens of tick species and warm or cold seasons was expected as the ticks are ectotherms. Such a correlation was also reported by Fourie *et al.*, (1996) during their study in south-western Free State. According to Rechav (1982), temperature is probably the main regulating factor in the seasonal patterns of the blue tick.

The presence of *R. evertsi evertsi* (red-legged tick) throughout the year with only small fluctuations in winter months, was also observed by Punyua *et al.* (1991), Fivaz and De Waal (1993), and Dreyer *et al* (1998b). In this study, the peak observed from March to May resembled the January to May peak on cattle in a Natal study (Baker and Ducasse, 1967), the April to May peak in a study by Rechav (1982) in the Eastern Cape, the March to May peak in a study on sheep in the Free State (Horak *et al.*, 1991), and the March to May peak on cattle in Botshabelo and Thaba Nchu (Dreyer *et al.*, 1998b). Results obtained in this study indicated that temperature, and specifically the winter temperature, was 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).

Rhipicephalus evertsi evertsi is one of the most important vectors of *Theileria equi* in horses in South Africa (Howell *et al.*, 1978), and transmits *B. bigemina* (De Vos

and Jorgensen, 1992) and *A. marginale* (Potgieter, 1979) in bovines. Certain adult *R. evertsi evertsi* strains can produce a toxin resulting in 'spring lamb paralysis' in lambs, kids and calves. High ear infestations with the immature stages of this species can result in ear irritation with secondary bacterial infections of ear canals (Howell *et al.*, 1978).

Red-legged ticks are most active in summer, though some specimens can be found all through the year. In Natal and the Eastern Cape the numbers of immature red-legged ticks start to increase early in November, are at their peak from January to April and then slowly decrease again. Adult numbers are highest from January to the end of May (Howell *et al.*, 1978). The tick is inactive in winter and spring. *Rhipicephalus evertsi evertsi* was the second most abundant tick species in Harrismith and Kestell. This is probably due to the availability of its other preferred hosts, such as sheep and goats, which normally graze together with cattle.

Seasonal fluctuations in the three localities were very similar but tick burdens in cattle in Qwa-Qwa were significantly higher compared to the other localities. A first possible reason for higher tick infestations in Qwa-Qwa was that the body condition of cattle in this locality were always poorer compared to the other two localities. This can result in a lowered tick resistance resulting in higher tick burdens (De Castro and Newson, 1993). A possible reason for the poor body conditions could have been the severe overgrazing of the veld in this specific locality. Farmers in this area appeared unmotivated and unwilling to herd the cattle to better grazing further away. A second possible reason for the higher burdens could have been the higher stocking densities, resulting in more contact with hosts (Hlatshwayo, 2000).

Another possible reason for higher tick burdens in Qwa-Qwa was that farmers in this area do not control ticks or tick-borne diseases. A possible reason for not controlling ticks is the fact that most of the cattle owners in this area are resource-poor farmers, and they do not have the necessary resources and knowledge to control ticks (Hlatshwayo, 2000).

5.4 CONCLUSION

Cattle production is important in the economic growth and stability of any country, and improved animal health is a vital component in this resource development. Although food production in Africa is increasing at a rate of approximately 33% per decade, it was unable to keep up with the population growth during the eighties. Thus food supply is a very important issue for the new South African government and not only commercial farmers are recognized as beef producers, but the many small-scale resource-poor farmers are now also seen as potential producers (Pinstrup-Anderson, 1993).

The eastern Free State, including the study area, is a good cattle farming province, and the improvement of farming conditions and the education of farmers in all the rural and urban areas of the province is now a major challenge for, and aim of, the Department of Agriculture and Environmental Affairs of the Provincial Government. The results obtained from studies conducted in the area were discussed in the previous chapters. The information gathered from these studies can now be used to formulate conclusions on the various problems regarding cattle farming in the area, with specific reference to tick-borne diseases and ticks. The following conclusions were therefore observed:

1. No blood parasites were detected from the Giemsa-stained thick and thin blood smears prepared from cattle during the entire sampling period.
2. A total of 94% of the studied animals were sero-positive for *B. bigemina* by IFAT. It is evident that cattle in the north eastern Free State fit into the group of infection rates that is indicative of an endemically stable situation.
3. The tested sera were sero-negative (100%) for *B. bovis*. As far as it is known, *B. bovis* is only transmitted by *Boophilus microplus* (De Vos, 1979; De Vos and Jorgensen, 1992), which did not occur in this area (Howell *et al.*, 1983).
4. The average sero-prevalence for *A. marginale* was 87% by ELISA indicating a situation of endemic stability. Possible explanation for the observed negative parasitological but high positive serological results, is that animals have been infected previously and developed immunity, thereby becoming healthy carriers of the diseases. Under these conditions it is difficult to recognize mild and subclinical infections since blood parasitaemias are extremely low and frequently do not rise to levels detectable by microscopy (Ross and Lohr, 1968).
5. There was no significant difference in the incidence of either anaplasmosis or babesiosis between the seasons in adult cattle sampled in the north eastern Free State.
6. There was no significant association on the occurrence of *Anaplasma* and *Babesia* infections when using the Likelihood Ratio of Chi-Square indicating that the two infections are independent of each other.
7. The General Linear Models Procedure showed no significant effect of site, month, breed, babesiosis and anaplasmosis on optical density (OD) values.
8. There was no significant effect of site, month, breed, babesiosis and

anaplasmosis on Packed Cell Volume (PCV) readings when using the General Linear Models Procedure.

