

A serological prevalence study of
important infectious diseases of cattle
in rural areas of
Kwa Zulu Natal, South Africa

By

Uta Hesterberg

Promoter: Prof. B. Gummow

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University of Pretoria**

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SUMMARY

In the rural areas of Kwa Zulu Natal small scale farming is the main agricultural activity, which is often carried out in addition to other employment taken on in another location by at least one member of the household. Although Veterinary services (VS) was aware of several diseases occurring in this area and has implemented a dipping strategy for tick borne diseases as well as a regional annual vaccination campaign for Rabies, Anthrax and Black quarter, uncertainty remained about the relative importance of other diseases such as brucellosis, leptospirosis and enzootic bovine leucosis. Further it was of interest to investigate the serological resistance of cattle against the tick borne diseases babesiosis and anaplasmosis. In addition to this veterinary services wanted to increase their understanding of the perceptions and practices of local cattle owners that were relevant for the context of disease control.

A serological survey of cattle was carried out between March 2001 and December 2003 to determine the prevalence of the above-mentioned diseases. The survey was designed as a two-stage survey, considering the diptank as the primary sampling unit.

The conduction of the questionnaire survey was based on a convenience sample and took place during the dipping procedure.

The apparent prevalence at district level was adjusted for clustering, and diagnostic test sensitivity and specificity and displayed in maps.

The mean true prevalence of brucellosis varied from zero to 15.8 percent in the north eastern region with the large majority of the districts being disease free. Enzootic Bovine Leukosis (EBL) was widely present in the province at generally low prevalences, except in the central region where the highest prevalence at district level

was recorded to be 70 percent. Leptospirosis also occurred frequently with the highest prevalence noted being 62 percent at district level. The southern regions showed a higher leptospirosis prevalence than other areas of the province, while in some of the northern and western districts a lower prevalence is noted. The encountered serovars were pomona, which occurred most frequently, tarrasovi, bratislava, hardjo, canicola and icterohaemorrhagica.

While *Babesia bovis* and anaplasmosis occurred at a high prevalence throughout the province, *B. bigemina* was found to be much less established and is absent from many of the northern districts.

Most prevalences calculated at district level do show large confidence intervals due to uncertainty that arose from the sampling frame and should be interpreted with care.

1 INTRODUCTION

The former homelands of South Africa are generally regarded as resource poor areas with a poor infrastructure and a high unemployment rate. Production indicators, such as herd mortality and calving rate are generally much below the results of commercial farmers. Before help can be implemented in an effective manner, areas where improvement is needed must be identified and the basic starting situation needs to be understood. From a veterinary point of view, this means the prevalence of infectious diseases, but also an understanding of the perceptions, culture and needs of the communities (Penrith and Thomson 2004).

Although many studies have been initiated in South Africa after the change of government in 1994 to improve the understanding of practices and perceptions in

relation to animal health (Getchell *et al.* 2002; Krecek *et al.* 1995; Makgatho *et al.* 2005; Masika *et al.* 1997a; Masika *et al.* 1997b; Masika *et al.* 2000; McCrindle *et al.* 1994; Mokantla *et al.* 2004), information from the rural areas of the country regarding the prevalence of important diseases however is often not available or of a poor quality. With the objectives of filling these knowledge gaps in mind, the Veterinary Services of Kwa Zulu Natal designed and implemented an extensive survey within these rural areas. This survey was divided into two major subsections: The first was a serological study of all relevant infectious diseases of all commonly kept types of livestock. Included in this survey were brucellosis, enzootic bovine leucosis, leptospirosis, bovine babesiosis and anaplasmosis. The second part of the study consisted of a questionnaire survey of cattle owners. The results of these surveys will be presented in this paper.

2 LITERATURE REVIEW

2.1 The Diseases

2.1.1 Brucellosis

2.1.1.1 The disease

Brucellosis is a disease caused by members of the bacteria genus *Brucella*, a facultative intracellular parasite, affecting many animal species and humans. In bovines Brucellosis is caused by *Brucella abortus* and is characterized by abortion in late

pregnancy and a subsequent high rate of infertility in females. The disease is of worldwide occurrence although several European and other first world countries have invested great resources into the eradication of the disease and are believed to be free from the agent, but eradication of the disease proves to be a difficult and expensive task (Hadjichristodoulou *et al.* 1999).

The primary sources of infection are foetal membranes and fluids as well as vaginal discharges, which are expelled by infected females when they abort or at parturition. *Brucellae* are also commonly shed in milk and semen (Godfroid *et al.* 2004). The source of the disease can be the neighbouring farm, and community pastures have been a means for herd transmission (Salman and Meyer 1984; Kerr 1960). Risk factors for infection in the African context have been proposed to be older cattle in large, and free grazing herds (Kadohira *et al.* 1997).

2.1.1.2 Serology of Brucellosis and serological Tests

Standardized and validated serological tests for Bovine Brucellosis listed in the OIE manual for diagnostic tests are: The serum agglutination test (SAT), the complement fixation test (CFT), an enzyme-linked immunosorbent assay (ELISA), the fluorescence polarisation assay (FPA), buffered Brucella antigen tests (BBAT) and the rose Bengal test (RBT) (OIE 2004). Vaccination with strain 19 vaccine and cross reactions with other agents can lead to false positive test results (Bernard *et al.* 2005). Two examples of such cross reactions are infections with *Yersinia enterocolitica* and *Vibrio cholera* with the former being the most significant cause of false positive reactors (Munoz *et al.* 2005; Nielsen *et al.* 2004). In an extensive literature review, Gall and Nielsen (2004) reviewed the performance of different serological tests used for diagnosis of bovine brucellosis and came to the conclusion that the CFT contrary to widely held belief of

being a highly sensitive and specific test, produced quite variable results. The authors suggested this was due to the complexity and labour of the test, a lack of standardisation, inability to test haemolysed samples, anti-complementary results, false positives due to vaccinated animals and cross reactions with other micro-organisms (Gall and Nielsen 2004). However, since 1995 the variability has decreased and some authors consider the test to be 100% specific in the absence of vaccination (Omer *et al.* 2001).

2.1.1.3 The disease in the African context

In the years from 1996 to 2004 between 291 and 457 outbreaks of bovine brucellosis were reported yearly by South Africa to the OIE (OIE 1996). The disease is known to have a high prevalence in southern Africa, especially in intensively farmed areas and has a strong economic impact: In a scenario where 14.7% of herds were infected in 1990, losses to farmers were estimated to be around 300 million Rand annually (Godfroid *et al.* 2004). Cattle from remote rural villages were found in one study to have a much lower sero-prevalence than cattle from peri-urban areas (Bakunzi *et al.* 1993).

In Kwa Zulu Natal an outbreak of *Brucella mellitensis* biotype 1 occurred in 1994. The goats were found to be infected after diagnosis of Malta fever was confirmed to have infected the herd owner. A consequent serological survey in goats in the province found positives in three districts (Reichel 1996). In March 1996 a test-and-slaughter eradication campaign was initiated and continued until the year 2000 resulting in a reduction of prevalence and at that time apparent eradication (Emslie and Nel 2002).

2.1.1.4 Zoonotic Aspects

Besides the impact the disease has on livestock, Brucellosis is also an important zoonosis. The disease caused by *Brucella mellitensis* is known as Malta Fever or Mediterranean Fever and the disease caused by *Brucella abortus* is known as Undulant Fever. Worldwide around 500 000 persons annually are estimated to be affected by brucellosis (Qazilbash and Bari 1997). Clinical signs include low fever, headache, weakness, cough, rheumatic pains, chills and backache (Qazilbash and Bari 1997) (Thakur *et al.* 2002). The established mode of transmission of *Brucella spp* to humans is usually direct contact with infected animals or their carcasses or through ingestion of infected unpasteurized milk or dairy products (Chomel *et al.* 1994; Qazilbash and Bari 1997). Consequently the risk for human infection appears to be related to occupational exposure as occurs when working as a farmer, herdsman, veterinarian or microbiologist (Al-Shamahy *et al.* 2000).

In southern Africa public awareness is very low and a lack of sufficient knowledge of the disease amongst physicians in addition to the absence of effective prevention and management strategies have attributed to the wide spread of the disease, but the actual rate of infections in humans is virtually unknown in this area (Godfroid *et al.* 2004; Thakur *et al.* 2002).

2.1.2 Leptospirosis

2.1.2.1 The disease

Leptospirosis is a world-wide zoonotic infection caused by *Spirochaetes*, occurring in domestic and wild mammals, but the agents have also been isolated from other vertebrates such as birds and amphibians (Alonso-Andicoberry *et al.* 2001). *Leptospira* are classified into serovars based on their antigenic characteristics and, more recently, into species based on genomic studies (O'Keefe 2002). In cattle the infection has been classified into two aetiological groups: one due to strains adapted to and maintained by cattle (*Leptospira interrogans* serovar *hardjo*) and a second group of incidental infections caused by strains maintained by other domestic and free living animals (Alonso-Andicoberry *et al.* 2001; Ellis 1994).

Clinical presentation following acute infection can range from transient febrile episodes to life threatening acute haemolytic disease. Infection may also be associated with abortion, neonatal mortality, infertility, uveitis, mastitis and renal failure. The syndromes vary depending on the host species (Hunter 2004; O'Keefe 2002). Where a maintenance host of a serovar is involved, acute infections may be mild and can result in the establishment of chronic infections, which may result in intermittent, sometimes lifelong shedding of *Leptospire*s in the urine and from the genital tract (O'Keefe 2002).

Losses in cattle mainly occur through decreased reproductive performance and milk production. Transmission of Leptospirosis can occur via the venereal, trans-placental, cutaneous, oral, respiratory or conjunctival routes (Hunter 2004). Wet environments are believed to aid the survival of *Leptospire*s. Seasonal fluctuations of the disease have been described and were hypothesized to be due to variations in stock management,

changes in age composition of the herd and seasonal changes related to disease transmission (Hathaway *et al.* 1986). In a study intended to investigate a possible difference in risk of infection with leptospirosis in different farm types, no significant difference of prevalence between commercial, small scale and communal farms in Zimbabwe could be established (Feresu 1987).

2.1.2.2 Serology and serological tests

The microscopic agglutination test (MAT) test, that was also applied in this study, is the standard serological test for Leptospirosis (OIE 2004) and uses a panel of leptospiral strains as antigens for detection of agglutinating antibodies. Significant experience is necessary to perform the test and inter-laboratory variations are high. When single samples are collected from clinically normal animals a result of 50% agglutination at a dilution of 1:100 is often considered significant (O'Keefe 2002). The MAT is not able to differentiate between infection, vaccination and cross reactions, which occur between closely related serogroups (Feresu 1987; Hodges and Day 1987). At individual animal level the test is described as most useful in acute infections, but of limited value for chronic infections (OIE 2004).

A study aimed to determine the potential of the test to correctly identify serovars in humans, estimated the ability of the test to correctly predict all serovars to be 46.4%. It was concluded from the study, that a reaction with an individual serovar that was selected for use as an antigen to represent a serogroup can not be taken to imply infection with the serovars tested but rather infection with antigenically similar serovars of the same serogroup (Levett 2003). The author states, that when multiple serovars from a single serogroup are included in the MAT, cross reactivity becomes the rule rather than the exception (Levett 2003).

Nevertheless, the MAT remains widely accepted as capable of producing serovar specific results and is still considered the gold standard (O'Keefe 2002).

2.1.2.3 The disease in the African Context

Leptospirosis in South Africa is considered important in the pig and to a lesser degree in the cattle industry (Hunter 2004). In South Africa *L. pomona*, *L. mini*, *L. canicola* and *L. hardjo* have been isolated from cattle (Herr *et al.* 1982; Hunter 2004; Te Brugge and Dreyer 1985). It appears that *L. hardjo* is the most important cause of bovine leptospirosis in South Africa and *L. pomona* of leptospirosis in pigs (Hunter 2004; Hunter *et al.* 1988). An abattoir survey in pigs in South Africa found a 37.2% prevalence in pigs from Kwa-Zulu Natal. The main serovars found in this study were *L. icterohaemorrhagica*, *L. bratislava* and *L. hardjo* (Potts *et al.* 1995). Leptospirosis in South African cattle occurs mostly in areas of high rainfall, - Mpumalanga, Kwa Zulu Natal and the coastal areas of the Eastern and Western Cape (Hunter 2004). In spite of the serologically documented presence of leptospirosis in South Africa, no outbreaks have been reported to the OIE since 1996 (OIE 1996). However leptospirosis is neither a controlled nor a notifiable disease in South Africa. In the neighbouring country, Zimbabwe, a study found the sero-prevalence of the disease to be an overall 27%. The author of this study suggested that outbreaks of the disease are most likely going to go unnoticed in the small scale rural setting (Feresu 1987).

2.1.2.4 Zoonotic Aspects

As a zoonosis the risk of *Leptospira* infection in humans is closely related to humans coming into contact with tissues or urine from infected animals. Other risk factors include contact with mud or water contaminated with animal excreta (Leal-Castellanos *et al.* 2003). The risk of infection increases in the presence of skin cuts or abrasions since *Leptospire*s rapidly invade the bloodstream after penetrating skin or mucous membranes (Leal-Castellanos *et al.* 2003).

In New Zealand *L. hardjo* serovars were found to be the most common serovars for human infections (Hodges and Day 1987).

The most frequent clinical sign of leptospirosis in humans is a self limiting systemic illness and since most cases present themselves with flu like symptoms the disease is often misdiagnosed or ignored (Leal-Castellanos *et al.* 2003).

In a smaller percentage of cases however, leptospirosis can also cause severe multi-systemic disease characterized by jaundice, acute renal failure and a mortality rate up to 15 % (Levett 2003).

2.1.3 Enzootic Bovine Leukosis

2.1.3.1 The disease

Enzootic bovine leukosis (EBL) is a retroviral infection of cattle and occasionally sheep caused by bovine leukaemia virus which, following a long incubation period, may lead to the development of lymphocytosis and lymphosarcoma in a small proportion of infected animals (Ferrer 1980; Werling *et al.* 2004). Cattle are the natural reservoir of the virus and serve as the immediate source of infection to other susceptible cattle

(Morris *et al.* 1996). Studies of EBL in Denmark showed that the occurrence and spread of bovine leucosis followed the pattern of an infectious disease (Gottschau *et al.* 1990). Clinical manifestations of the disease depend on the organs involved, the rate of tumour growth and the degree of dissemination (Bendixen 1965). The typical clinical picture is one of emaciation, weight loss, anaemia, decreased milk production and enlargement of one or more of the peripheral lymphnodes (Ferrer 1980). As stated above, clinical signs do not always manifest and covert infection, characterized by seroconversion, but no clinical signs is also one of the possible disease scenarios (Werling *et al.* 2004). The main modes of transmission are iatrogenic (syringes, needles, and instruments contaminated with blood) and direct animal contact (Werling *et al.* 2004). It was suggested, that the purchase of infected animals at sales could play an important role in the transmission of the disease (Morris *et al.* 1996), but in another study in Tanzania it was found, that some cattle with high herd sero-prevalence came from remote areas and had not been in contact with the hypothesized risk factor of infected imported dairy cattle. Consequently the author considered the possibility that EBL was already present in indigenous cattle populations (Schoepf *et al.* 1997).

2.1.3.2 Serology and Serological tests

After infection, the production of antibodies against Bovine Leucosis Virus (BLV) grows rapidly and after a maximum of 15 days it generally reaches a level where all diagnostic procedures are effective (Mammerickx *et al.* 1980); (Mammerickx *et al.* 1984). Two serological tests are commonly in use, the agar gel immunodiffusion (AGID) test and the ELISA test. The ELISA is the recommended serological test for this disease, as the AGID, was found not to be sensitive enough to differentiate between the forms of the disease caused by BLV and the other non-infectious causes of

lymphomas (Klintevall *et al.* 1993; Klintevall *et al.* 1991). In a study on cattle, the ELISA test was compared with the AGID test and proved to be very sensitive and also showed to be able to detect diluted antibodies (Mammerickx *et al.* 1984).

2.1.3.3 The Disease in the African Context

The only OIE record of EBL in South Africa dated from 1996 where 182 outbreaks were notified, but as the disease is not under official control and not even notifiable in South Africa, it is under-reported, and thus no reliable figures or details are available for more recent time periods (OIE 1996). In the only South African limited serological survey found in the literature, 3.67% of dairy cows tested positive on farms where EBL had been previously diagnosed. On 3 other farms not associated with the affected farms, the sero-prevalence was 8.64% (Morris *et al.* 1996), but apparently the sero-prevalence can reach up to 90 % in dairy cattle (Werling *et al.* 2004).

A serological study applying the ELISA test in Tanzania found prevalences ranging from 16.2% in a coastal region to 50 % in another region (Schoepf *et al.* 1997). Other serological studies applying the AGID test had yielded rather low infection rates e.g. 4.2 % in Nigerian cattle, 1.5 % in Sudanese cattle, and 7.8 % in Botswana cattle (Schoepf *et al.* 1997).

2.1.4 Babesiosis

2.1.4.1 The disease

Bovine Babesiosis, which is also known as Redwater, is a tick-borne disease caused by the red blood cell parasitizing protozoan *Babesia bovis*, *Babesia bigemina* and other *Babesia* species (Jongejan *et al.* 1988; de Vos *et al.* 2004). The disease plays an important role in the livestock industry of Africa, Asia, Australia, and Central and South America and about 300 million cattle in tropical and subtropical regions of the world were estimated to be at risk of infection with *Babesia bovis* and *Babesia bigemina* (Regassa *et al.* 2003).

Babesia bovis is only transmitted by *Boophilus microplus*, whereas *B. bigemina* is transmitted by both, *Boophilus microplus* and *Boophilus decoloratus* (de Vos *et al.* 2004). Clinical signs occurring in the acute form of the disease are high fever, haemolysis, haemoglobinuria, and in some cases death, as well as nervous signs in the case of infection with *B.bovis*. In general, *Babesia bovis* is thought to be more pathogenic than *Babesia bigemina* or the other *Babesia spp.* (de Vos 1979). The risk of the development of clinical disease is dependent on several factors such as breed and presence of the disease. Calves up to six to nine months of age do show an innate immunity to clinical disease and an acquired immunity occurs at around 6 months of age for *B. bigemina* (de Vos *et al.* 2004; Mahoney and Ross 1972). As Babesiosis is transmitted by ticks, the distribution of the disease is linked to its vector, which is widespread in the tropics and subtropics. The vector *B. microplus* is quoted to have replaced *B. decoloratus* in certain areas and appears to have a competitive advantage over *B. decoloratus*, but tick infection- and transmission rates were stated to be higher

for *B. bigemina* than for *B. bovis* (Estrada-Pena 2003; Tonnesen *et al.* 2004). As a result, *B. bigemina* infections were stated to be usually more prevalent in those herds where both species are present and less readily affected by factors such as climate or management, which reduce tick numbers (de Vos *et al.* 2004). If both the disease and the parasite are present in an area, but no measurable losses or clinical disease occur, the situation is defined as endemic or enzootic stability (de Vos *et al.* 2004; Mahoney and Ross 1972; Norval *et al.* 1983). To achieve an endemic stability, calves have to be exposed to sufficient numbers of infected ticks before losing their innate immunity, a situation that is influenced by many factors such as the level of parasitaemia in the cow ensuring infection of ticks, vector reproduction and survival and subsequent exposure of cattle to infected ticks (Mahoney and Ross 1972). Factors affecting this process are displayed in Figure 20. On several occasions the reduction of regular dipping has led to an increased serological prevalence of babesiosis and development towards enzootic stability - in some cases without an increase in clinical cases (Tice *et al.* 1998) and in other occasions with an increase of clinically affected animals (Norval *et al.* 1983; Rikhotso *et al.* 2005). Although evidence of resistance of indigenous breeds to babesiosis has been quoted to be ambiguous (de Vos *et al.* 2004) a resistance of indigenous Nguni cattle to the vectors has been proved (Spickett *et al.* 1989).

2.1.4.2 Serology and Serological tests

The two serological tests for Babesiosis listed in the OIE diagnostic manual are the indirect fluorescent antibody (IFA) and the enzyme-linked immunosorbent assay (ELISA)(OIE 2004).

Although the IFA test is used to differentiate between *B. bovis* and *B. bigemina*, cross reactions do occur in the form of positive test results for *Babesia bigemina* if infected with *B. bovis* (Bessenger and Schoeman 1983; Echaide *et al.* 1995). Further problems associated with the IFA were that the test is subject to operator error and low throughput (Bidwell *et al.* 1978); (OIE 2004).