9. There was no significant association between PCV readings and Optical Density values when using the Pearson Correlation Coefficients.

10. The findings of the present study suggest that only two ixodid ticks, namely *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi* infect cattle in the north eastern Free State. These conclusion may nevertheless be premature as complete body searches were not conducted on cattle, for especially immature tick stages.

11. An integrated approach to tick control should be considered in Harrismith, Kestell and Qwa-Qwa. With the adoption of this integrated tick management plan (ITMP) in this area, a more flexible approach to tick control will be maintained. Control measures would vary according to the local interactions of the farming cultures, hosts, ticks and environment. An ITMP for the present control of ticks and tick-borne diseases in the eastern Free State might include the following:

    (a) The selection of tick-resistant individuals or cattle breeds.

    (b) The use of biological and traditional tick control methods.

    (c) The use of acarides only when economically justified to limit the damage done by tick *per se* (Hlatshwayo, 2000).

CHAPTER 6

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APPENDICES

Appendix I: Sample of IFAT serological result book

Appendix II: Materials and buffers used in the enzyme-linked immunosorbent assay (ELISA)

1. Chemicals

Sodium bicarbonate	$NaHCO_3$
Magnesium Chloride	$MgCl_2.6H_2O$

PBS Dulbecco

Conjugate- Vecta Stain ABC Kit (Vector Laboratories)

p-Nitrophenol phosphatase (Merck)

2. Plastic ware

Nunc C96 Pollysorp 96 well plate	
Measuring cylinders	100 ml
Ehrlenmeyer flask	100 ml
Pipette	10 ml
Micro-pipette tips	0-200μl

3. Apparatus

ELISA reader		
Printer		
Shaking platform		
ELISA plate shaker		
Chemical balance		
Micro pipette	Single	5-200μl
	Multi	25-250μl
		20-1000μl

Incubator	37^0C

4. 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
$MgCl_2.6H_2O$	0.24 g
Distilled water (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)

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
$MgCl_2.6H_2O$	10 mM	0.203 g
D-H_2O		100.00 ml

pH 9.5

D-Washing Buffer

PBS	1000.00 ml
Tween 20	1.00 ml

Vecta Stain ABC

Portion A (anti-mouse antibodies)

Dilute 1:3000 with dilution buffer (solution B)

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	(portion B)	7.50 μl
Biotin	(portion C)	7.50 μl
Washing buffer	(solution D)	11.00 ml

Incubate for 30 minutes at 37 after mixing.

Substrate

p-Nitrophenol phosphatase	0.01 g
Substrate buffer (solution C)	11.00 ml

Appendix III: Sample of ELISA data and time sheet

ELISAANA

ANAPLASMA – ELISA

DATE :_____ PLATE: _____ ANTIGEN: _____

NOTES:_____

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	1		9		LPC	17		25		33	
B	NC	2		10		NC	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	

TIME SHEET:

ANTIGEN: Time_IN_____OUT_____Temp:_____

WASHING: Times:_____

BLOCKING:Time in :_____Time out:_____ Conc.:_____TEMP_____

SERA: Time in:_____ Time out:_____Conc.:_____

MONOCL. ANTIBOD : Time in:_____ Time out:_____ Conc.:_____

WASHING: Times:_____

ANTI – MOUSE: Time in:_____Time out:_____ Conc:_____

WASHING: Times:_____

CONJ A & B: Time in:_____ Time Out:_____Conc:_____

(CONJ B INCUBATION:_____ Conc:_____

WASHING: Times:_____

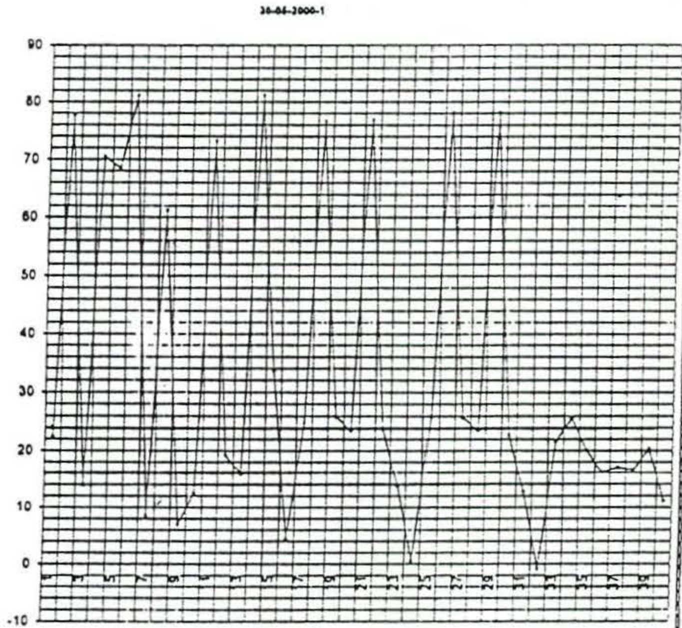
SUBSTR.: Time in:_____ Time Out:_____Conc:_____