In a study evaluating serological tests for the detection of antibodies to *Babesia bovis*, the IFA showed a sensitivity between 92.8% and 100% (Echaide *et al.* 1995). The specificity is also considered to be quite high for the serological diagnosis of *B. bovis* but lower for *B. bigemina* in case *B. bovis* is also prevalent in the tested population due to the above mentioned cross reactions.

An internationally validated enzyme-linked immunosorbent assay (ELISA) for the diagnosis of *B. bovis* infection has been developed (Echaide *et al.* 1995; Molloy *et al.* 1998; Waltisbuhl *et al.* 1987) but is not yet available for *B. bigemina* and consequently present ELISA tests have a poor specificity for antibodies to *B. bigemina* (OIE 2004).

Due to the variation in climatic conditions, a single cross sectional study of seroprevalence of the disease can only serve as an indicator of the probability of endemic stability. Tice 1998 (Tice *et al.* 1998) demonstrated considerable variation between the years in seroprevalence of tick borne disease in South Africa: At one site in their study prevalence varied over three years from 47% to 20% to 96%.

2.1.4.3 The disease in the African context

Bovine babesiosis is widespread in South Africa. While the distribution of *B. bovis* is limited to the higher rainfall areas, including Kwa Zulu Natal because of the limited occurrence of its sole vector *B. microplus* (de Vos *et al.* 2004; Regassa *et al.* 2003), *B.*

bigemina, which is transmitted by both vectors *B. decoloratus* and *B. microplus*, exists throughout most of the country except in the drier parts of the Western and Northern Cape, Limpopo and Western Free State (de Vos 1979). A study on the appearance of ticks in the different altitude zones of Natal found that *B. microplus* was mainly confined to the coastal areas, but also appeared up to 300 m above sea level (Baker *et al.* 1989). The author of the same study found between 80 and 900 ticks per animal in Natal in September and January to April respectively (Baker *et al.* 1989). In a recent survey of calves in Mozambique 39% of all calves tested were sero-positive for *Babesia bovis* (Alfredo *et al.* 2005). A serological study carried out in Zimbabwe found a mean serological prevalence of 32.3% for *B. bovis* and 52.4% for *B. bigemina*. At none of the tested diptanks was an endemically stable disease situation encountered (Katsande *et al.* 1999).

2.1.5 Anaplasmosis

2.1.5.1 The disease

Anaplasmosis in cattle is an arthropod-borne disease caused predominantly by *Anaplasma marginale* and is caused by obligate intra-erythrocytic rickettsial organisms of the genus *Anaplasma* (Potgieter and Stoltz 2004). *A. marginale* is distributed worldwide in tropical and subtropical regions of Europe, Africa, Asia and Australia and has been shown to be transmitted to cattle by a variety of 19 different ticks (Kocan *et al.* 2004). The ticks considered to be involved in the epidemiology of anaplasmosis in South Africa are *Boophilus decoloratus*, *Boophilus microplus*, *Rhipicephalus simus*, *Rhipicephalus evertsi evertsi* and *Hyalomma marginatum rufipes* (Mbatia *et al.* 2002)

(Potgieter and Stoltz 2004), but mechanical transmission by instruments and biting insects is also known to occur (Potgieter and Stoltz 2004). Although *A. marginale* has a global distribution, the prevalence and incidence are highest in regions where the tropical cattle fever tick *Boophilus microplus* is endemic (Futse *et al.* 2003). The clinical disease is generally characterized by fever, progressive anaemia and icterus, as well as weight loss, and death (Potgieter and Stoltz 2004; Reyna-Bello *et al.* 1998). The severity of clinical signs increases with age, but similar as described above for babesiosis, calves under 6 months are resistant to clinical disease and cattle that were raised in endemic areas do develop a naturally acquired immunity (Potgieter and Stoltz 2004). Persistent infections develop in ticks and mammalian hosts which can consequently serve as reservoirs (Kocan *et al.* 2004).

2.1.5.1 Serology and Serological Tests

A complement fixation test (CFT) and several ELISA tests are commonly in use for the serological diagnosis of anaplasmosis. While the CFT is considered of an unacceptably low sensitivity in persistently infected cattle (OIE 2004), the competitive, indirect or dot ELISA tests appear to be of high sensitivity (Nielsen *et al.* 1996; Reyna-Bello *et al.* 1998). Also internationally approved is the IFA test (OIE 2004). Antibodies in calves infected with the agent were shown to be detectable after 20 days and reached a maximum level at 28 days post infection (Reyna-Bello *et al.* 1998)

2.1.5.1 The disease in the African context

Anaplasmosis is present in most cattle farming areas in southern Africa (Potgieter 1979; Potgieter and Stoltz 2004) and has been shown to be widespread in Kwa Zulu Natal (Du Plessis *et al.* 1994). A study in Mozambique produced quite variable findings

of sero- prevalence in cattle ranging from 34 to 87 % (Alfredo *et al.* 2005). In Zambia anaplasmosis was found to be present throughout with sero-prevalences between 75 and 86 % (Jongejan *et al.* 1988).

A study investigating tick prevalences at different altitude levels in the south of Natal, found *R. appendiculatus* and *R. evertsi*, which are recognized vectors of the disease, in appreciable numbers at all altitude levels up to 1500 m, while *B. decoloratus* and *B. microplus* occurred more at lower altitude levels. *Rhicicephalus* numbers were found to be higher on commercial farms, which was hypothesized to be due to a higher occurrence of scrubs. The presence of vector *R. simus* was consistently low in the study area (Baker *et al.* 1989).

2.2 Survey design and sample size calculation

2.2.1 Introduction

Surveys are a common way of information collection and in the veterinary field they are frequently conducted at various geographical levels ranging from local to national. The objectives of veterinary surveys are usually to determine the prevalence of a specific disease, prove the absence thereof to trade partners, or to detect an exotic disease. The difference between surveys compared to so-called passive surveillance is, that here data is collected in an active, structured fashion. Conducting a survey for any of the above objectives, consists of three stages: the first being the calculation of the required sample size, the second the practical process of sample collection and the third the interpretation of the results. To get the most value from the collected data, statistical

requirements need to be satisfied during the process of designing and conducting a survey.

2.2.2 Methods

As in most situations when a survey is conducted, it is not feasible to sample the complete population; most surveys rely on a sample taken from the population defined through a sampling scheme. The validity of any sampling scheme is based on the assumption, that a population is divided into representative subunits from which characteristics of the population can be estimated (Thrusfield 2005). When a sample is taken the result is always subject to uncertainty (Cameron *et al.* 2003). Uncertainty can arise from the sampling design, from diagnostic tests used and from normal random variation. Bias is introduced if the units sampled are not representative of the population. A balanced sampling design attempts to minimize bias and by this derive a good estimate that represents the population on which the survey was conducted. The precision of the estimate from the survey is a measure of how variable the estimates of repeated samples would be. This means that if a survey with the same design were to be conducted again and again, the results are unlikely to be exactly the same. Precision expresses how different the results of these repeated surveys would be, and is usually expressed by the 95 % confidence interval which states, below or above or between which value 95% of the results of surveys with the same design should fall (Cameron *et al.* 2003). The number of samples collected has the most influence on the precision of the estimate. To calculate the required sample size for a survey many approaches exist - ranging from a very simple formula to complex simulation: In 1982 Cannon and Roe

published a manual for veterinarians, which has found widespread application (Cannon and Roe 1982). The stated sample size, calculated by the approximate formula, depends on the estimated prevalence in the population, the confidence level desired and the size of the population. The formula underlying the presented tables is as follows:

For the detection of disease (as taken from Thrusfield (2005)):

$$n = (1 - (1 - p)^{1/d}) (N - d/2) + 1 \quad (\text{Eq 1})$$

Where N is the population size, d the minimum number of affected animals in the population and p the probability of finding at least one case in the sample.

For the estimation of prevalence:

$$n = ((1.96)^2 \times p(1-p)) / L^2 \quad (\text{Eq 2})$$

Where p is an estimate of the prevalence of the disease, n is the number of units that need to be sampled, 1.96 is the value corresponding to a 95% confidence interval and L is the allowable error.

The appeal of this approach is its simplicity. However, this approach assumes that test sensitivity is perfect, that the population the samples are taken from is infinite and that samples are collected on a random basis (Cameron and Baldock 1998a).

Perfect diagnostic test sensitivity and specificity hardly ever occur and for this it is important to gather knowledge on the test performance and integrate it into the design

of health surveys. A formula, based on the normal approximation of the binomial distribution, exists that does allow the integration of test sensitivity and specificity (Rogan and Gladen 1978); (MacDiarmid 1988).

For sample size determination for prevalence surveys this formula has been adapted as follows:

$$n = \frac{(1.96/d)^2 \times ((Se \times P) + (1-Sp)(1-P)) \times ((1-Se \times P) - (1-Sp)(1-P))}{(Se+Sp-1)^2} \quad (\text{Eq 3})$$

Where n is the required sample size, d the precision, Se the test sensitivity, Sp the test specificity, P the estimated prevalence and 1.96 the value corresponding to a 95 % confidence interval (Humphry *et al.* 2004; Thrusfield 2005). All the above formulae are based on the approximation to the binomial distribution and the population for sampling is assumed to be infinite. This is because the binomial distribution assumes sampling with replacement, meaning that an animal that was selected can be selected again. To overcome this, and to extend this method for the application where populations are not assumed to be infinite, Cameron and Baldock presented a probability formula for the calculation of the sample size for surveys to prove freedom from disease, that is based on the hypergeometric distribution (Cameron and Baldock 1998a). This formula calculates the exact probability of detecting diseased animals in surveys designed to prove the freedom from disease:

$$P (T^+ = x) = \sum_{y=0}^d \frac{\binom{d}{y} \binom{N-d}{n-y}}{\binom{N}{n}} \sum_{j=0}^{\min(x,y)} \binom{y}{j} Se^j (1-Se)^{y-j} \binom{n-y}{x-j} x (1-Sp)^{x-j} Sp^{n-x+y+j}$$

(Eq 4)

Where Se stands for test sensitivity, Sp for test specificity, T^+ for test positive animals, $P()$ for the probability of the event in brackets, x for the number of T^+ in a sample, y for the number of diseased animals in the sample, n for the sample size, N for the population size and j for the number of true positives.

However, the above presented formula is quite complex to be used in practice and the authors also present a more computationally convenient approximation to this formula, which performs well in most circumstances.

To facilitate calculation of sample sizes for surveys for disease freedom, the authors put their above work in the practical application of a software program named Freecalc, which calculates the sample size that produces a given probability by a process of trial and error (Cameron and Baldock 1998a; Cameron and Baldock 1998b).

Humphry *et al.* 2004 published the formula described in (Eq 3) where he uses the formulae described by Cameron and Baldock (Cameron and Baldock 1998a) to calculate the sample size required for a prevalence survey at herd level, rather than a survey intended to substantiate disease freedom (Humphry *et al.* 2004). He uses the equation described in (Eq 3) to determine the number of herds required to be sampled for an estimated prevalence and takes the test imperfection into account. The animal sample size for each herd is then determined by applying Freecalc for a desired herd

sensitivity and specificity. Application of this method shows that if ignoring herd sensitivity, the calculated sample sizes are much lower.

To account for finite populations the hypergeometric distribution is used here rather than the binomial distribution at the within herd level. However, a limitation of this approach is that it is not applicable to small herds and the authors suggest that only large herds should be selected for inclusion in the survey (Humphry *et al.* 2004).

All the above described methods except the last one, assume that samples are collected on a random basis and violations of this assumption invalidate the results of the formulae (Cameron and Baldock 1998a). Besides this the above methods are only applicable to the scenario, where the determined sample number is taken as a simple random sample from the whole population. However in the veterinary scenario this is not often possible as animals are aggregated in, for example herds, or other units and taking a random sample from the population is rarely feasible as this implies maintaining a register of each animal. To take this into consideration sampling schemes such as cluster sampling or multi-stage sampling exist. In a multi-stage sampling scheme the higher unit of aggregation i.e. diptanks is chosen in a process of random selection and subsequently the determined number of animals to be sampled from these units is established. To account for this sampling design, the number to be sampled is often doubled in practice (University of Pretoria 2003), but if data on the total number of animals in the population as well as the total number of cluster units is known, then formal ways of adapting the sample size are available (Thrusfield 2005).

Cameron and Baldock published the application of the formula presented in (Eq 4) to two-stage sampling, considering that collection of samples is likely to cluster at herd level (Cameron and Baldock 1998b). This concept works on a herd-level prevalence

basis, considering the classification of a herd as diseased or not as a diagnostic test itself. Parameters required for the calculation of the sample size are the test sensitivity and specificity, the prevalence amongst herds and the prevalence within herds, the desired confidence and the total number of herds in the population. The number of required herds and required animals to be tested can then be calculated in two steps.

2.2.3 Analysis of survey results

The apparent prevalence derived from the survey, which is very plainly the number of test positive animals over the total number of animals tested, does not take the possibility of false positive and false negative test results into account and does not consider any uncertainty of the survey result. For the calculation of the underlying ‘true’ prevalence, the most simple formula adjusts for the sensitivity and specificity of the diagnostics test and calculates the result from the number of positive animals out of all the animals tested and the known test sensitivity and specificity (Rogan and Gladen 1978):

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1} \quad (\text{Eq 5})$$

Where TP is the true prevalence, AP the apparent prevalence, Se the test sensitivity and Sp the test specificity. This formula though can produce negative results if the apparent prevalence is very low and the test specificity below 100 %. Normally negative values are corrected to zero (Audige L. *et al.* 2003). The amount of sampling variation is related to the numbers of samples taken and is an important factor of uncertainty when estimating the prevalence of a disease. Even in a balanced sample design the real prevalence is unlikely to be exactly the previously estimated prevalence, so results from

a survey should be reported with confidence intervals (Thrusfield 2005). In a simple random survey design, the normal approximation to the binomial distribution can be used for this purpose:

$$Pe - 1.96\sqrt{\frac{Pe(1-Pe)}{n}}, Pe + 1.96\sqrt{\frac{Pe(1-Pe)}{n}} \quad (\text{Eq 6})$$

Where Pe is the apparent prevalence and n the number of animals sampled. If the survey was conducted using a two stage sampling design, the variance increases once more as ignoring the clustering of disease at different aggregation levels leads to a violation of the assumption of independence and by that to a downward bias of the variance (Wagner B. *et al.* 2003). Thrusfield (Thrusfield 2005) accounts for this by applying the following formula instead of the confidence intervals described above:

$$Pe - 1.96\left\{\frac{c}{T}\sqrt{\frac{V}{c(c-1)}}\right\}, Pe + 1.96\left\{\frac{c}{T}\sqrt{\frac{V}{c(c-1)}}\right\} \quad (\text{Eq 7})$$

$$V = Pe^2(\sum n^2) - 2Pe(\sum nm) + (\sum m^2) \quad (\text{Eq 8})$$

Where Pe is the apparent prevalence, c the total number of clusters in the samples, n the number of animals sampled in each cluster, and m the number of diseased animals in each cluster. T is the total number of animals in the sample. However this approximate confidence interval is valid for a distribution of prevalences at cluster level that follow a normal distribution, or at least assimilates a normal distribution only (Thrusfield 2005).

In 1993 Donald introduced an empirical estimator for the herd level prevalence, which in this Kwa Zulu Natal study equates to the diptank level prevalence, taking into account the diagnostic test sensitivity and specificity, as well as clustering (Donald A. 1993). Cluster and population size need to be fixed to estimate the cluster prevalence here. Another parameter required in order to apply this method is the intra-cluster correlation coefficient for the probability that an animal is infected as well as for the sensitivity and specificity of the test. The cluster coefficient describes the probability that a second element drawn from a positive cluster is also positive given that the first tested element was positive (Donald A. 1993). The requirement of the cluster coefficient for the sensitivity and specificity was criticised by Jordan *et al.* (1998) as being not very intuitive for veterinarians and lacking published information (Jordan and McEwen 1998). To evaluate if a region is likely to be disease free, Cameron and Baldock have implemented the exact probability formula described above in Eq 4 in the software programme Freecalc (Cameron and Baldock 1998a; Cameron and Baldock 1998b). The programme tests against the null hypothesis, which in this case is that the disease *is present* in the population. It then calculates the exact probability of observing the present survey result if the disease was present at an assumed minimum prevalence. If this probability is below 5%, the null hypothesis that the disease is present can be rejected at the 95% confidence level and the alternative hypothesis, that the region is disease free can be accepted. The program allows for the integration of test sensitivity and specificity and the underlying population size. Required inputs for the implementation are the sample size, the number of animals that tested positive; the minimum expected prevalence, the desired Type I and Type II errors, and the test sensitivity and specificity. Bayesian methods, that allow for the integration of

uncertainties about any input parameters can be used if some prior information on these parameters are available from the literature, from previous studies or from expert opinion. Using stochastic simulation with many iterations, these approaches use a probability distribution rather than a definite input value for the parameters and sample from these various prior distributions to derive a posterior value for the outcome variable. Modern computer technology has made these approaches feasible. Credible intervals can be produced from their output, which differ from confidence intervals in that they can be directly probabilistically interpreted.

Branscum *et al.* (2004) described two such Bayesian approaches previously developed by Hanson *et al.* (2003) for a cross sectional sampling design with the scenario where individual animals were tested within a single herd with a single diagnostic test with binomial and hypergeometric sampling and for the scenario of estimating the herd level prevalence under binomial sampling (Hanson *et al.* 2003a; Hanson *et al.* 2003b). The difference between binomial and hypergeometric sampling is that the virtual sampling performed when the model is run is carried out with replacement in the application of the binomial sampling, meaning an animal that has been selected and whose result has been recorded is placed back and has a chance of being selected again. The hypergeometric sampling works without replacement. Consequently the binomial sampling is only applicable to situations where the animal sample collected is much smaller than the animal numbers at the herd/diptank where it is collected from. Uncertainty about the test sensitivity is accounted for by using a beta distribution instead of a fixed value.

The prevalence of a disease at the animal or herd level can also be modelled by using a beta distribution function. The beta distribution is the conjugate prior to the binomial

likelihood function in Bayesian inference and as such is often used to describe the uncertainty about the probability of the occurrence of an event, given a number of trials n and a number of successes (Vose 2001).

$$P = \text{Beta}(s+1, n-s+1) \quad (\text{Eq 9})$$

In the scenario of a survey, s stands for the number of animals tested positive and n for the number sampled. A special computer program though is needed to run these distributions. The use of this distribution allows for the display of uncertainty of the result related to the sample size. However in the use as described above, the test imperfection is not accounted for. One extension to integrate this is to use the beta distribution in the following way (Decision Analysis Risk Consultants 2000):

$$\text{Beta}((p/Se) + 1, n - (p/Se) + 1) \quad \text{EQ 10}$$

Where p is the prevalence and Se is the diagnostic test sensitivity.

The disadvantage of this approach is, that it does not consider test specificity and assumes it to be 100 percent, which leads to an estimate, where no false positives occur. This can be a problem in the situation where a country is trying to prove freedom from disease but has false positive reactions. Another feature important in the attempted proof of absence of disease is that the distribution is bound between 0 and 1 and consequently the prevalence resulting from it cannot be 0. Because of this it was proposed (Branscum *et al.* 2004) to use the above mentioned approach by Hanson (Hanson *et al.* 2003a; Hanson *et al.* 2003b), using a mixed model to allow for the

outcome that a herd is not infected. This is done by modelling the prevalence with a beta distribution with the probability that a herd is infected and a zero prevalence in the herd with $1 -$ the probability that a herd is infected. However, this approach needs input priors on the probability of herd infection and an estimate of infection prevalence in the case of infection that has to be retrieved from previous surveys or expert opinion and consequently its value depends on the quality of available input information.

Suess *et al.* (2002) describes a similar Bayesian model for the determination of the within herd prevalence and the proportion of infected herds within a country and illustrates it using the example of Newcastle disease in Switzerland. Here the animal level test outcomes are modelled with the Bernoulli distribution and instead of using a cut-off value to declare a herd as infected or not in the model, the underlying populations of infected and not infected herds are simulated. Similar input parameters as for the method of Branscum *et al.* (2004) above are required. One assumption in their model is that an equal sample size of animals has been collected in each herd.

2.3 Geographic Information Systems (GIS) and spatial analysis

A GIS system has been defined as an “automated systems for the capture, storage, retrieval, analysis and display of spatial data.”(Clarke *et al.* 1996). A GIS system consists of a database with spatial references to the collected data often retrieved through the use of a global positioning system (GPS) and spatial or map information. For the display of the collected data, these tables need to be linked within the system. In

the last 20 years through the increasing availability of the required technical equipment and data, the use of GIS has increased drastically and by now plays an important role in the public and animal health sector. Maps of disease data are used to generate new hypotheses about disease risk factors and to identify areas of high risk, so that action can be taken in disease control (Lawson *et al.* 2000), or, in the scenario of an outbreak, they can help to indicate the extent and spread of disease (Moulton *et al.* 1994). Examples of their application in the veterinary field are the display of surveillance information, assessing the spread of disease within wildlife populations, detection of space and time interaction and clusters, prospective surveillance of endemic diseases, modeling of infection and possible control of diseases and risk factor analysis (Smith 2006).

Spatial analysis has been categorized into visualization, exploratory data analysis, and model building. These range in complexity from simple map overlay operations to statistical models such as spatial interaction and diffusion models (Clarke *et al.* 1996). The three different tasks will be described in more detail below.

Visualization and display of collected information in maps allows an overview of the data that could not be gained just by the assessment of a data table, but as only a limited part of the information available can be displayed in one map the method chosen to represent disease intensity on the map -colour scheme or symbolic representation - can dramatically affect the resulting interpretation of disease distribution and should therefore be carefully considered (Lawson *et al.* 2000).

Regarding the symbolic representation, data that is available in point form, can be directly displayed as such, allowing the user to identify clusters of points easily by visual inspection in case the results were gained from reporting. Displaying the information in this way though does not take the underlying population into account, and for this might give a false impression of clustering, where in fact only the population itself is clustered. Besides one should consider that if the data is available in a point format (as it is often the case when, as in this KZN study, the diptank location is recorded by means of a GPS), but is in fact area data (because the herds served by a diptank originate from a much larger area), the point data should be transformed into area data for more accurate display. This can be achieved by creating a buffer zone around the points, if it can be assumed that all points have the same size of 'catchment area'. If this cannot be assumed, then Thiessen Polygons can be applied to transform the data. Thiessen Polygons are constructed by determining the bisector of straight lines joining pairs of points. Due to this though, Thiessen Polygons cannot take into account non-agricultural land (Sanson 2004) and consequently require manual 'clipping' of areas that do not represent the actual catchment area of a location.

The display of the area data then is most frequently realized in choropleth maps. In a choropleth map, the data values that fall within a specific class interval are assigned a unique colour, shade or pattern. A key issue in choropleth maps is the choice of intervals as changing the interval scheme can fundamentally change how the map looks (Cromley and McLafferty 2002). For this, one can choose between dividing the data in equal intervals, create classes that represent equal frequencies of the measured values, or apply a method that searches for natural breaks in the data. In sampling situations

where the samples were not obtained in a structured survey but by means of case reporting or scanning surveillance, one would often like to display areas of high unusual risk. For this type of visualisation though, several problems and potential biases need to be considered: One is that the population at risk needs to be accounted for in displays of rates of disease. Another is, that the number of holdings within administrative districts may vary and small populations will produce more unstable risk rates than larger populations, if plain rates are used. Alternatively, if instead of rates the significance of the difference from the observed risk to the expected risk is mapped, only areas with large populations are able to produce significant results (Xia and Carlin 1998). This problem is addressed by smoothing the rates, most simply by localised averaging, and in the most complicated form by integrating the information into complex models. The Standard morbidity ratio (SMR) calculates an expected disease rate based on attributes of the population and compares it to the expected value. However, SMR's do not perform well for rare diseases, because there will be a near zero expectation and they do not account for the lower information value from small local populations (Lawson A.B. 2005). Bayesian smoothing methods work for aggregated data and are a bit 'smarter' in the sense that they do not just work on a local weighted average, but differentiate between the types of information available: the posterior distribution of risk is estimated from a weighted combination of observed data such as the local risk and prior information such as the neighbourhood risk. Essentially, it is supposed that the observed spatial variation of measures consists of an underlying spatial trend and local variation or error. The aim is to make the underlying trend more visible by smoothing local variation. The relative weights given to the local data and the prior information depend on the local population: if the local population is large the local data will receive a

stronger weighting in the calculation process than the neighbourhood data. If the local population is small, the weighting will be smaller and the rate will be more strongly shrunk towards a mean. The particular choice of mean can be decided by the user: If the estimate is shrunk towards the mean of neighbouring regions the shrinkage is called ‘local’; if the rate is shrunk towards the overall mean the shrinkage is called ‘global’ (Pfeiffer 2004). Weighting by local population size allows Bayesian estimates to cope with small or zero local populations at finer scales, unlike SMRs (Clayton D.G. and Bernardinelli L. 1992).

Spatial exploratory analysis or cluster analysis attempts by means of hypothesis testing to detect spatially abnormally distributed rates and test for their significance. These methods can either test for these rates throughout the study region (global cluster tests), localise clusters (general cluster tests), or test if there are elevated rates in the vicinity of a hypothesised risk source (focused cluster tests) (Besag J. and Newell J. 1991). To test for abnormal rates, cluster tests need to account for the spatial distribution of the population at risk and require either spatially referenced data on the population at risk or suitable control data. These tests were initially developed for non-infectious chronic diseases and when applying and interpreting these tests in the infectious disease surveillance setting one should consider that a ‘significant cluster’ might only confirm that the disease is infectious. The term significant also loses its meaning in this setting in the sense that one can expect an infectious disease to cluster, unlike the distribution of cancer. However, cluster tests are valuable screening methods, ideally combined with an adjusted disease map, but significant findings should be accompanied by an epidemiological investigation (Pfeiffer 2004). Examples of cluster tests include the The

K-function (global test) (Diggle and Morris 1996) Cuzick and Edwards test (general)(Cuzick J. and Edwards R. 1990), Kulldorf's scan statistic (Kulldorff *et al.* 2005) (general) and Stone's test (Stone RA. 1998).

Modelling of spatial data, the third task of spatial analysis attempts to explain or predict the occurrence of disease. These models can be constructed from a set of spatially referenced data on cases and controls or case reports and the underlying population at risk as well as spatial data of potential risk factors (Pfeiffer 2004). Two main methods for the creation of risk maps are available : Generalized linear mixed models (GLMM) and Bayesian estimation methods (Pfeiffer 2004). When applying these models, it is of great importance to obtain all spatial data at the same or very similar spatial resolution as otherwise spurious associations can be identified (Pfeiffer 2004).

In summary, initial visualization of surveillance data is very valuable to get an overview of disease occurrence and formulate hypotheses regarding its occurrence. Initial visualization can be achieved with any spatially referenced case or survey data, some basic mapping data and relatively straightforward manipulation. Further exploration though requires either spatial information on the population at risk, or suitable controls as well as spatially referenced data on suspected risk factors which either needs to be collected with the disease data, or obtained from other sources from which it is often very difficult or expensive to obtain. However such data is becoming increasingly available and obtaining it might not be an obstacle anymore in the future.

3 MATERIALS AND METHODS

3.1 The Survey

3.1.1 Survey design

Samples of the provincial survey were taken from March 2001 until December 2003. At the time of the survey the province of Kwa Zulu Natal was divided into 52 magisterial districts according to the existing political boundaries. This division has been applied for the design of the survey and required sample sizes were calculated on a district basis. Since rural communities are not present in all the districts, only 33 districts were included. These districts are displayed in Figure 1. Diptanks were the primary sampling unit and were selected on a random basis. The sampling design intended that animals were also selected on a random basis provided their owners approved of the collection of samples. Samples were only taken from fully-grown animals and no stratification according to age or sex was applied. Table 1 displays the total number of animals and diptanks sampled. Details of the number of diptanks and animals sampled in each district can be accessed through the Appendix for each disease.

Table 1 Number of animals and diptanks sampled in the 2002/03 KZN survey

Number sampled:

Diptanks	Cattle	Pigs	Sheep	Goats	Donkeys	Horses
446	46025	916	838	4092	975	652

- 5) Details of vaccinations and parasite control measures presently in use and the role of traditional medicines
- 6) Perceived health problems of owned animals and related management problems
- 7) Health problems in cattle
- 8) Most frequent management problems

3.1.2 The survey conduction

Animal health technicians collected the serum samples at the selected diptanks. The animals were bled according to standard procedure from the jugular vein and the samples returned to Allerton Laboratory. The samples when received from the field, were logged and identified, centrifuged for serum, placed into cryo/micro tubes, and placed in the fridge at 4 degrees until dispatch or testing.

For enzootic bovine leukosis, anaplasmosis, babesiosis and leptospirosis, the samples were sent to the Onderstepoort Veterinary Institute (OVI) for testing, which was completed once a month. Testing for brucellosis was conducted at the Allerton Laboratory once a week. Standard laboratory methods were used for analysis. The diagnostic tests applied and their references are displayed in Table 2.

Table 2: References for the diagnostic tests carried out on samples collected during the 2001-2003 KZN survey

Disease	Reference
Bovine Anaplasmosis	ELISA from Illry commercial test kit, based on the following reference: (Visser <i>et al.</i> 1992)
Bovine Babesiosis	IFA test based on: (Leeflang and Perie 1972)
Enzootic Bovine Leukaemia	Idexx commercial test kit, based on: (Miller <i>et al.</i> 1981)
Brucellosis	RBT based on: (Herr <i>et al.</i> 1979) CFT: (Herr <i>et al.</i> 1985)
Leptospirosis	MAT (no further info available)

For the conduction of the questionnaire survey, the thirty-two AHT's employed by Veterinary Services were given a day of training before the survey and were instructed to conduct the interviews personally in Zulu. Answers were recorded on a data capture sheet. Since livestock owners were approached during the routine dipping, the sampling method constituted a convenience sample. Completed questionnaires were send to VS in Allerton for storage and collation. In total 315 questionnaires were returned.

3.1.3 Analysis of the survey

3.1.3.1 The information used

Once all samples had been tested, the results were entered into the spreadsheet program Excel and Access (Microsoft Corporation 1997). Results at diptank level were linked with a database on the geographic location of diptanks, which was collated by the State Veterinary Services in Kwa Zulu Natal. As not all diptanks recorded in the results tables could be identified in the GIS database, only those diptanks that could be matched with certainty in the GIS database were used for the creation of diptank maps. Information captured on the 315 questionnaires was entered into and analysed with the software program Epi-Info (CDC Atlanta 2005).

3.1.3.2 Processing and analysis

All maps were created in ArcView 3.3 (Esri Redlands 2002). As the density of the distribution of diptanks throughout the study area varied and with that their catchment area, it was decided to use Thiessen Polygons for a more accurate display of the diptank area, using the spatial analyst extension of ArcView (Esri Redlands 2002). As areas that do not have neighbouring diptanks (because there is no communal agricultural land in that area), become artificially enlarged using this procedure, the polygons concerned were 'clipped' manually and by that reduced in size. Figure 2 illustrates Thiessen Polygons that were derived from point data before clipping.

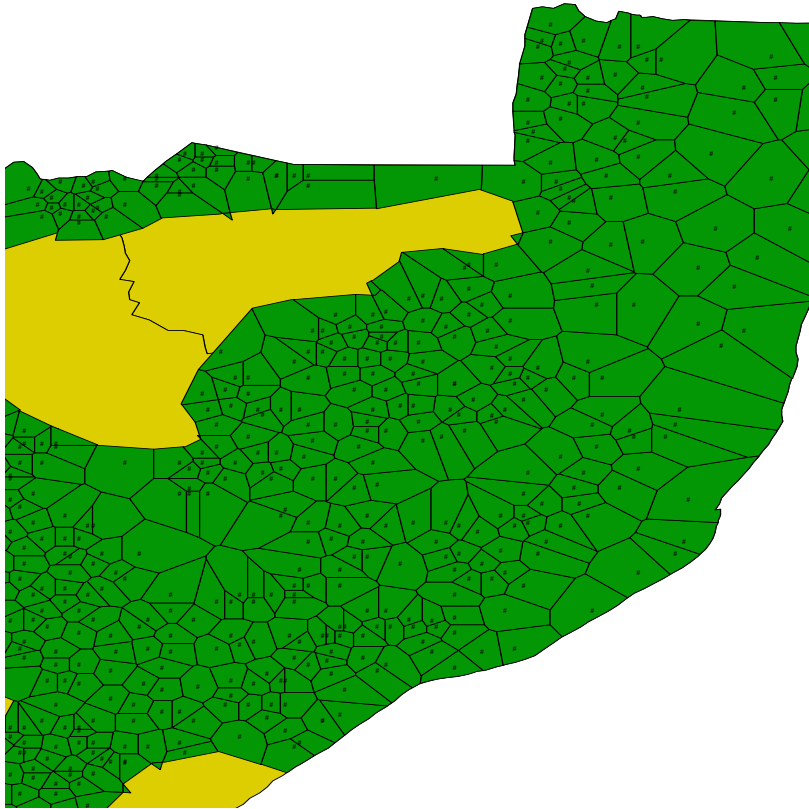


Figure 2 Thiessen Polygons derived from point data of diptanks collected during the 2001-2003 KZN survey. (Diptank point location displayed as dots with surrounding lines showing the Thiessen Polygons)

Calculation of the district-level prevalence was carried out using the Excel add-in @Risk (Palisade Corporation New York 2005). The prevalence at district level was calculated in the following way:

First the apparent prevalence was adjusted for the test sensitivity and specificity using the formula presented in Eq 5 above. Negative results were adjusted to 0 and results above 1 to 1. To allow for uncertainty around the sensitivity (Se) and specificity (Sp) of the diagnostic test, these values were entered into the formula as a probability distribution. The assumed test sensitivities and specificities and the utilized distributions are displayed in Table 3 below.

Table 3 Probability distributions and their references applied for the adjustment of prevalence

Disease	Distribution	Reference
Bovine Anaplasmosis	Se ELISA: Triangular (0.93, 0.96, 0.99) (min, most likely, max)	(Morzaria <i>et al.</i> 1999) (Van Donkersgoed <i>et al.</i> 2006)
	Sp ELISA: Triangular (0.9, 0.94, 0.98)	(Nielsen <i>et al.</i> 1996) (Reyna-Bello <i>et al.</i> 1998)
Babesia bigemina	Se IFA: Triangular (0.95, 0.97, 0.99)	C. Katsande, pers. comm.
	Sp IFA: Triangular (0.8, 0.85, 0.9)	(Echaide <i>et al.</i> 1995)
Babesia bovis	Se IFA: Triangular (0.95, 0.97, 0.99)	C. Katsande, pers. comm.
	Sp IFA: Triangular (0.95, 0.97, 0.99)	
Enzootic Bovine Leukaemia	Se ELISA: (Triangular (0.86, 0.97, 0.98)	(Monti <i>et al.</i> 2005)
	Sp ELISA: (Uniform (0.96, 0.975) (min, max)	(Buzala and Deren 2003) (Trono <i>et al.</i> 2001)
	Sensitivity CFT (Se1): Beta (61, 13)	
Brucellosis	Sensitivity RBT (Se2): Triangular (0.688, 0.81, 0.936)	(Paweska <i>et al.</i> 2002) (Gall and Nielsen 2004)
	Combined sensitivity: (Se1xSe2)	(Stemshorn <i>et al.</i> 1985)
	Specificity RBT: Beta (1052, 1)	
Leptospirosis	Sensitivity MAT: Uniform (0.9, 0.98)	(Chappel <i>et al.</i> 1992)
	Specificity MAT: Uniform (0.9, 0.98)	(Bajani <i>et al.</i> 2003)

Eq 7 and Eq 8 were used to derive 95% confidence intervals adjusted for the clustering effect that has occurred through the two stage sampling design of the survey. However, instead of the apparent prevalence, the true prevalence was inserted into the formula. A

simulation of 10 000 iterations was then run for the point values of the lower and upper confidence interval in Eq. 7 respectively and the lower 95% confidence interval of the simulation output for the lower 95% confidence interval point of Eq. 7 was used as the lower 95% confidence interval displayed in the Appendix. The same was done for the upper confidence interval, just that here the upper 95% confidence interval of the simulation was chosen. The maps were produced using the mean true prevalence and the upper 95% confidence intervals in the case of brucellosis, leptospirosis and EBL and the lower 95% confidence interval for the tick borne diseases. The lower confidence interval for tick borne diseases was chosen as here the worst-case scenario is a situation of low serological resistance to the diseases and hence is better reflected by the lower confidence interval. The intention of displaying the confidence intervals was to give an impression of the possible 'worst case scenario'. A map was produced for each disease displaying the animal prevalence at diptank and at district level. Further maps were produced to display the number of diptanks sampled in each district. For districts where no serological reactors were discovered, calculations were carried out in Freecalc (Cameron and Baldock 1998a; Cameron and Baldock 1998b) described above to evaluate how certain one can be regarding the absence of the disease. The software programme Freecalc bases its calculations on the formula described in Eq (4) and allows for integration of test sensitivity and specificity. These calculations were done assuming a minimum prevalence of 1 percent in the case the disease was present.

Uni- and bivariate descriptive statistical analysis was used to analyse the collected information from the questionnaire survey by making use of Epi Info (CDC Atlanta 2005). The results were analysed together and for each surveillance zone separately, and

whenever there was a significant difference in the results between the individual districts and the overall results, it is mentioned.

4 RESULTS

4.1 Results of the serological survey

The results are presented in the form of maps and/or summarized tables. For more detailed tables the reader is referred to the Appendix. All district prevalence maps show the mean true prevalence and the upper 95% confidence interval (except for the tick-borne diseases where the lower 95% confidence interval is displayed) after adjustments for clustering, diagnostic test sensitivity and specificity and uncertainty around these parameters have been made.

4.1.1 Bovine Brucellosis:

4.1.1.1 The prevalence at district level:

The number of diptanks sampled and the number of animals sampled in each district are displayed in Figure 3. The number of animals sampled is reflected by the colour shade of the map and the number of diptanks sampled is displayed as written labels on the districts. Figure 4 shows the mean true prevalence and the upper 95% confidence interval (CI) of the true animal prevalence at district level. Figure 4 shows that bovine brucellosis is most prevalent in the north-eastern areas of the province, the highest mean prevalence found was 15.6% and the highest upper 95% CI was 28%, while apparently absent from most of the rest of the province.

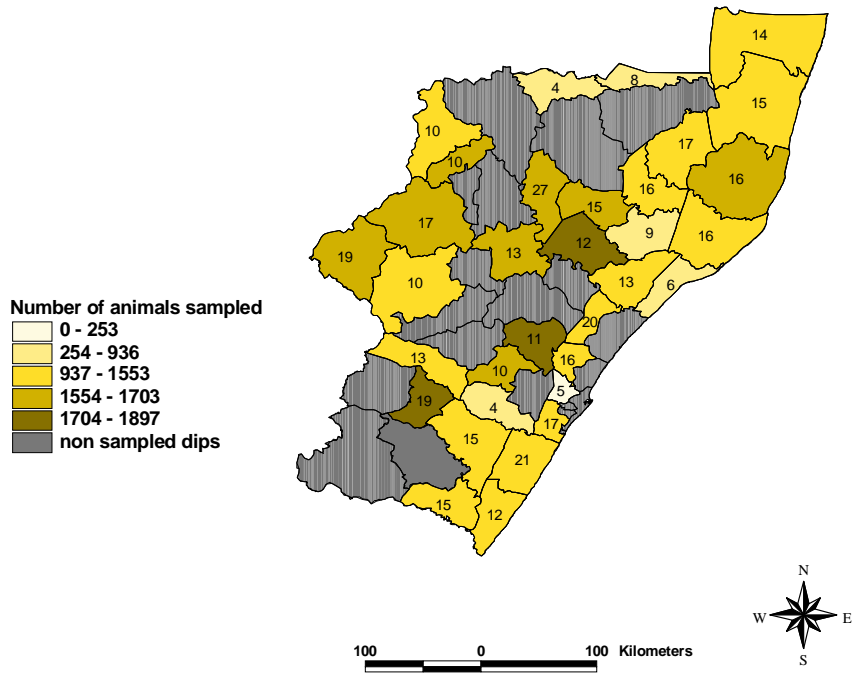


Figure 3 Number of animals (colour intensity) and diptanks sampled (actual number) for bovine brucellosis in the serological survey of KZN 2002/3

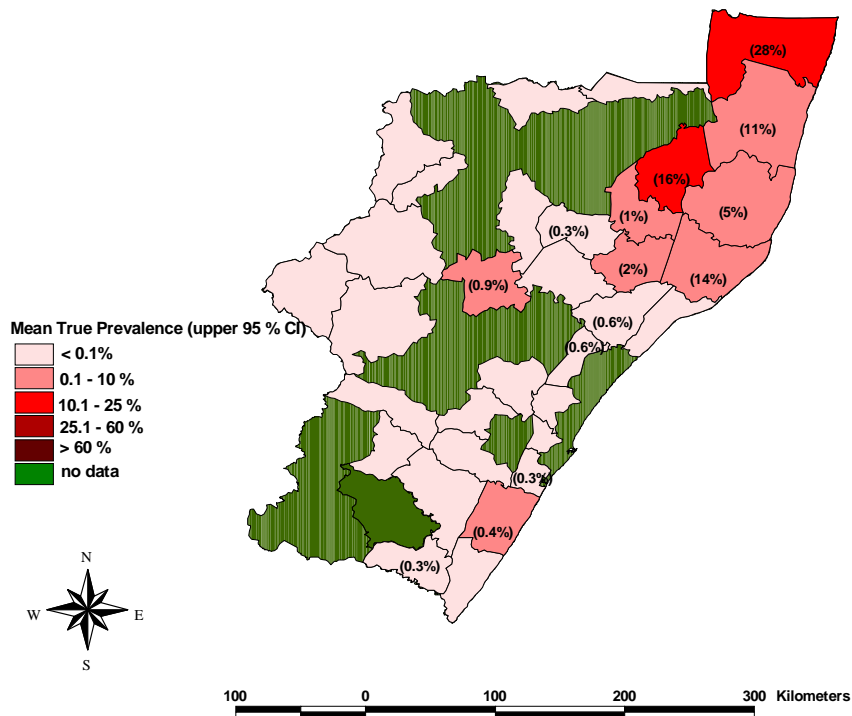


Figure 4 Mean (colour) and upper 95% confidence interval (label) of the true animal prevalence of bovine brucellosis in the serological survey of KZN 2002/3 at district level

No serological reactors occurred in the districts listed in Table 4. To evaluate how certain one can be regarding the absence of the disease in these districts, calculations were carried out in Freecalc as described in the materials and methods section. With the exception of two districts where the sample size was too low, one can be 99% confident, that the districts without serological reactors have a prevalence of less than 1%.