Appendix IV: Sample of OD values and the analysed results on an Excel computer program

			ANAPLASMA - ELISA			Upper accep CV		0.12			DATE	00/05/30			
		Plate No	1	Antigen: b41 50/50 4UL			Operator	E'BU	min						
	Mean =	1.81528571	SD =	0.1431964	X-(3SD) =	1.37870	COP% =	24	PC Mean =	0.9305	POSC% =	41			

	1	2	3	4	5	6	7	8	9	10	11	12
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	[illegible]	[illegible]	[illegible]	[illegible]	1.421	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	[illegible]	[illegible]	[illegible]	[illegible]	1.112	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

REF	REF	Sample	Mean	%Inhibition	Result	CV	Pass/Fail	REF	REF	Sample	Mean	%Inhibition	Result	CV	Pass/Fail
20116	A 2,3	1	1.407	22	-ve	3.55%	Pass	3327	E 7,8	21	0.419	77	Positive	4.90%	Pass
20131	B 2,3	2	0.405	78	Positive	1.73%	Pass	3387	F 7,8	22	1.347	24	-ve	0.72%	Pass
20127	C 2,3	3	1.563	14	-ve	1.02%	Pass	MT	G 7,8	23	1.566	14	-ve	0.47%	Pass
2002	D 2,3	4	0.537	70	Positive	3.45%	Pass	MT	H 7,8	24	1.805	1	-ve	2.27%	Pass
2048	E 2,3	5	0.572	81	Positive	4.11%	Pass	MT	A 9,10	25	1.345	26	Positive	5.17%	Pass
2039	F 2,3	6	0.343	81	Positive	1.17%	Pass	MT	B 9,10	26	0.398	78	Positive	5.16%	Pass
2064	G 2,3	7	1.660	9	-ve	0.60%	Pass	MT	C 9,10	27	1.348	26	Positive	0.85%	Pass
2068	H 2,3	8	0.704	61	Positive	8.52%	Pass	MT	D 9,10	28	1.368	24	-ve	1.77%	Pass
20139	A 4,5	9	1.689	7	-ve	0.21%	Pass	MT	E 9,10	29	0.397	78	Positive	0.13%	Pass
20174	B 4,5	10	1.589	12	-ve	0.31%	Pass	MT	F 9,10	30	1.403	23	-ve	2.46%	Pass
20169	C 4,5	11	0.487	73	Positive	6.18%	Pass	MT	G 9,10	31	1.584	13	-ve	3.09%	Pass
2075	D 4,5	12	1.466	19	-ve	2.39%	Pass	MT	H 9,10	32	1.831	-1	-ve	2.08%	Pass
20175	E 4,5	13	1.526	18	-ve	4.08%	Pass	MT	A 11,12	33	1.422	22	-ve	8.47%	Pass
2051	F 4,5	14	0.343	81	Positive	0.44%	Pass	MT	B 11,12	34	1.350	26	Positive	12.12%	Fail
20137	G 4,5	15	1.205	34	Positive	7.60%	Pass	MT	C 11,12	35	1.452	20	-ve	5.20%	Pass
2066	H 4,5	16	1.735	4	-ve	3.69%	Pass	MT	D 11,12	36	1.522	16	-ve	7.56%	Pass
3664	A 7,8	17	1.364	25	Positive	1.39%	Pass	MT	E 11,12	37	1.505	17	-ve	7.38%	Pass
53327	B 7,8	18	0.424	77	Positive	0.12%	Pass	MT	F 11,12	38	1.514	17	-ve	7.86%	Pass
3387	C 7,8	19	1.345	29	Positive	0.71%	Pass	MT	G 11,12	39	1.446	20	-ve	12.10%	Fail
3664	D 7,8	20	1.381	23	-ve	0.90%	Pass	MT	H 11,12	40	1.812	11	-ve	11.90%	Pass



Appendix V: Sample of a data sheet

Data Sheet

A Sero-epidemiological Survey of Parasites in Cattle in the North Eastern Free State.

Researcher : .....
Location : .....
Date : .....
Species : .....

Species No.	Breed	Sex	Color	Age	Physical condition	Tick spp	Mite spp	Helminths	Temp	PCV	Microscopy	Serology