Table 4 The probability of districts in KZN with an apparent prevalence of brucellosis of zero having a true prevalence of more than 1% in 2002/03

Name of district	Population size	Sample size	Probability of the true prevalence being more than 1%
Bergville	32105	1650	<0.01
Dannhauser	11054	1631	<0.01
Estcourt	25118	1500	<0.01
Ixopo	29580	1500	<0.01
Klip River	47730	1672	<0.01
Mpendele	24433	1300	<0.01
Mtunzini	9462	809	<0.01
Ndwedwe	20645	1504	<0.01
Newcastle	12366	1544	<0.01
New Hannover	11000	1742	<0.01
Nkandla	78520	1770	<0.01
Nqutu	44488	1605	<0.01
Paul Pietersburg	15300	348	0.07
Pietermaritzburg	17734	1565	<0.01
Pinetown	7000	253	0.14
Polela	38275	1897	<0.01
Port Shepstone	12124	1503	<0.01
Richmond	1336	616	<0.01
Simdlangentsha	8000	710	<0.01

4.1.1.2 The prevalence at animal level:

Figure 5 displays the mean of the true prevalence at diptank level. The survey results at diptank level are shown in the Appendix.

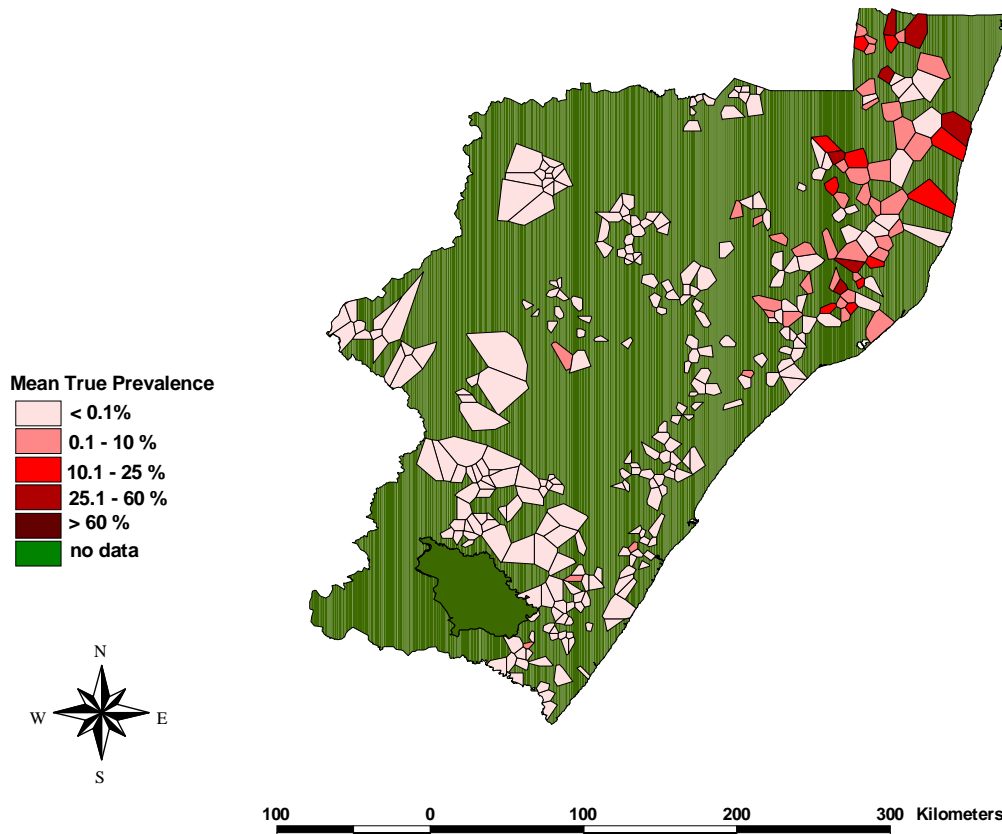


Figure 5 Mean true prevalence of bovine brucellosis at diptank level in the serological survey of KZN 2002/3

The figure above shows, that most infected diptanks are in the north-eastern region of the province, only very few singular diptanks showed reactors in the other districts. Some variation of diptank prevalence occurs and several diptanks without any reactors are found neighbouring diptanks with a higher prevalence.

4.1.2 Leptospirosis

4.1.2.1 The prevalence at district level

The number of cattle and diptanks sampled is displayed in Figure 6.

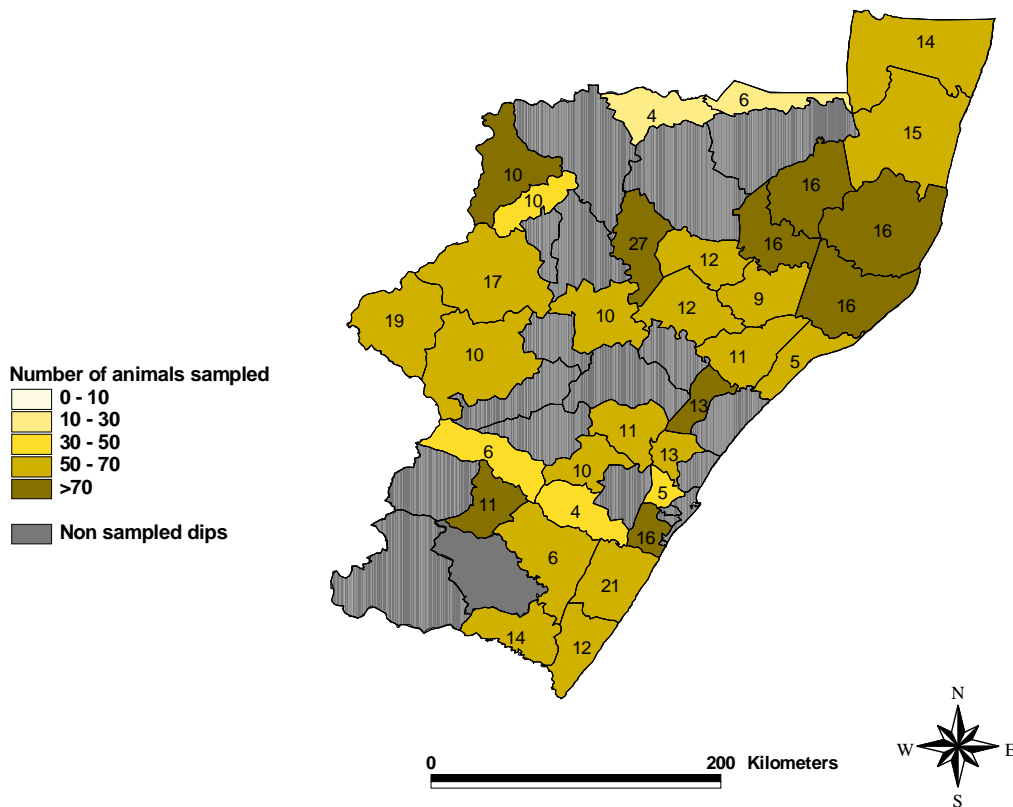


Figure 6 Number of animals and dips sampled for bovine leptospirosis in the serological survey of KZN 2002/03

Figure 7 displays the mean and the upper 95% confidence interval (CI) of the true animal prevalence of bovine leptospirosis at district level. Bovine leptospirosis occurs throughout the province with the exception of the two districts Babanango and Dannhauser. In these two districts the sample sizes were sufficient to be 99% confident, that the districts are disease free if the minimum animal level prevalence is assumed to be 1 percent (Table 5). The southern regions showed a higher prevalence than other areas of the province, while in some of the northern and western districts a lower prevalence is noted.

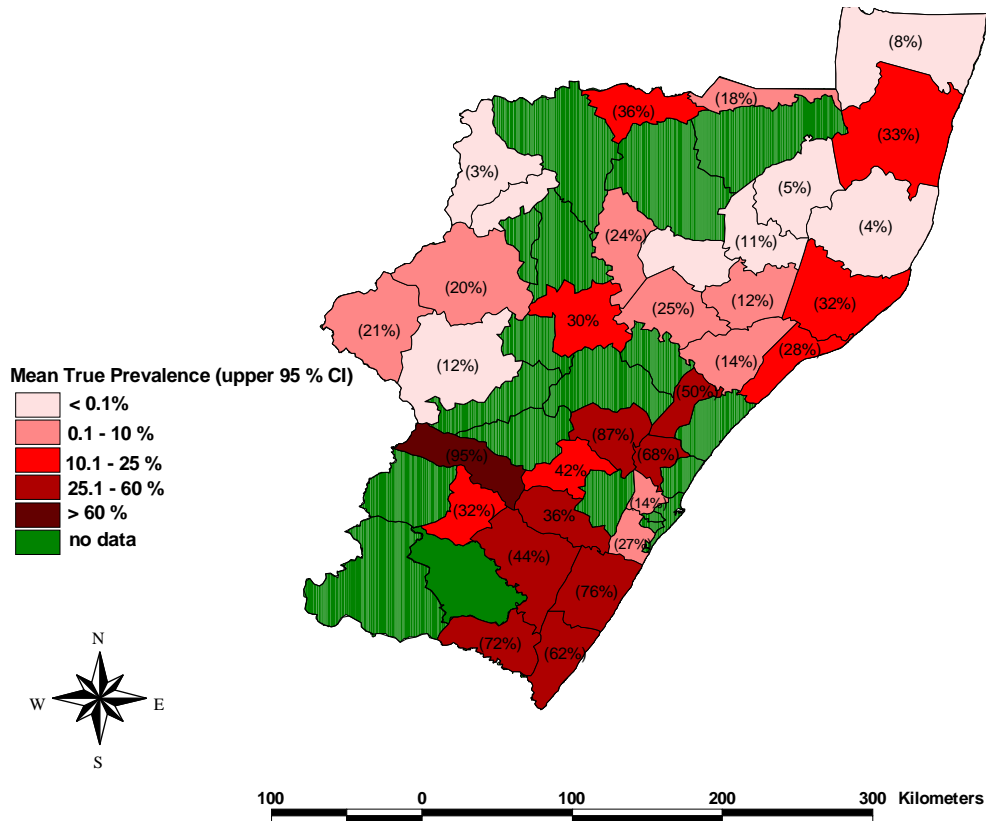


Figure 7 Mean (colour) and upper 95% confidence interval (label) of the true animal prevalence of bovine leptospirosis in the serological survey of KZN 2002/3 at district level

Table 5 The probability of districts in KZN with an apparent leptospirosis prevalence of zero having a true prevalence of more than 1% in 2002/03

Name of district	Population size	Sample size	Probability of the true prevalence being more than 1%
Babanango	14772	60	0.014
Dannhauser	11054	50	0.014

Several serovars were detected by the MAT and although serovar *pomona* occurred most frequently, *tarrasovi bratislava*, *hardjo*, *canicola* and *icterohaemorrhagica* were also frequently identified. The proportions of detected serovars are displayed in Figure 8.

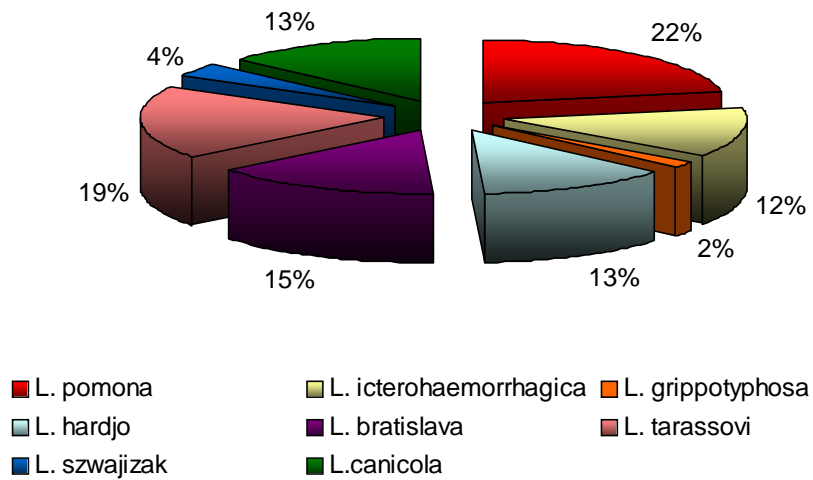


Figure 8 Serovars of *Leptospira* detected in cattle in the 2002/03 survey in the rural areas of KZN

4.1.2.2 The prevalence at diptank level

Figure 9 displays the true prevalence at diptank level for bovine leptospirosis. A similar pattern as in Figure 7 can be seen: The highest density of diptanks with higher proportion of reactors occurs in the southern areas of the province.

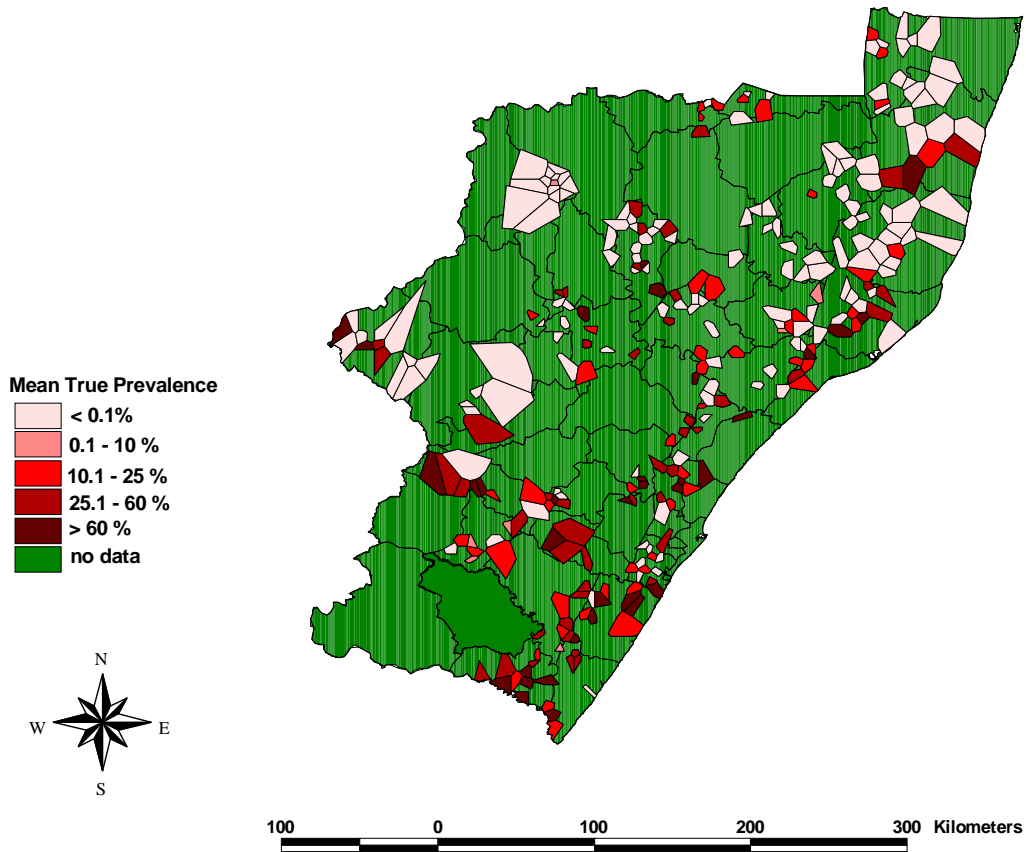


Figure 9 True prevalence of bovine leptospirosis in the serological survey of KZN 2002/3 at diptank level

4.1.3 Enzootic bovine leucosis (EBL)

4.1.3.1 The prevalence at district level

The number of animals and diptanks sampled for EBL are displayed in Figure 10.

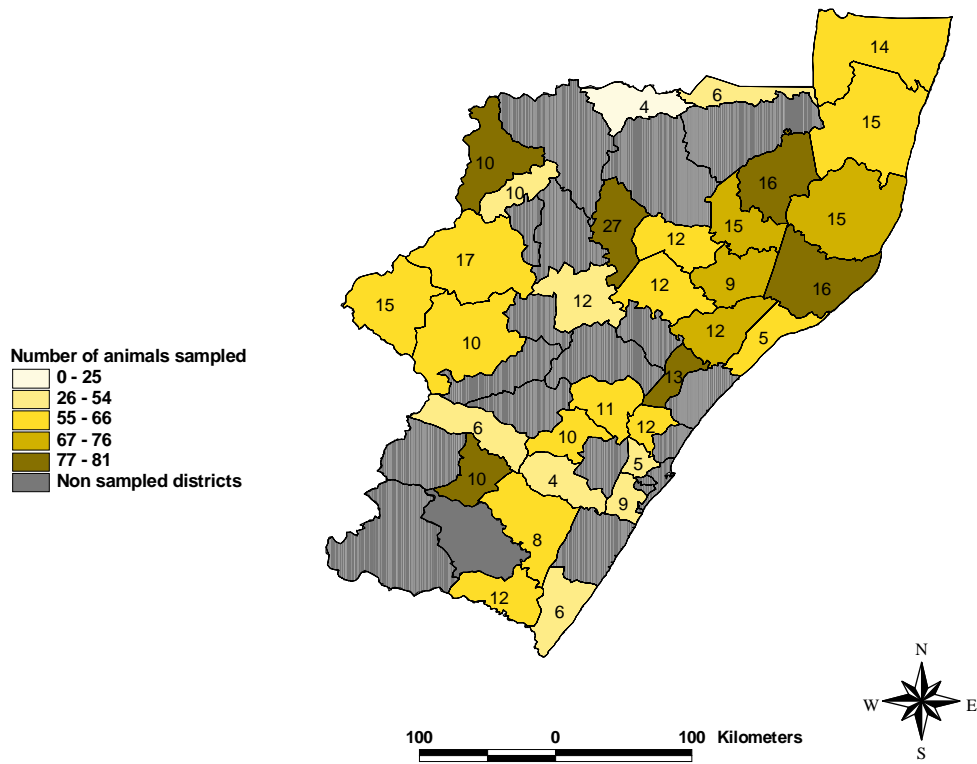


Figure 10 Number of animals and diptanks sampled for EBL in the serological survey of KZN 2002/03

Figure 11 shows the mean and upper 95% confidence interval (CI) of the true animal prevalence of EBL at district level.

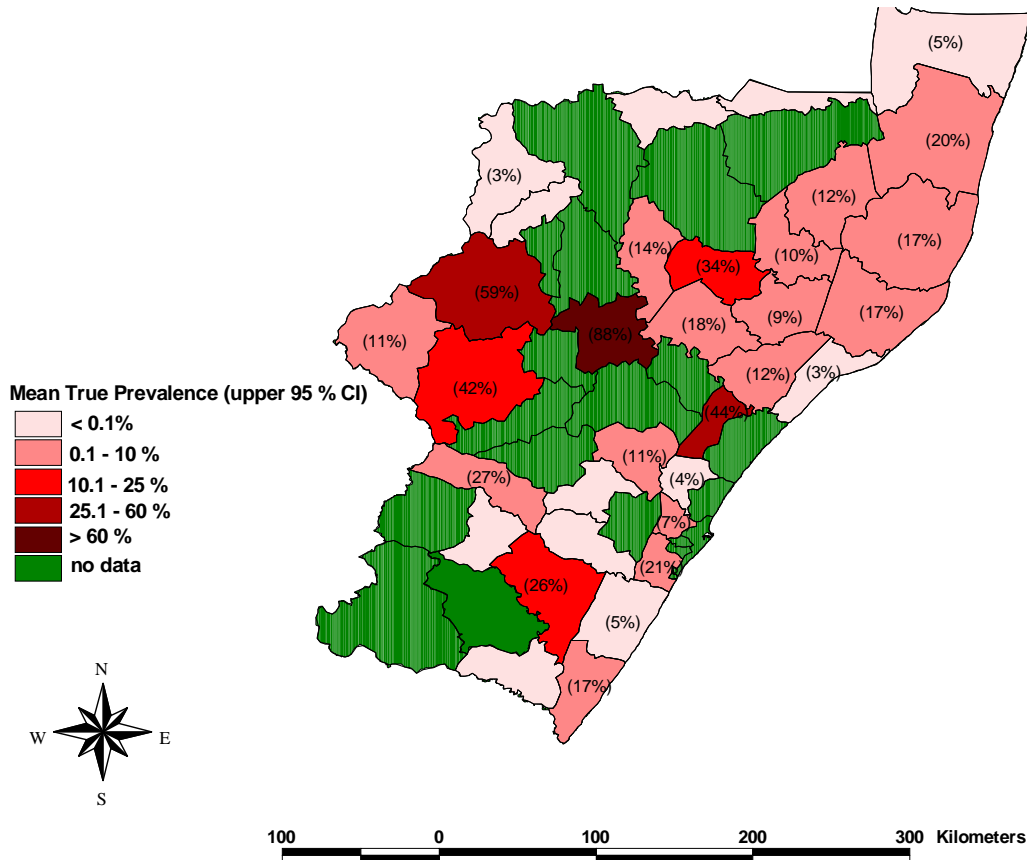


Figure 11 Mean (colour) and upper 95% confidence interval (label) of the true animal prevalence of EBL in the serological survey of KZN 2002/3 at district level

In seven districts no serological reactors were found, but the sample size was insufficient to have a higher than 95% confidence of disease freedom, except in Polela where one can be 96% confident that the true prevalence is below 1% (Table 6). In the majority of districts the mean prevalence is below 10%. The highest prevalence occurred in Msinga, where 32 out of the 48 animals tested positive. The centrally located districts appear to have a higher prevalence than the remaining districts.

Table 6 The probability of districts in KZN with an apparent EBL prevalence of zero having a true prevalence of more than 1% in 2002/03

Name of district	Population size	Sample size	Probability of the true prevalence being more than 1%
Alfred	23818	60	0.09
Dannhauser	11034	50	0.14
Paul Pietersburg	15300	25	0.37
Pietermaritzburg	17734	60	0.09
Polela	38275	80	0.04
Richmond	1336	48	0.15
Simdlangentsha	8000	29	0.31

4.1.3.2 The prevalence at diptank level

Figure 12 displays the mean the true prevalence at diptank level for EBL. The diptanks in the central areas of the province have the highest prevalence and many affected diptanks occur in close proximity, a pattern that also appears to be present in the north eastern area but further infected diptanks occur sparsely scattered throughout the province.

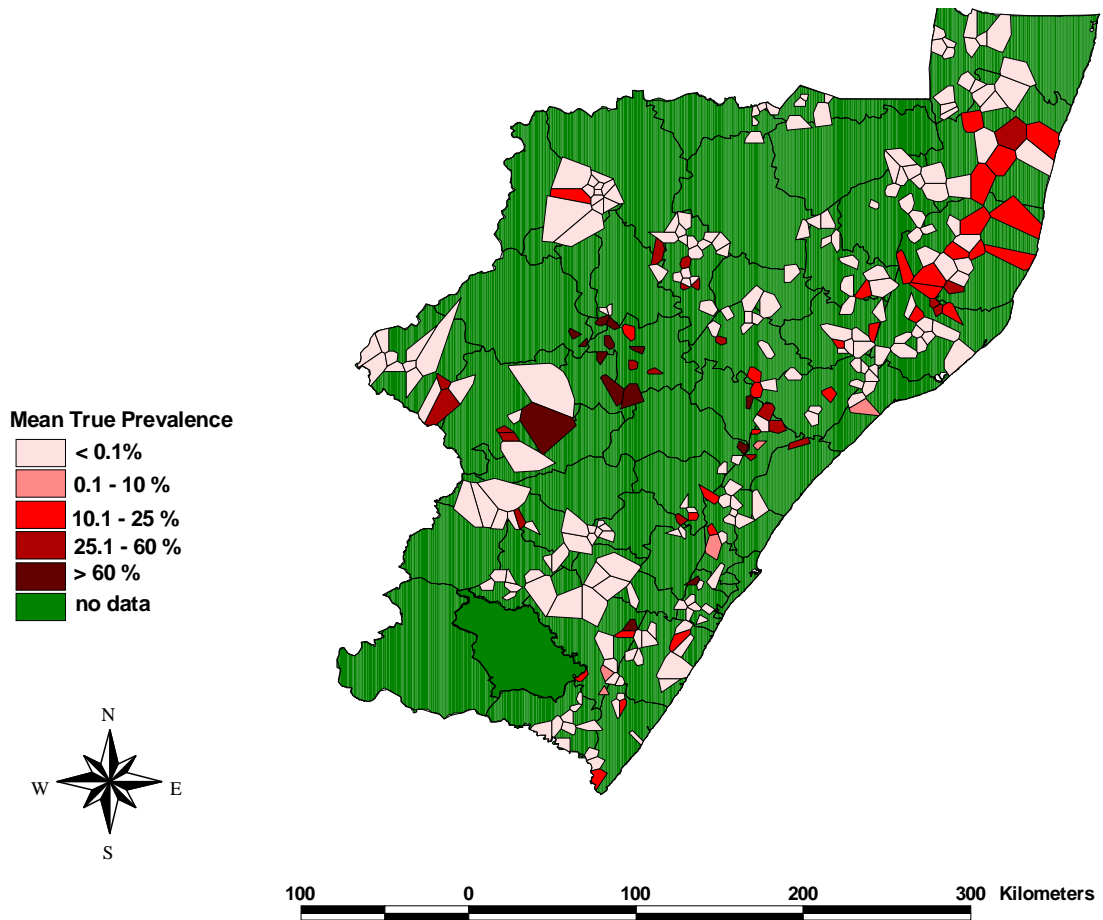


Figure 12 Mean true prevalence of EBL in the serological survey of KZN 2002/3 at diptank level

4.1.4 Bovine Anaplasmosis

4.1.4.1 The prevalence at district level

The number of diptanks and animals sampled for all tick-borne diseases (TBD) are displayed in Figure 13.

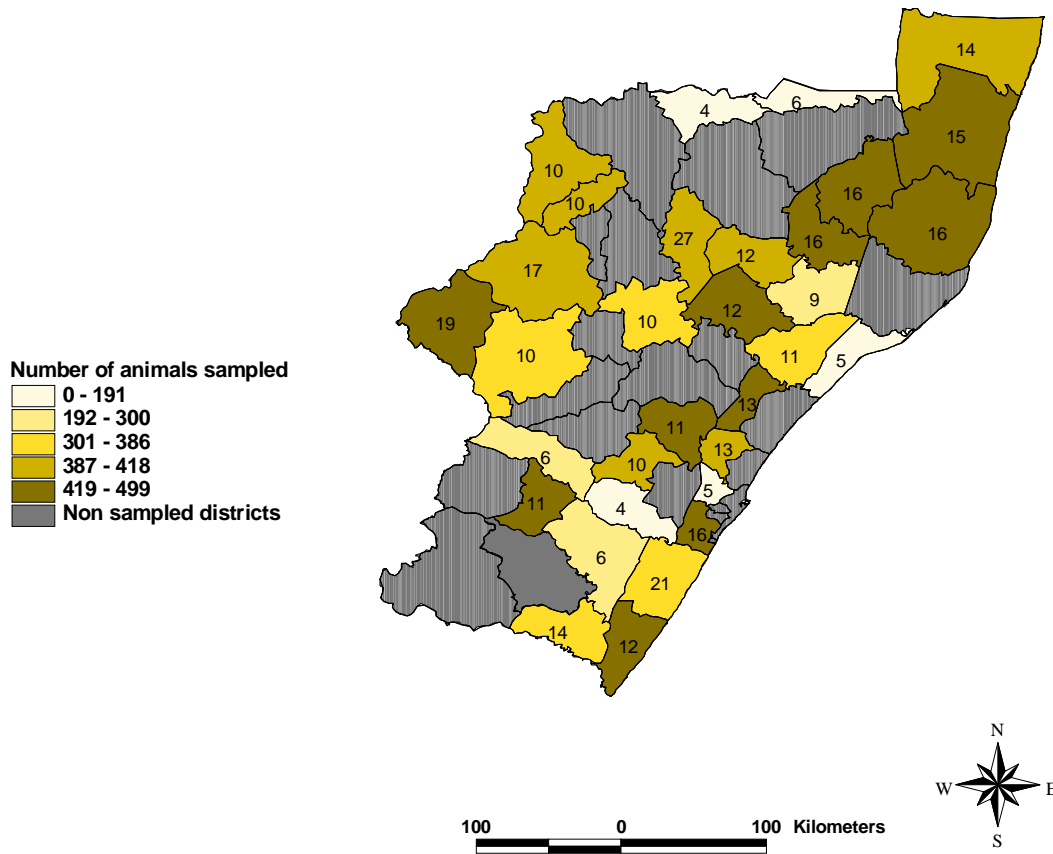


Figure 13 Number of animals and diptanks sampled for tick borne diseases (TBD) in the serological survey of KZN 2002/03

The mean and lower 95% confidence interval (CI) of the true animal prevalence of bovine anaplasmosis at district level is displayed in Figure 14.

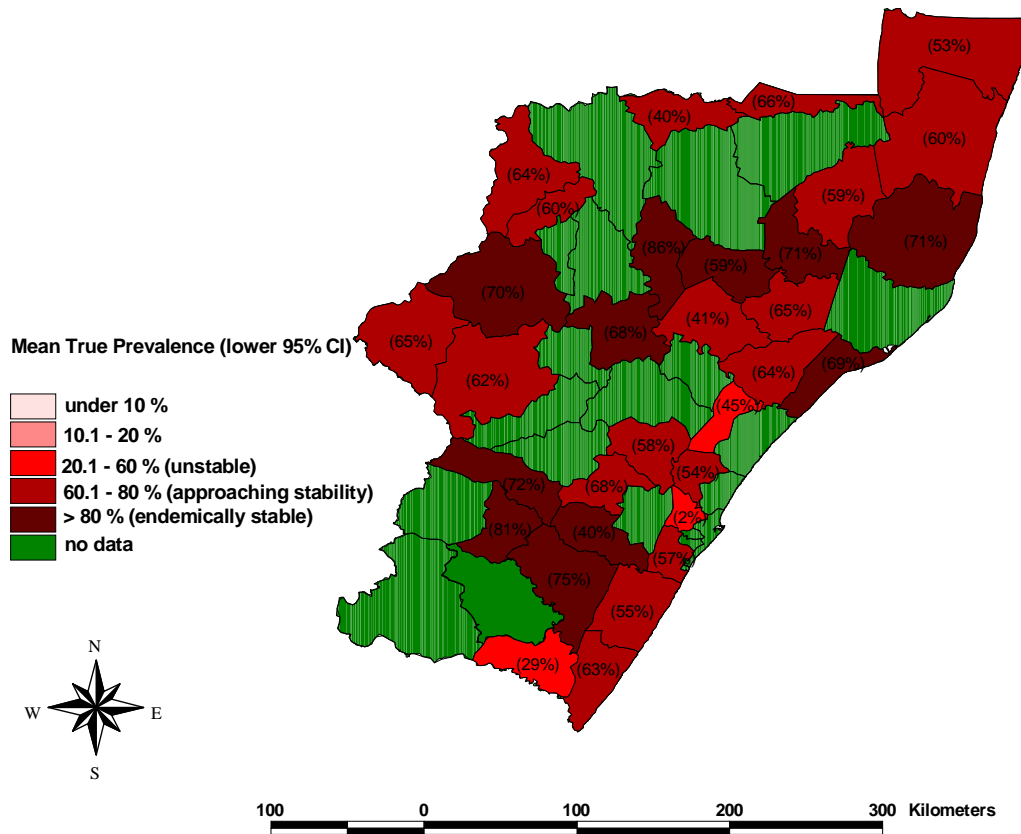


Figure 14 Mean (colour) and lower 95% confidence interval (label) of the true animal prevalence of bovine anaplasmosis in the serological survey of KZN 2002/3 at district level

A high seroprevalence of anaplasmosis occurs throughout the province and most districts show an endemically stable serological profile.

4.1.4.2 The prevalence at diptank level

Figure 15 displays the mean true prevalence at diptank level for bovine anaplasmosis after adjustments for test sensitivity and specificity have been made.

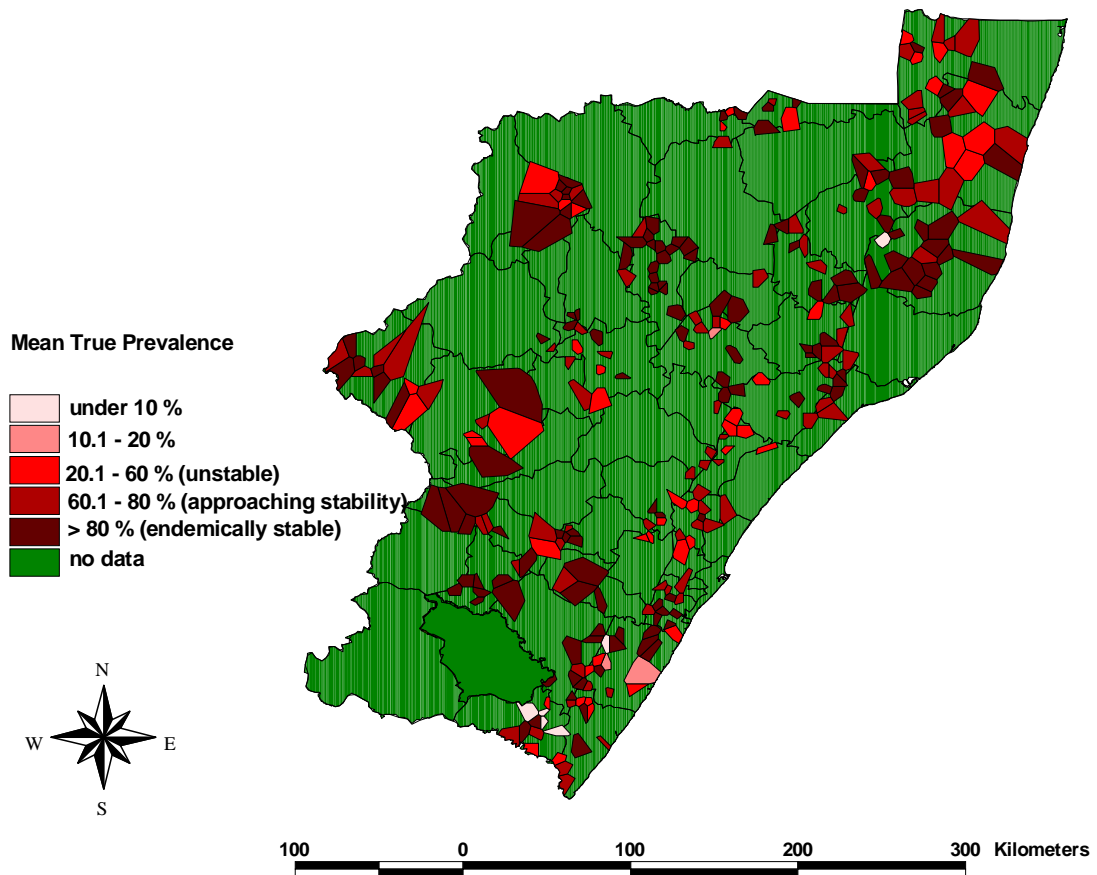


Figure 15 Mean true prevalence of bovine anaplasmosis in the serological survey of KZN 2001/03 at diptank level

Although the district map (Figure 14) above showed that most districts have reached or are approaching endemic stability, as can be seen from Figure 15 above, there is variation in between dips and many diptanks do show unstable sero-prevalences.

4.1.5 Bovine Babesiosis

4.1.5.1 The prevalence at district level

The collected samples were tested for *Babesia bovis* and *Babesia bigemina*.

The mean true animal prevalence of *B. bigemina* and *B. bovis* at district level is displayed in Figure 16 and 17 respectively. The classification of categories of endemic stability applied in the maps has been done according to (Norval *et al.* 1983). Throughout the prevalence of *Babesia bigemina* is very low and the disease appears to be totally absent from many districts especially in the northern half of the province. In none of the coastal districts did the mean prevalence exceed 20%.

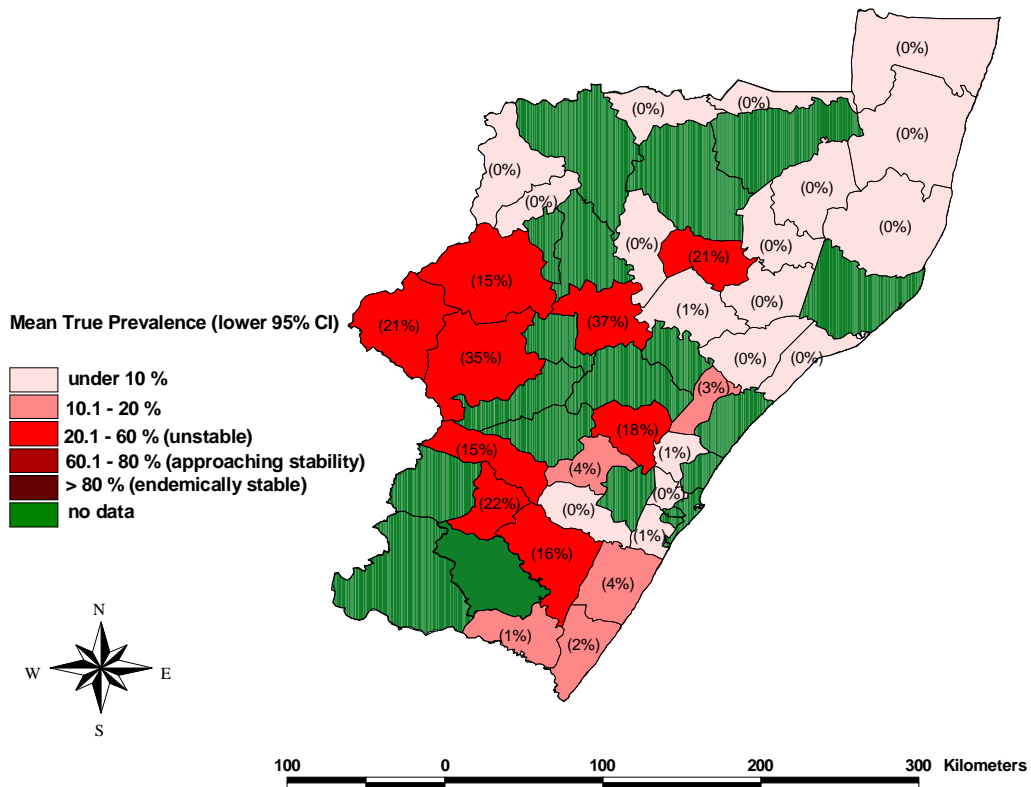


Figure 16 Mean true prevalence (colour) and lower 95% confidence interval (label) of the true animal prevalence of *Babesia bigemina* in the serological survey of KZN 2002/3 at district level

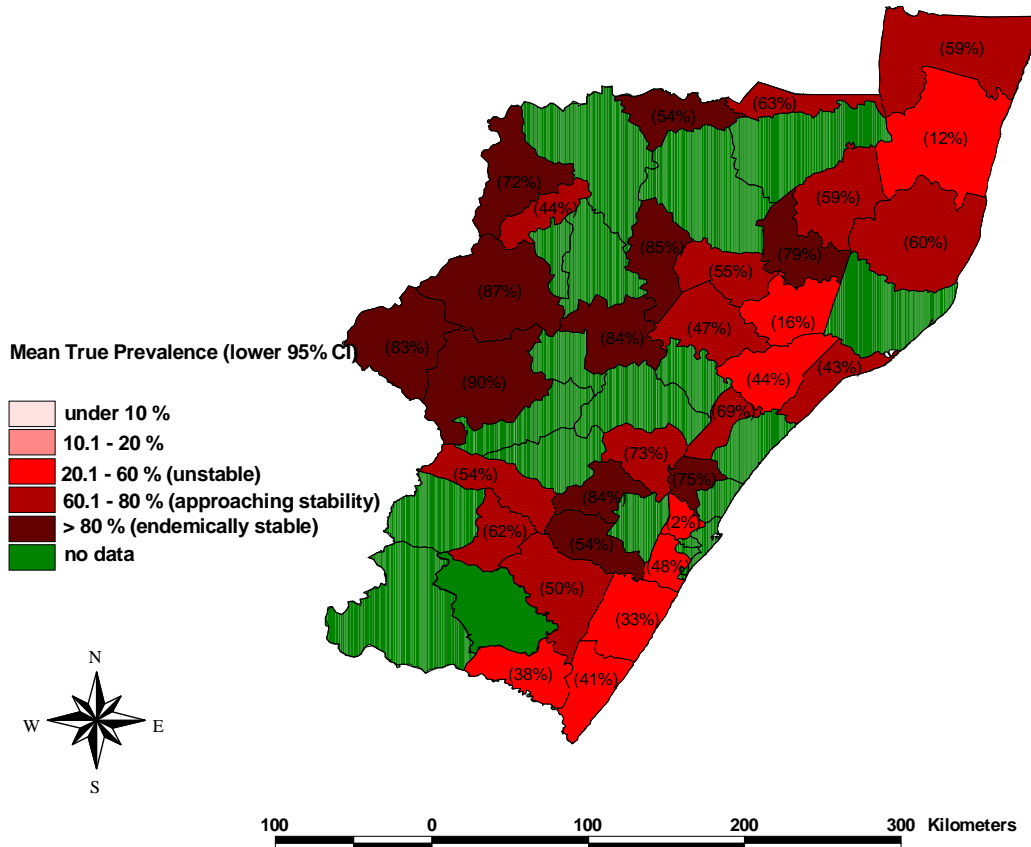


Figure 17 Mean (colour) and lower 95% confidence interval (label) of the true prevalence of *Babesia bovis* in the serological survey of KZN 2002/3 at district level

Different to *Babesia bigemina*, *Babesia bovis* appears to occur at high prevalence throughout the province, with the highest proportion of serologically positive animals occurring in the central and western districts (Figure 17).

4.1.5.2 The prevalence at diptank level

Figure 18 and 19 show the mean true prevalence of *Babesia bigemina* and *Babesia bovis* respectively at diptank level. In the north eastern area of the province a lower serological prevalence appears to be present for both *Babesia spp.* Only very few

diptanks are approaching endemic stability for *B. bigemina*, the large majority are unstable with a prevalence below 10%. The prevalence of *B. bovis* (Figure 19) is generally high with two small areas in the north east of the province and the southern coastal area where several dips in close proximity do show an unstable disease situation.

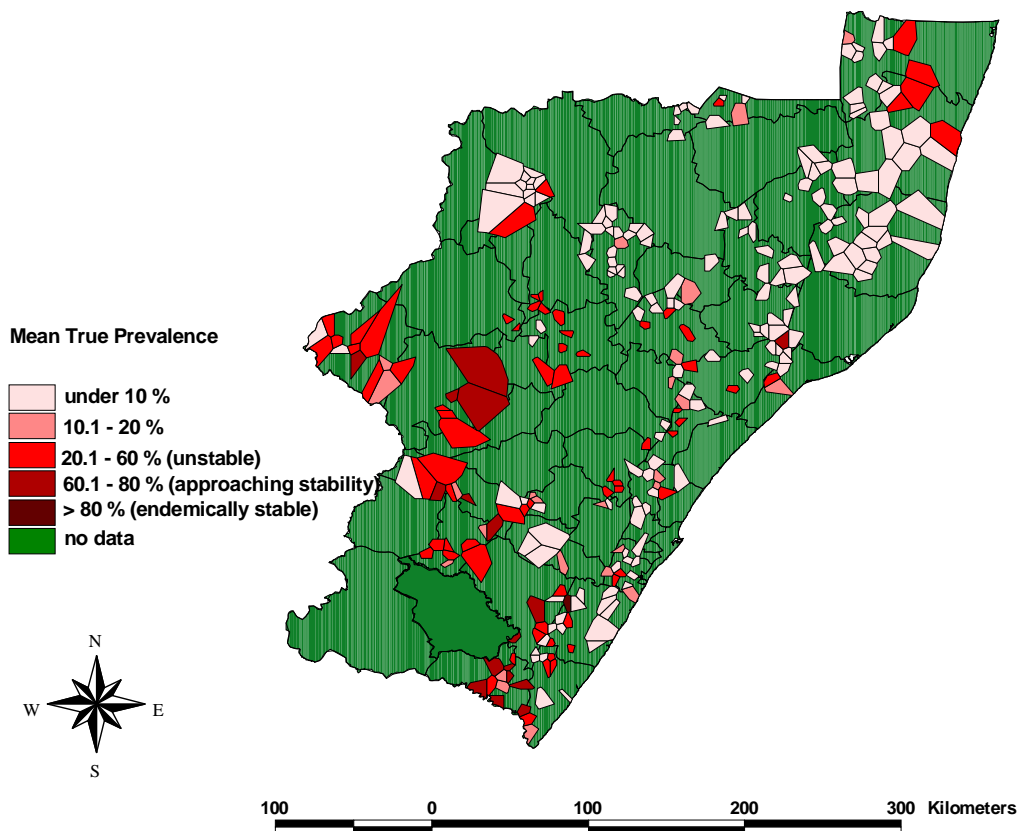


Figure 18 True prevalence of *Babesia bigemina* in the serological survey of KZN 2002/3 at diptank level

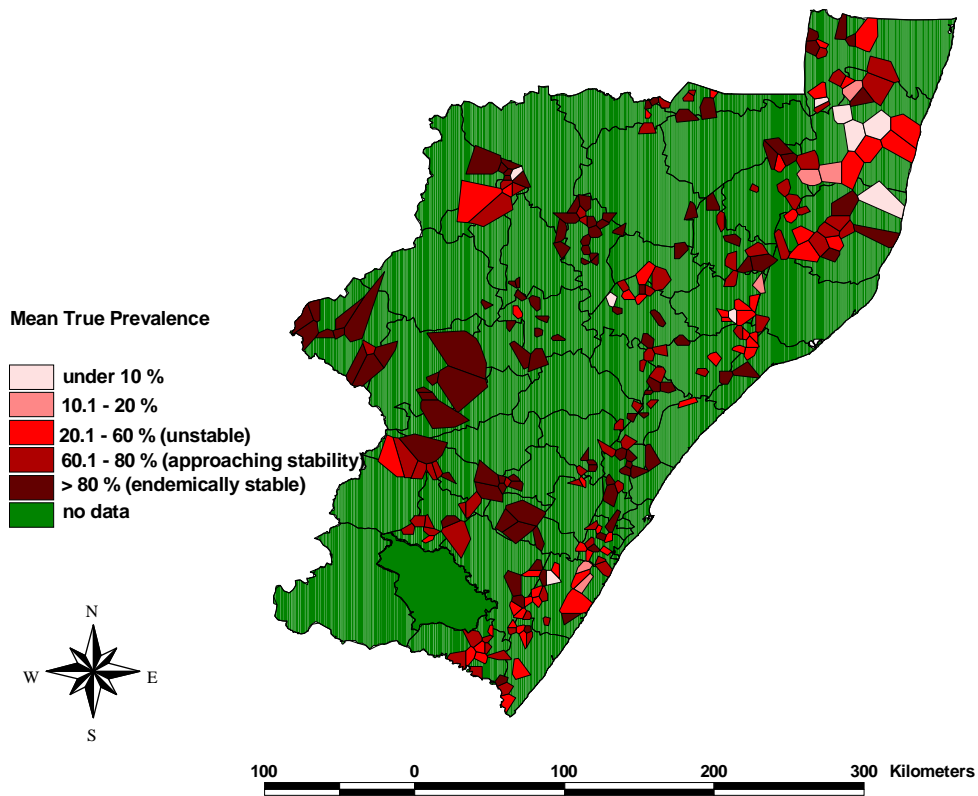


Figure 19 True prevalence of *Babesia bovis* in the serological survey of KZN 2002/3 at diptank level

4.2 The results of the questionnaire survey

4.2.1 Demographics

The collected information on general demographics of livestock owners and their stock are displayed in Table 7.

Table 7 General demographics and livestock ownership information collected in the 2002/03 survey in KZN

	Age of inter-viewees	People in household	Number of							
			Cattle	Donkey	Chicken	Dogs	Goats	Horses	Pigs	Sheep
Mean	58.46	10.2	12.2	3.27	20.3	2.8	9.9	2.8	6.1	16
Standard deviation	13.43	5.98	12.76	3.32	21.02	2.14	12.39	1.73	17.0	26.76
No of observations	309	291	276	22	223	192	180	30	17	46

4.2.3 Contact person for advice

The AHT was found to be the most frequent source of advice for 71.6 % of the interviewees, although in two districts (Estcourt and Ixopo), the coop, a local farmers cooperative which is a retail outlet for remedies commonly required by framers, appears to fulfil the main advisory role. Generally private veterinarians do not appear to play a pivotal role in health, being contacted by only 19.9% of the people. The districts Hluhluwe and Estcourt were an exception and around half of the owners seek advise from private veterinarians. The actual percentages for each district are displayed in Table 8.

Table 8 Contact person for advice in the 2002/03 KZN survey

District	Main Advisory contact in %						No of interviewees
	AHT	Chemist	Coop	Extension officer	Private Vet	Other	
Dundee	66.7	0	11.9	4.8	19	14.8	42
Durban	45.5	0	9.1	0		9	11
Estcourt	16.7	0	66.7	11.1	50	5	18
Hlulhuwe	88.6	0	2.9	5.7	40	85	35
Ixopo	50	0	64.3	28.6	14.3	7.1	14
Pmburg	89.7	0	38.5	0	30.8	3.3	39
Port Shepstone	69.5	6.1	25.6	2.4	15.9	2.4	82
Verulam	80	2.9	0	0	0	20	35
Vryheid	90		10	0	5		20
Overall	71.6	2	22.3	4.1	19.9	16.5	296

4.2.4 The perceptions of Veterinary Services

The majority of interviewees (82.1%) knew what veterinary services are and (81.1%) said they knew what veterinary services do. Almost all (90.5%) of the respondents knew their animal health technician. This result was reflected in all the surveillance zones. Fewer people appeared to be informed how veterinary services are organized (47.3%). Ninety percent had a good opinion of veterinary services, 2.4% said their opinion of VS was bad. Most people agreed with Veterinary Services bleeding their animals for surveillance purposes (77.4%). Only 19.3% said they had objections to bleeding. Only Hlulhuwe was significantly different ($p < 0.005$) and 77.1% refused to have their animals bled. Most of the owners (51.4%) did not agree with the procedure because they did not believe it could lead to improved health of their animals, a smaller proportion (11.4%) objected because they were not given advanced warning.

4.2.5 Extension of Veterinary services that were estimated to be useful

The opinions on useful extension services differed a lot between residents of the different surveillance zones. The results are displayed in Table 9.

Table 9 Extension services estimated to be useful in the 2002/03 KZN survey

District	Useful extension services in %					No of observations
	Clinics	Demos	Farmers days	Pamphlet	Radio	
Dundee	33.3	14.3	52.4	14.3	9.5	42
Durban	27.3	45.5	54.5	27.3	36.4	11
Estcourt	0	5.6	11.1	50	88.9	18
Hluhluwe	34.3	5.7	74.3	2.9	25.7	35
Ixopo	90.9	84.8	51.5	48.5	15.2	33
Pmburg	64.1	0	15.4	0	23.1	39
Port Shepstone	0	14.6	56.1	6.1	40.2	82
Verulam	2.9	0	17.1	2.9	85.7	35
Vryheid	15	35	80	50	10	20
Overall	27.9	19.4	46.7	16.2	35.6	315

4.2.6 Vaccines used in cattle

In general vaccination of stock appears to be common practice (84.8% of interviewees), and varied only slightly throughout the districts (71.4% in Ixopo – 94.4% in Estcourt). Only 6.8% do not vaccinate and 8.4% did not answer the question. Black quarter was the vaccine most frequently used throughout the study area (77.6%) followed by vaccination for Anthrax (47.9%). The detailed answers to this question are displayed in Table 10.

Table 10 Frequencies of vaccines used by cattle owners in percent in the KZN survey 02/03

District	Vaccine used in %				No. of Observations
	Anthrax	Black Quarter	Brucellosis	Lumpy Skin Disease	
Dundee	69	97.6	21.4	19	42
Durban	40	10	10	10	10
Estcourt	0	100	0	5.6	18
Hluhluwe	8.8	76.5	29.4	26.5	34
Ixopo	57.1	71.4	57.1	0	14
Pmburg	51.3	59	17.9	2.6	39
Port Shepstone	58.8	85	43.8	15.0	80
Verulam	67.6	73.5	14.7	0	34
Vryheid	26.3	68.4	31.6	10.5	19
Overall	47.9	77.6	27.9	11.7	290

4.2.7 Deworming

Deworming is carried out by 79.8% of the interviewed owners and thereof 63.1% used only commercial remedies, 10.7% used only traditional and 6% used both.

A significant association occurred between cattle owners deworming their animals and noticing worms as a problem in their stock ($p < 0.005$).

Intervals between drenching are frequently large: 40.2% deworm their animals every 6 months, 41.5 % with intervals of over one year and only 15.2 % in three months intervals.

4.2.8 Traditional medicines

Over half of the respondents (58.9%) used traditional medicines for their stock.

Utilization of traditional remedies is more common in Durban and Hluhluwe.

The conditions most frequently treated with these medicines were worms and diarrhoea. Around a third of the participants stated they do believe that witchcraft plays a role in causing diseases and death of their animals. However the proportion believing in the role of witchcraft in disease causation varied from 11.4% in Verulam to 57.3% in Port Shepstone. The detailed results for each district are displayed in Table 11.

Table 11: Livestock owners use of traditional medicines in the 2002/03 KZN survey (n=296)

District	Use traditional medicine (%)	Believe witchcraft plays role in causing disease (%)	Conditions traditional medicines were used for in (%):				
			Worms	Diarrhoea	Reproductive problems	Skin problems	Respiratory
Dundee	53.8	19	47.6	57.1	4.8	4.8	0
Durban	87.5	18.2	57.1	14.3	14.3	0	0
Estcourt	61.1	33.3	36.4	81.8	45.5	0	0
Hluhluwe	85.7	28.6	70	46.7	10	33.3	0
Ixopo	30.8	21.4	100	75	25	0	25
Pmburg	43.2	28.2	87.5	62.5	12.5	6.3	0
Port Shepstone	52.5	57.3	76.2	38.1	4.8	14.3	2.4
Verulam	79.4	11.4	92.6	59.3	14.8	0	3.7
Vryheid	45	25	22.2	44.4	0	22.2	0
Overall	58.9	32.4	69.5	50.9	11.4	12.0	1.8

4.2.9 Control of external parasites

In general, the majority of people (61.1-100%) dip their animals weekly in summer. In Durban, Dundee and Vryheid and Hluhluwe, mainly a two-week dipping strategy is followed. Only very few people (4.4%) dip their animals with monthly intervals in summer.

In winter, 32.8% of owners dip their cattle in two weekly intervals, 25.3% monthly and 20.6% do not dip their cattle. 35.2% of respondents do apply additional control of

external parasites such as pour-ons. These dipping intervals are the intervals Veterinary Services determined for the different areas of the province. Details on parasite control means additional to dipping are displayed in Table 12.

Table 12 Methods other than dipping used for controlling external parasites in the 2002/03 KZN survey

Additional means of external parasite control in %					
District	Pour-on	Tick grease	Injections	Spray	No of Observations
Dundee	40.5	0	4.8	14.3	42
Durban	9.1	27.3	0	9.1	11
Estcourt	0	0	5.6	0	18
Hluhluwe	22.9	2.9	5.7	28.6	35
Ixopo	21.2	12.1	21.2	9.1	33
Pmburg	12.8	0	0	7.7	39
Port Shepstone	64.6	0	11	0	82
Verulam	48.6	8.6	0	2.9	35
Vryheid	15	20	15	10	20
Overall	35.2	4.8	7.6	8.3	315

Disrepair of the diptank was the most frequently mentioned problem encountered in the dipping procedure (33.3%). In Hluhluwe this appears to be particularly the case with 80% of interviewees quoting this problem. Unavailability of dipping agent was a problem for every fifth owner overall, but was particularly noted in Verulam (85.7%). The third most frequently mentioned difficulty was the duration of the dipping procedure (18.4%). Especially in Estcourt (50%) and Port Shepstone (41.5%), where this appears to be a problem. Another problem frequently stated was the unavailability of water. Details are shown in Table 13 below.

Table 13 Problems experienced with the dipping procedure in the KZN survey 2002/03

District / Answers in percent	Disrepair	No dip	Takes too long	Too crowded	Other	No of observations
Dundee	19	14.3	11.9	2.4	Water, AHT arrives late	42
Durban	27.3	0	0	0	Not effective, Intervals too long	11
Estcourt	16.7	0	50	22.2	No problem	18
Hluhluwe	80	14.3	11.4	14.3	No water	35
Ixopo	27.3	15.2	0	0	No problem, no water	33
Pmburg	41	5.1	0	2.6	No problem	39
Port Shepstone	24.4	23.2	41.5	14.6	No problem (23.4%)	82
Verulam	22.9	85.7	0	0	Too weak	35
Vryheid	50	15	30	0	Too weak No water	20
Overall	33.3	22.2	18.4	7.3	21.6 (11.1 No problem)	315

4.2.10 Health problems in cattle

In all districts with the exception of Estcourt, ticks and worms were by far the two most important problems, followed by diarrhoea. In Estcourt lameness appears to be an important health problem in cattle (88.9%). The detailed results are displayed in Table 14.

Table 14 Health problems noted by cattle owners in the 2002/03 KZN survey

District	Health problems in cattle noted in %									No of observations
	Respiratory	Diarrhoea	Ticks	Lameness	Mastitis	Nutrition	Reproduction	Skin	Worms	
Dundee	4.8	31	66.7	9.5	0	26	19	11.9	38	42
Durban	30	20	80	30	30	40	30	30	70	10
Estcourt	5.6	50	27.8	88.9	5.6	16.7	27.8	0	44	18
Hluhluwe	0	2.9	41.2	0	8.8	2.9	0	8.8	47.1	34
Ixopo	9.1	33	81.8	15.2	6	15.2	3	9	48.5	33
Pmburg	2.6	51.3	94.9	7.7	0	12.8	12.8	15.4	74.4	39
Port Shepstone	0	30	96.3	0	35	7.5	10	28.8	68.8	80
Verulam	0	5.9	94.1	0	5.9	5.9	2.9	0	79.4	34
Vryheid	0	10.5	63.2	10.5	15.8	15.8	5	5	52.6	19
Overall	3.2	27.2	77.7	10.7	13.6	12.9	10.4	14.2	59.5	309

4.2.11 Most frequent management problems encountered

Overall, diseases (56.1%) and lack of knowledge (51%) were the most frequently mentioned problems. Poor facilities (36.8%) and socio-political (theft) (31.8%) can also be considered important problems. Difficulties associated with a lack of markets (15.5%) or which fell under the category 'economic' (16.9 %) appeared to be less strongly felt. However, variation occurred among the districts, for example in Verulam (91.4%) and Estcourt (88.9%) diseases were considered to be a major problem, while in Pietermaritzburg (PMB) and Ixopo diseases were only noted as a problem by 23.1% and 28.6% of cattle owners respectively. Theft (socio –political) appeared to occur mainly in Estcourt (88.9%), Ixopo (92.9%) and Vryheid (65%) otherwise with the exception of Verulam (42.9%) it was mentioned by less than a third of the interviewed persons. On average two problems were mentioned per person overall, ranging from 1.2 problems per interviewee in Pietermaritzburg to 3 problems in Port Shepstone. The detailed results can be viewed in Table 15 below.

Table 15 Management problems noted by livestock owners in the 2002/03 KZN survey

District	Management problems encountered in%						No of observations
	Diseases	Economic	Lack of Knowledge	No markets	Poor facilities	Socio-political	
Dundee	54.8	9.55	42.9	11.9	19	26.2	42
Durban	45.5	36.4	54.5	27.3	36.4	27.3	11
Estcourt	88.9	27.8	22.2	11.1	16.7	88.9	18
Hluhluwe	74.3	14.3	28.6	0	17.1	20	35
Ixopo	28.6	21.4	42.9	0	28.6	92.9	14
Pmburg	23.1	15.4	25.6	12.8	17.9	23.1	39
Port Shepstone	62.2	24.4	86.6	34.1	68.3	26.8	82
Verulam	91.4	0	57.1	0	2.9	42.9	35
Vryheid		15	30	15	25	65	20
Overall	56.1	16.9	51	15.5	31.8	36.8	296

Milk is not sold by the large majority of respondents: only 2.7 % stated that they sell their milk.

5 DISCUSSION

5.1 The diseases

5.1.1 Brucellosis

Brucellosis does not occur in the majority of the districts included in this survey, only in seven of the north eastern districts was the disease detected. Considering that no formal control programme is in place, that only very few vaccinations are carried out each year and that other surveys in sub-Saharan Africa frequently encountered a prevalence in excess of 10% (McDermott and Arimi 2002), the low prevalence or even absence of the disease here, is surprising, although a previous study in a analogous South African rural setting found a comparably low result (Bakunzi *et al.* 1993; Bishop 1984). The two districts with a mean true prevalence below 2 percent could be disease free, as a very high test specificity of 99.9% has been assumed (Stemshorn *et al.* 1985) which was considered appropriate based on these survey results, but much lower specificities of the RBT have been reported (Gall and Nielsen 2004) and in case a lower test specificity applied to the test used in the survey, the positive animals found in the relevant districts may actually be false positives. In the infected districts in the north eastern area, the mean prevalence ranged from 2.4% (5% upper 95%CI) to 15.5% (28% upper 95% CI). This impression gained from serology is supported by further indications of the presence of the disease in this area: fairly frequent reports of abortions and additional serological evidence that was obtained outside this survey. Although it has been stated that the majority of infected animals aborts only once (Godfroid *et al.* 2004) similar

clinical observations such as abortions, hygromas and infertility were made under comparable circumstances (McDermott and Arimi 2002).

Brucellosis was known to occur in the commercial herds in many areas of the province, but was thought to be eradicated through intensive vaccination and test and slaughter programmes that were carried out in the past. While in the north eastern area beef and game farming predominate, dairy farming is the main animal based farming activity in the south-eastern area and a mix of the two occur in the west of the province. Commercial dairy cattle are tested on a monthly basis and farmers generally follow a vaccination and control strategy, but commercial beef cattle are tested at most every 5 years and consequently the brucellosis status is much more uncertain and far less controlled in areas of beef farming.

As owners within the community do not generally want to sell their cattle, surrounding commercial farms are the main source of new stock. Purchase of infected stock is the major risk factor for the introduction of the disease (Crawford *et al.* 1990; Salman and Meyer 1984) and this could provide a possible explanation for the occurrence of brucellosis in this rural setting. Other known risk factors for the between herd transmission are the proximity to infected herds and grazing patterns that allow sharing of pastures with infected herds (Crawford *et al.* 1990).

Cattle in the rural areas in KZN are generally grazed on communal pastures and do move over distances of several kilometres which could lead to contamination of large areas as calving is not restricted to a specific place, such as a pen. This could function as a source of infection for other groups of cattle within that community utilising the same pasture, a factor that has been found to be important in the risk of infection in Zambia (Muma *et al.* 2006). Although in general the contact of herds from different diptanks is

thought to be limited, in the findings of this survey, many infected diptanks do neighbour other infected diptanks. However, several diptanks do also have uninfected neighbours and it is impossible to differentiate from the results of this survey if neighbouring infections are caused by disease spread or multiple introductions of disease through purchase of infected animals.

For the within herd transmission, the practice of extensive grazing reduces the infection rate, as through the nature of this management a smaller proportion of cattle within a herd would be exposed to contaminated pasture. Since an outbreak of *Brucella mellitensis* occurred in goats in KZN (Emslie and Nel 2002), it could be possible that, in case that the disease was not completely eradicated, serological reactions in cattle occurred due infection with *Brucella mellitensis*. However, this possibility appears fairly unlikely, as all goats that were tested within this survey tested negative for brucellosis on serology (unpublished data).

At a small number of diptanks outside the north-eastern area serological reactions occurred, albeit on a very limited scale. These reactions could either represent additional false positives not accounted for in the adjustment for the specificity of the test, or the presence of the disease in an initial phase and would need to be followed up by further investigation to allow a differentiation.

Although a rapid spread of the disease from the affected area is not very likely, because cattle are not often traded or moved over large distances, and the actual importance of brucellosis compared to other impacts on production parameters such as calving percentage might be limited (Mokantla *et al.* 2004), the implementation of a control strategy for brucellosis is advisable, mainly because of the zoonotic potential of the disease. At present, if a serological reactor is found, the case is not followed up further,

primarily due to technical hitches in the identification of individual animals and the absence of a control and compensation scheme for detected cases. Further difficulties for the implementation of a control strategy are the frequent resistance of owners to vaccination against brucellosis. This is thought to originate from the time period when an official control program was still in place and cattle were earmarked after vaccination, which triggered the belief in local farmers, that the state marked the cattle to later confiscate them. The above suggests that a lot of cooperative research and work would have to be carried out with the local community before the implementation of a control strategy. Mc Dermott and Arimi (2002) recommend the conduction of a cost benefit analysis considering the economic losses and the potential additional returns before deciding on a control strategy (McDermott and Arimi 2002). However, several specifications required for this analysis are not readily quantified, such as the years of human life lived with disability, and more epidemiological research of the disease in the circumstances in KZN is needed before such an analysis can be carried out.

As the budget of veterinary services is already limited and is very unlikely to increase in future times, it is highly improbable that a compensation scheme for a test and slaughter protocol could be re-introduced on a large scale basis, also its acceptance in the community would be highly questionable. Since treatment of brucellosis is not an acceptable option, vaccination remains the only option for a control strategy. However, since animals remain lifelong carriers, the following requirements have to be fulfilled for any potential success of this approach:

- All cattle owners in the affected area need to be convinced of the necessity to have their heifers vaccinated.

- Sufficient funds for vaccination need to be ensured even for future years due to the carrier status and the long life expectancy of cattle in these circumstances.
- It should be excluded by means of prior research that cattle are frequently purchased from the neighbouring infected province Mpumalanga, which is under the authority of different provincial veterinary services. Otherwise a continuous risk of new introduction of the disease impacts on the chance of a successful control campaign.
- Sufficient human resources need to be available to enable a large-scale long-term vaccination program.

Until it can be ensured that these requirements can be satisfied and that such an approach would be cost effective, efforts can be made to decrease the impact of the disease on the local population through awareness campaigns. This approach could be initiated through training of animal health technicians on the routes of infection and preventive measures such as boiling of milk before consumption and avoidance of contact with aborted material and placentas. With the support of the technicians, this campaign could then be extended to include the topic in farmer days and distribution of information pamphlets. Local radio proved to be the most effective means of communicating with the local population in a recent classical swine fever outbreak in the area and the initiation of a regular program that informs farmers on issues such as general disease prevention and stock management might be a valuable component of such a campaign. Due to the relatively unspecific symptoms in humans and a frequent

lack of information on zoonotic diseases (Coulibaly and Yameogo 2000), it is important to inform and collaborate with the human health services to increase the likelihood of correct diagnosis and treatment as well as to advocate the prevention of the disease through the measures mentioned above in addition to the work that can be done by animal health technicians.

5.1.2 Leptospirosis

This survey is the first large-scale serological study of Leptospirosis in the rural areas of South Africa. Figures 7-9 illustrate, that generally the disease occurs throughout the province, albeit it was not detected at all diptanks. The north eastern region appears to be less affected and two districts (Dannhauser and Babanango) are free of the disease. The displayed prevalence though, should be interpreted with care, as a high uncertainty exists around the mean and the confidence intervals are very large (up to 67% in the case of Mpendele). This is mainly an effect of the sample size collected: around 5 -10 animals were sampled at each diptank and epidemiological research suggests, that a sample size of 10 animals per herd merely provides evidence of the presence or absence of the disease in a herd (Hathaway *et al.* 1986). This conclusion was drawn because antibody levels decline fast and remain above the diagnostic threshold only for a limited amount of time and in an endemic situation a positive serological reaction is often not associated with the disease (Hathaway *et al.* 1986). In addition to this, a sample size of five or ten animals allows the minimum prevalence of the disease to be 20 or 10 % respectively at diptank level and might give the impression on a map that no low prevalence of the disease occurs. Additional widening of the confidence intervals was

caused by uncertainty on the sensitivity of the MAT: Since no published information was available for these parameters - as this test is often used as the gold standard test against which other tests are evaluated-, some information available from a study in humans (Bajani *et al.* 2003) and in pigs (Chappel *et al.* 1992) was used to derive a relatively uninformed uniform probability distribution with the minimum sensitivity and specificity set to 90% and the maximum to 98%.

Another confounder in the serological interpretation of survey results can be vaccination against leptospirosis (Hodges and Day 1987; Stringfellow *et al.* 1983), but this is thought to play a minimal or no role in this setting, as the general opinion of state vets and animal health technicians in the area is, that vaccination is not practiced for this disease in the communal areas.

The widespread presence of the disease reflects the results of other studies that found Leptospirosis in cattle, pigs and game in South Africa (De Lange *et al.* 1987; Hunter *et al.* 1988; Myburgh and Otto 1990; Potts *et al.* 1995).

The highest density of affected diptanks occurs in the south-eastern region of the province, while the north-eastern region appears to be less affected by the disease. Considering that *Leptospira spp.* are excreted in the urine and can survive especially well under moist conditions (up to 94 days in river water) (Kaasschieter *et al.* 1992) (Ellis 1986; Okazaki and Ringen 1957) and that exposure to contaminated water sources as well as inadequate husbandry (Alonso-Andicoberry *et al.* 2001) are important risk factors, the conditions for the occurrence of leptospirosis appear very favourable in the setting of the rural community in KZN. This is especially so in the southern part of the province where a high air humidity and mist combined with high rainfall are frequently encountered. The favourable conditions in the south of the

province are furthered by the frequent practice of local commercial farmers to irrigate pastures with slurry and the increased amount of species mixing such as with pigs, which are not kept in the northern areas, where mainly beef and game farming under extensive conditions is practiced.

The main serovar identified in cattle in this survey was *Leptospira pomona* (22%), but a wide variety of serovars was detected: (*tarassovi* (19%), *bratislava* (15%), *canicola* (13%), *hardjo* (13%), *icterohaemorrhagica* (12%), *szwajizak* (4%) and *grippotyphosa* (2%)). Generally *L. pomona* is thought to cause only sporadic infections in cattle in SA while *L. hardjo* is considered the most important cause of bovine leptospirosis (Hunter 2004). The second most frequently identified serovar was *tarassovi* which is maintained by pigs (Alonso-Andicoberry *et al.* 2001) but was not detected in a previous study on south African pigs (Potts *et al.* 1995). However *tarassovi* is known to also occur in cattle and was in combination with *hardjo* and *wolffi* among the most frequent serovars in a survey on bovines carried out in Mexico (Moles-Cervantes *et al.* 2002) and also occurred frequently in cattle in Zimbabwe (Feresu 1987).

The serovar *bratislava*, that here was found to be the cause of 15% of the serological reactions, was frequently identified to be an important serovar in infections in pigs (Boqist *et al.* 2002; Cisneros-Puebla *et al.* 2002). This serovar though is maintained by many free living species (Alonso-Andicoberry *et al.* 2001; Hathaway *et al.* 1986), and pigs (Alonso-Andicoberry *et al.* 2001; Ellis 1989), horses (Alonso-Andicoberry *et al.* 2001; Ellis *et al.* 1983) and probably dogs (Van der Broek *et al.* 1991; Alonso-Andicoberry *et al.* 2001) can serve as maintenance hosts. The serovar *hardjo*, which is thought to be the most important cause of leptospirosis in bovines (Hunter 2004) and which is adapted to and maintained by cattle (Alonso-Andicoberry *et al.* 2001),

accounted for 13% percent of the serological reactions. However, caution in the interpretation of the serovar specifications is advisable, as especially if antibodies to more than one serovar are present, the MAT test may not correctly specify them (Levett 2003). The wide variety of serovars and the widespread prevalence of leptospirosis in Kwa Zulu Natal suggest, that the disease is endemic and that the epidemiology of the disease is multi-factorial. The involvement of many species in the transmission combined with the frequent mixing of species that occurs in the context of the communal lands, the role of carrier animals in the infection, the warm and humid climate and husbandry practices that could not prevent contact with rodents, dogs and other animals will complicate any control of the disease. Theoretically a vaccination program could be considered, but it would have to be ensured that all serovars are included in the vaccine and that the vaccine is applied in a large scale, to all involved domestic species present and for long periods of time. Before it is decided to implement such a control program it may be advisable to investigate the clinical importance of leptospirosis for the impact on production and its role as a zoonosis further. Since the practice of applying slurry from infected commercial farms is known to propagate the occurrence of the disease, the implementation of legislation, which prevents farmers from using slurry if infection has been confirmed on the property, may be considered. Besides these measures, a campaign could be started to decrease the zoonotic impact of the disease through informing owners of the routes of infection and measures of protection that have been found useful, such as wearing footwear, the use of disinfectants when in contact with animal excreta and to cover abrasions when handling animals (Leal-Castellanos *et al.* 2003). Additional to this it might be useful to inform the health services practising in this area of the presence of the disease to increase its

diagnosis, as the flu-like symptoms of the disease make mis-and under-diagnosis probable, if no knowledge on the occurrence of the disease exists.

5.1.3 Enzootic Bovine Leukosis (EBL)

This survey appears to be the first published large-scale study on the serological prevalence of EBL in SA. A previous study, that investigated the occurrence of the disease in farms that had and had no contact with an initially identified infected farm, found EBL to occur in both study groups at a 3.67 and 8.64% prevalence respectively (Morris *et al.* 1996). The presence of the disease in other countries of southern Africa is demonstrated through a limited number of serological studies that detected the disease also in Namibia (Kaura and Hubschle 1994), Tanzania (Schoepf *et al.* 1997) and Botswana (Mushi *et al.* 1990). If present, in this study the upper 95% confidence interval of the prevalence at district level varied from 3 to 84%. This high variation of prevalence was equally found in Namibia where the prevalence varied from zero to 52.6% in different State Veterinary Districts (Kaura and Hubschle 1994). However the majority of the included districts showed mean prevalences below 10%, which can be considered low. Care should be taken when interpreting the prevalence displayed in the maps, as due to the small sample size the uncertainty around the mean is large. Seven Magisterial districts did not show any serological reactors, however if one would like to be 95% confident, that the district is disease free, under the given assumptions, only in Polela was the sample size sufficient to arrive at this conclusion.

From Figure 11 it can be noticed, that the highest serological occurrence of the disease was found in the central and mid-western area of the province. It is known from incidental findings of State vets, that EBL does cause problems in several commercial

dairy herds in this area. State vets did not notice any obvious signs of the clinical form of EBL in the communal herds though. However, some of the typical clinical signs, such as emaciation, might be difficult to differentiate from other problems present such as feed shortage and parasite infections. Furthermore, clinical signs have been observed to only occur in a low percentage of infected animals and after a long incubation period (Schoepf *et al.* 1997) increasing the difficulty of clinical diagnosis. At diptank level, the small number of samples collected (3-12 animals), does not allow any other interpretation than that the disease is present at that diptank. In the central area of the province more affected diptanks occur in close neighbourhood, an effect that appears to be also present in the north-eastern area of the province, albeit less pronounced. In the remaining part of the province the infected diptanks appear to be distributed in a more scattered manner. The close neighbourhood of infected diptanks could either indicate the spread of the disease from diptank to diptank, multiple introductions from an infected source or a mix of the two. While cattle from the same diptank are often in close contact for example during dipping, which would facilitate the transmission (Werling *et al.* 2004) which occurs through the exchange of blood and effusions through, for example, abrasions (Sargeant *et al.* 1997), cattle from different diptanks rarely come into close contact. Considering this and the fact that cattle are the only maintenance hosts of EBL (Burny *et al.* 1985) (Werling *et al.* 2004), an additional possible route of transmission, that could play a role in this context is iatrogenic transmission through for example use of the same needle when TB testing several diptanks (Hopkins and DiGiacomo 1997; Werling *et al.* 2004). Since the disease is so widely present in the communal areas, doubt will remain regarding the original source of the disease. While it appears likely that the disease was introduced into the rural

herds through the purchase of infected commercial cattle, a slight possibility remains, that the disease was already present in that population, as Schoepf *et al.* (1997) for example, detected EBL reactions in herds of indigenous cattle in Tanzania, that were not known to have had any contact with commercial cattle.

Considering the prevalence and importance of other diseases such as rabies, brucellosis and the clinical importance of tick-borne diseases, a provincial control strategy for EBL is not likely to be very feasible and would appear lower down on the priority list of disease control based on criteria such as clinical impact, efficacy of control measures and production benefits of control. However, cheap measures that would reduce the spread of the disease are likely to have some beneficial impact. Such measures could be for example to advise animal health technicians to disinfect, or change needles when carrying out TB testing or taking serological samples.

5.1.4 Bovine Anaplasmosis

Bovine Anaplasmosis was included in the survey to investigate the enzootic stability of the disease in the rural areas. From Figures 14 and 15 it becomes apparent, that even when considering the lower 95% confidence intervals, the majority of diptanks is either approaching enzootic stability or has achieved it.

This finding is not surprising considering that *Anaplasma* is transmitted by several vectors including biting flies (Potgieter and Stoltz 2004). From reports of State Vets and Animal Health Technicians in the area it appears that clinical cases due to the disease hardly occur, a finding which supports the serological evidence of a enzootically stable situation. Other studies carried out in countries of southern Africa also encountered high

sero-prevalences in the majority of their study area to the disease (Alfredo *et al.* 2005; Jongejan *et al.* 1988). Considering these findings, no control strategy appears to be required for anaplasmosis.

5.1.5 Bovine Babesiosis

As in the other diseases discussed above, the mean prevalence displayed in the maps should be interpreted in conjunction with the large confidence interval around them, which are displayed in the Appendix. The actual sero-prevalence is the result of many influences in the host- vector- disease –environment interaction. The variety of processes impacting on whether infection occurs and is consequently detectable through serology are summarized in Figure 20 for better illustration. Although there is no immediately striking spatial pattern, three processes can be noted in the maps: The serological prevalence of *B. bigemina* is generally notably lower than the prevalence of *B. bovis*, cattle from a small area in the north eastern area of the province showed less serological reactions than cattle originating from other districts for both diseases and it appears that the prevalence of both diseases is lower in the coastal districts.

Based on the mean true prevalence of the analysis at district level, *B. bigemina* appears to be absent from 8 districts; although in some of them serological reactions occurred at a limited number of diptanks. This finding is somewhat surprising considering that *B. bigemina* has several vectors while *B. bovis* is only transmitted by *B. microplus*. However, a corresponding observation of a lower prevalence of *B. bigemina* in relation to *B. bovis* was made by Stevens *et al.* (in press) and was postulated to be caused by the replacement of the vector *Boophilus decoloratus* with *Boophilus microplus*, a process

which had been recently reported from the Limpopo province (Tonnesen *et al.* 2004). However, considering that *B. microplus* can transmit *B. bigemina* as well, there are likely to be some additional factors involved in this process. To give an impression of other factors involved in the host-vector-disease relationship, Figure 20 displays their role in the pathway from the initial tick bite to a serologically positive animal and may add to the understanding of a higher sero-prevalence of *B. bovis*:

Firstly *Boophilus* ticks are one host ticks and larvae attach to the animal after hatching. Contrary to *B. bigemina* which is transmitted in the later nymphal and adult stages, *B. bovis* can already be transmitted at the larval stage, so if intensive dipping is practiced, or the general conditions for survival are adverse, the larva might not survive long enough to transmit *B. bigemina*. Secondly, the period a cow stays infective for a tick is much longer in the case of infection with *B. bovis* (years) than with *B. bigemina* (4-7 weeks) (de Vos *et al.* 2004) and this could lead to a higher infection rate of *B. bovis* in ticks. However, although plausible, this is not supported by existing literature, where it is thought that if both *Babesia* species are present, the prevalence of *B. bigemina* infections prevails over that of *B. bovis* in ticks (de Vos *et al.* 2004). No literature was found that investigates a possible competitive relationship for the establishment of infection in *B. microplus* ticks and the consequent impact on transmission. Such a relationship is known to occur for anaplasmosis, where infection with the milder vaccination strain prevents establishment of infection of the more virulent field strain. Further research would need to be conducted to find out if *B. bovis* has a potential competitive advantage in the tick, and if an established infection with *B. bovis* could possibly prevent infection with *B. bigemina* and whether this could be an additional explanation for the higher prevalence.

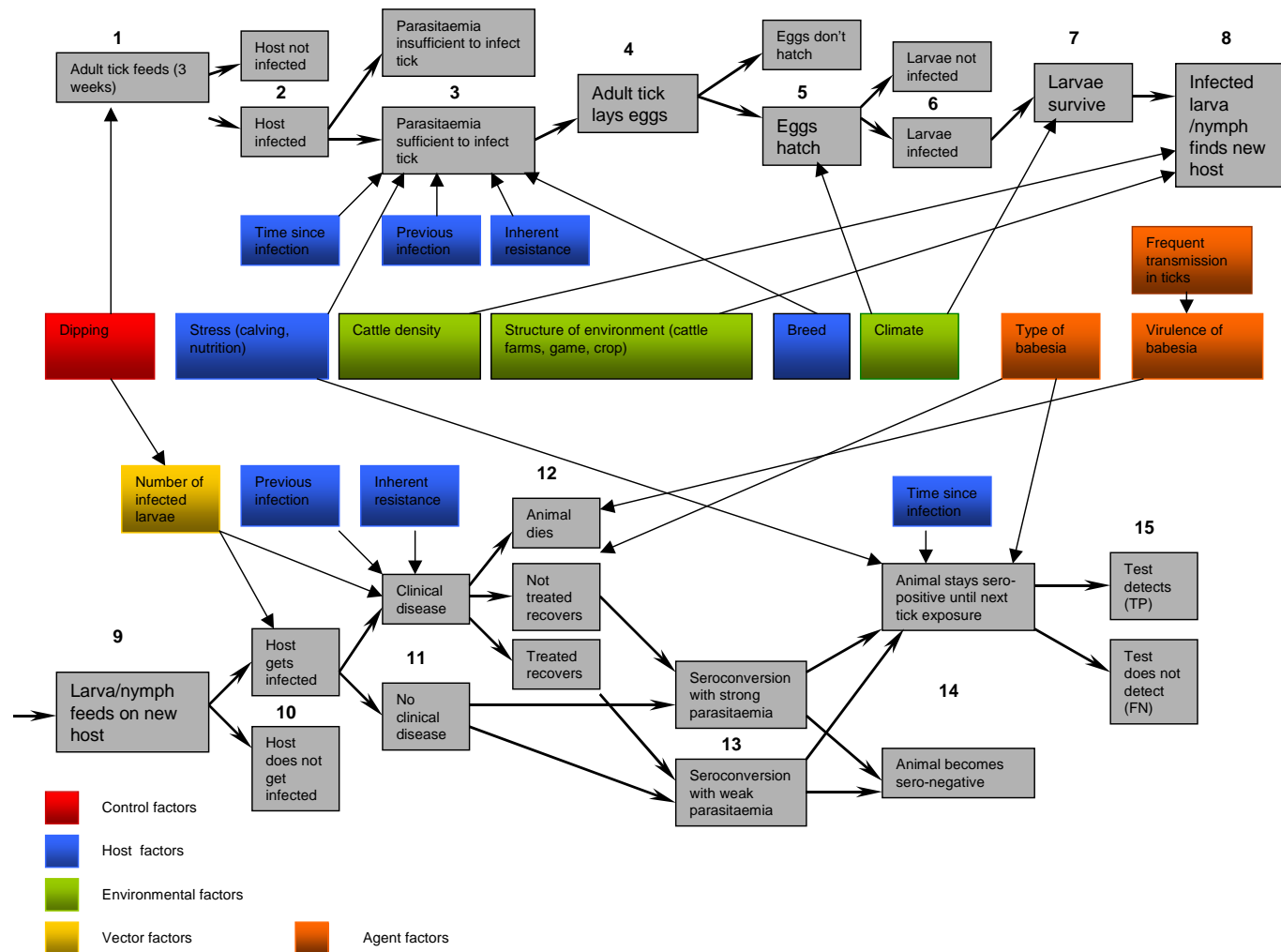


Figure 20 Diagram of the infection pathway from tick bite to serologically positive animal in bovine babesiosis

The cause of the observed lower prevalence in the north-eastern area in the province, which was also reflected in the serological results for anaplasmosis, could possibly be, that farming in those areas consists mainly of extensive beef and many game farms, which may make it more difficult for the larvae to find their host, this effect is likely to be more important in the occurrence of *Boophilus microplus* as this tick uses cattle as virtually its only host (Norval and Horak 2004). Less rainfall occurs in this area than in other areas of the province which could also contribute to the explanation of a lower prevalence. More intensive dipping is less likely to be an explanation for this observation, as problems with the functionality of the dip are very frequently encountered in this region.

The lower prevalence in the coastal regions for both *Babesia spp.* though could possibly be explained by the dipping strategy applied by veterinary services. Due to the occurrence of *Amblyomma* ticks in these areas cattle are dipped at weekly intervals in summer contrary to other regions where this interval is generally doubled. For the control of one-host ticks such as *Boophilus*, an interval of three weeks for dipping is generally considered sufficient and the short intervals used in the coastal area might have a large impact on this tick population and consequent infection of cattle with babesiosis.

Although for *B. bovis* the majority of diptanks achieved an apparently endemically stable situation with sero-prevalences over 80 percent, many diptanks are only approaching stability or are unstable. This scenario of instability is much more evident for infections with *B. bigemina* where endemic stability was not achieved in any of the diptanks. However, it is difficult to draw conclusions on the endemic stability from this

sero-prevalence, as a sterile immunity for babesiosis exists, where even in the absence of detectable antibodies, cell-mediated protection is present (Callow *et al.* 1974; Johnston *et al.* 1978) and it has been found that results may vary strongly when prevalence studies are repeated in the case of tick born diseases (Tice *et al.* 1998).

Clinical cases of redwater are frequently observed in all areas of the province. These cases tend to occur mainly in cattle that are in peri-parturient stress or during winter, when nutritional stresses may allow a resurgence of the infection. Apart from the above, it has been observed that younger cattle that have just lost their immunity are frequently affected. Consequently tick borne diseases are regarded as being very important amongst livestock owners, a fact that needs to be taken into consideration when deciding on control strategies. Any change in the tick control needs to be carefully reflected, as dipping has been the main focus area of veterinary services for many years and many factors will be impacted on, if a change to the regimen is initiated. Table 16 aims to illustrate some advantages, disadvantages and complications of various elements of control strategies in the specific context of the communal areas in KZN.

Table 16 Advantages, disadvantages and complications of various control measures for babesiosis in the context of the rural areas of Kwa-Zulu Natal

Control strategy	Advantages	Disadvantages	Complications in the implementation
Measure 1 Abandon dipping to allow endemic immunity to establish	<p>Reduced cost, which would allow for the significant resources to be used for other purposes such as vaccinations and knowledge communication</p> <p>In an endemically stable situation cattle would be resistant and disease is not expected to occur (Norval <i>et al.</i> 1983)</p>	<p>Since the climatic conditions are very favourable, tick burdens might become overwhelming</p> <p>This practice might allow the further spread of <i>Boophilus microplus</i> and consequently the more virulent <i>B. bovis</i> to neighbouring provinces</p> <p>Damage from other ticks such as <i>Hyalomma spp.</i> and <i>Amblyomma spp.</i> may become great</p> <p>Dipping is also used to control TseTse flies in endemic areas and an alternative TseTse treatment would need to be found.</p> <p>An increase of clinical cases and death is to be expected before endemic stability would establish (Rikhotso <i>et al.</i> 2005)</p>	<p>It is unlikely that such an approach would be acceptable in the communities and extensive knowledge communication would be needed up front</p> <p>Dipping also serves other purposes such as passive surveillance through inspection and collection of samples and is generally the only contact point between livestock owner and veterinary services</p>

Control strategy	Advantages	Disadvantages	Complications in the implementation
<p>Measure 2</p> <p>Intensify dipping to reach a tick and disease free status</p>	<p>Desirable by livestock owners (Emslie 2004)</p> <p>Increased contact to livestock owners</p> <p>Decreased number of clinical cases if tick free status can be achieved</p>	<p>Already one million Rand, which is more than three quarters of the provincial veterinary services budget, is spent on dipping and additional financial and human resources are unlikely to be available.</p> <p>Favourable climatic conditions for ticks make it unlikely that a tick free state can be established, especially if no similar programs are implemented in neighbouring areas</p> <p>Resistance to acaricides</p>	<p>Staffing shortages would hardly allow this option</p>
<p>Measure 3</p> <p>Vaccinating cattle in endemically unstable areas</p>	<p>Definite decrease in clinical cases</p> <p>Might be more cost effective than intensive dipping especially when considering that cattle are not normally sold and do have a long live expectancy in those areas</p>	<p>Vaccine is dispatched frozen and cold chain is difficult to maintain in this setting (de Vos <i>et al.</i> 2004)</p>	<p>The endemic state needs to be constantly reassessed which might require large financial and human resources</p> <p>Vaccine is not entirely safe and reactions in adult animals can occur (de Vos <i>et al.</i> 2004)</p>
<p>Measure 4</p> <p>No dipping of calves under 6 months</p>	<p>Calves are exposed to infection while still protected by immunity</p> <p>Would allow for a gradual change in procedures</p>	<p>potential limitations in weight gain if tick numbers are overwhelming</p> <p>exposure to infected ticks later in life needs to be ensured to maintain immunity</p>	<p>Owners believe that calves need to be taught to dip early as they consider them more difficult to dip at later stage if not trained early</p>

Control strategy	Advantages	Disadvantages	Complications in the implementation
<p>Measure 5</p> <p>Strategic acaricides application on udders and extremities of adult cattle</p>	<p>Would allow for the control of damage caused by <i>Amblyomma</i> and <i>Hyalomma spp</i> while exposure and consequent resistance to <i>Boophilus spp</i>.</p>	<p>More labour intensive</p>	<p>If this is combined with a reduction in dipping similar complications as mentioned above in Measure 1 may complicate implementation</p>
<p>Measure 6</p> <p>Use of resistant cattle breeds</p>	<p>Most cattle in communal areas are based on indigenous breeds and should have a higher resistance against ticks</p>	<p>Cattle with a significant zebu content can lose the infection within two years (Johnston <i>et al.</i> 1978) and this may contribute to endemic instability if insufficient infected ticks are available to ensure exposure of calves</p>	<p>Cattle are mainly purchased from commercial farms, which leads to a dilution of indigenous breeds with European breeds, as cattle are not generally sold within the communal areas and it is unlikely that this practice is easily influenced or changed</p>
<p>Measure 7</p> <p>Treatment of clinical cases</p>	<p>Loss could be prevented and immunity established in affected animals</p>	<p>Labour intensive</p> <p>If wrongly applied could sterilize infection and prevent immunity from developing</p>	<p>At present impossible to implement as no trained personnel is available for the administration of treatments and there is hardly any local availability of treatments</p>

From a purely epidemiological and economic point of view a beneficial control strategy in an endemic area could make use of several of the above stated measures in the following way:

- Continued dipping of adult cattle, as the present situation is unstable
- No dipping of cattle under 6 months, consequent marking of these cattle and if exposure to infected ticks is sufficient, no further plunge dipping of these animals
- Gradual replacement of plunge dipping with strategic application of acaricides to control *Amblyomma*, *Hyalomma* and other multi-host ticks as well as Tse Tse flies. Since no communal structure such as dip tanks are needed for this, responsibility of dipping and the costs thereof can be gradually shifted to cattle owners
- Promotion of the use of more tick resistant indigenous breeds. Although probably no difference in the purchase of cattle can be achieved, practices such as use of bulls that have a high indigenous breed content and castration of other bulls, could be promoted through knowledge communication.
- Enhancement of knowledge on the treatment and the availability of non-sterilizing drugs in the community.

However, besides epidemiology and economics, other cultural and sociological influences are likely to play a large role and need to be carefully considered before any change in the dipping regimen should be implemented. This would require extensive resources for further research into the perception of cattle owners and the knowledge communication on the advantages of this change. To achieve any success through a change of strategy, the following requirements need to be satisfied:

- More information needs to be collected to see if sufficient numbers of infected ticks can be achieved to establish an endemic stability if dipping is stopped. This could be addressed through a research project that would over a period of time such as 1-3 years, periodically investigate the serological status as well as numbers and species of *Boophilus* ticks and their infection rate on calves in the absence of dipping.
- Another structure of contact with and communication to livestock owners for veterinary services needs to be established. This could possibly be aided through the creation of mobile outreach clinics for primary veterinary care and advice and organization of cattle owners into livestock associations, as it is already practiced in some magisterial districts.
- Further research should be conducted to see if communities would find a change in strategy acceptable.

Dipping of cattle in South Africa started in an effort to control and eradicate East Coast Fever in the '30's and 40's. By the '50's the disease was under control and eradicated from SA. However as the dipping infrastructure was already there the dipping continued for the control of Babesiosis, Anaplasmosis and in some areas of course for Heartwater. Since dipping has been an integral part of disease control for such a long time in these areas, owners have come to depend on this service and expect this service to be continued. Extensive knowledge communication campaigns need to be undertaken to increase knowledge of tick borne diseases and to make the communities understand why the dipping strategy is changed, as dipping is regarded as a very important and established measure and lack of understanding could lead to mistrust of the communities towards veterinary services.

5.2 The Questionnaire survey

Several sources of bias did occur in this survey:

The convenience selection of interviewees during the dipping procedure constituted the first source of bias, as this approach limited the response group to cattle owners, who cooperate with veterinary services and consider dipping important. However, even the owner status of respondents can not necessarily be assumed, since frequently owners are employed outside the communal lands and parents and/or their children take care of the livestock. The high average age of the persons interviewed, might indicate, that they were possibly the parents of the actual stockowners. Due to the length of the questionnaire and the implicated amount of time necessary to answer the questions, it is possible, that persons with a more positive attitude to veterinary services were included. Considering all of the above the findings of this survey may not be representative of the rural communities in Kwa Zulu Natal. During the analysis the impression was gained, that further bias was attributable to variation between the technicians conducting the survey, which might have been enhanced through the individual translation of questionnaires, as they were designed in English and translated into Zulu during the interview. Some discrepancies occurred in the answers which highlight that care should be taken when interpreting the results. An example of this was, that several owners stated in the beginning of the interview, not to own dogs, but answered at a later stage, that their dogs were vaccinated against rabies within the last year. (The answer to this question was not included in this thesis).

Since unequal numbers of questionnaires were collected in the different magisterial districts, some districts are represented more strongly than others in the overall result.

Since the change of government took place in 1994, increased attention has been paid to the communal farmers that were previously poorly represented in research projects. Several studies that investigated the needs, perceptions and present disease and parasite control practices in the rural areas of South Africa have been conducted since then: (Dreyer *et al.* 1999; Getchell *et al.* 2002; Hlatshwayo and Mbatlali 2005; Krecek *et al.* 1995; Makgatho *et al.* 2005; Masika *et al.* 1997a; Masika *et al.* 1997b; McCrindle *et al.* 1994). Although they confer a similar picture, different weightings of problems and perceptions are likely to occur in the various rural communities and the contribution of this survey is to allow an initial insight into the perceptions of the rural farmers in Kwa Zulu Natal, who were until recently not included in any of the research undertaken.

Considering the demographics, parallels to other studies are present. For example the average number of 12 cattle owned in this study does not differ much from that found by Dreyer *et al.* (1999) and also the species owned and their relative frequency is very similar to findings from other South African rural areas (Dreyer *et al.* 1999; McCrindle *et al.* 1994). The declared frequent use of the blackquarter vaccine is not surprising, as this vaccine is administered free of charge to a certain proportion of owners on a yearly basis by veterinary services. Since the clinical signs are very dramatic and the disease is known to occur frequently, awareness is high and so is the desire to have the stock vaccinated. Deworming appears to be in frequent practice, but efficacy may be questionable, as owners deworming their animals, were also significantly ($p < 0.05$) more likely to state that worms were a health problem in their cattle. Long intervals between worm treatments found here and the fact that worms were quoted to be an important health problem in cattle in this study, support the likelihood of inefficacy of internal parasite treatment, an effect that was also observed by Getchell *et al.* (2002) who ,

besides long intervals also found, that the medication is often underdosed. To further investigate the efficiency of deworming practices, more information on these practices should be collected, faecal egg counts conducted. Then advice on appropriate treatment, as well as management practices should be integrated in the program of farmer's days and other information pathways.

Many owners used additional external parasite control such as the use of pour-ons. The application of such measures could indicate that tick control by means of dipping is considered insufficient. Indeed it was observed during another study in this area, that owners preferred to have their cattle relatively free of ticks (Emslie 2004), a finding that was repeated in a study in a different rural area, where owners went through great efforts to render their cattle completely tick free (Masika *et al.* 2000). This intention is likely to have contributed to the endemic instability for babesiosis that was observed in the serological part of this survey. Ticks were also mentioned as the most important health problem in most of the districts, mirroring the findings of Dreyer *et al.* (1999). Unfortunately, seen under the light of the above, it remains unclear for both studies, whether this refers to the ticks themselves, resulting abscesses or associated diseases.

The results of another study that found the killing of ticks to be the most important reason for tick control followed by disease control and damage to teats give the impression that the ticks themselves might be considered the problem (Hlatshwayo and Mbatlali 2005). Not much additional information could be gained from the answers given to the question relating to dipping intervals, as they appear to follow the frequency with which veterinary services implements dipping in the various districts. The high proportion of owners dipping their cattle, although corresponding with findings of another study (Masika *et al.* 1997b), should be interpreted with care, due to the above

mentioned bias of selecting interviewees during the dipping procedure, which occurred in both studies. However, since dipping is free of charge and owners appear to desire low tick counts, this proportion might well reflect reality, irrespective of the bias that may have occurred. Dipping of cattle in South Africa started in an effort to control and eradicate East Coast Fever in the '30's and 40's. By the '50's the disease was under control and eradicated from SA. However as the dipping infrastructure was already there the dipping continued for the control of Babesiosis, Anaplasmosis and in some areas of course for Heartwater.

Since dipping has been an integral part of disease control for such a long time in these areas, owners have come to depend on this service and expect this service to be continued.

Similar to the findings of another study (Masika *et al.* 2000) traditional medicines appear to play an important role in the treatment of animals in this context. Knowledge of herbal medicine, that is likely to have accumulated over many years bares further exploration, as previous research has shown that many plants in use in South Africa do show marked pharmacological activity (Fourie *et al.* 1992). No association was found between the use of traditional medicine and believing in witchcraft, which may indicate that use of traditional remedies and traditional believes are not necessarily linked. As in other rural areas (Masika *et al.* 1997a) diseases, together with lack of knowledge are considered to be an important management problem, while the lack of markets was less strongly felt in this context. Since many owners do work in different occupations such as mining outside the rural area, it is likely that cattle are mainly kept for cultural and status related reasons and that an enhanced production and marketing of cattle is not necessarily desired, although the loss of animals due to diseases is strongly felt. Since

the answers to this question were recorded in a multiple choice fashion, it is possible that other problems such as the lack of water and missing of fences that were experienced as very important in another study (McCrindle *et al.* 1994) were present, but not captured through this survey as they were not included as options in the answer. Since any animal health initiative is known to be effective only if it is supported and understood by the local farmers and reflects their needs, (Penrith and Thomson 2004) further research on where knowledge gaps exist and what additional knowledge would be perceived as beneficial, is essential before more specific programmes for extension work can be designed.

Participatory workshops and focus groups have been found very useful in this context (Kaasschieter *et al.* 1992; McCrindle *et al.* 1994) and could be initiated by taking the results of this survey back into the community for discussion. General subject areas of such workshops and further research could include knowledge of risk factors for the diseases that were found to occur here, external and internal parasite management, and matters outside the animal health arena, such as a desire to increased livestock production and whether this could be sustainable by the environment.

The knowledge accumulated through such an approach, could then be transformed and further focused training of animal health technicians and community based health workers, which could advise cattle owners on management and health questions. Such extension work is also very important to limit the impact of diseases and to increase the awareness of risk reduction for infection with the present diseases through husbandry and management measures. It appears that any development and disease control measures initiated can only be effective if they are approached in an holistic manner and include and cooperate with other disciplines such as agriculture and sociology

(Kaasschieter *et al.* 1992; Penrith and Thomson 2004). Preferences for the channels of communication of such extension measures varied among the districts in this survey, but this variability might have been caused by the fact that owners preferred types of extension work they were already exposed to and which were not uniform throughout the province. Animal health technicians were shown in this survey to play an important role in providing advise to local farmers, but their involvement in knowledge communication is presently largely dependent on their own motivation and their priorities determined by individual state vets. This would need to be addressed through provincial measures if an equal impact of extension work on all districts within the province is desired.

6 RECCOMENDATIONS

Recommendations for the individual diseases have been made during the discussion. However considering the variety of diseases that proved to be present in this survey and taking into account that other diseases such as rabies and classical swine fever also cause significant problems in the province it appears of value to prioritize diseases to be controlled and to develop a provincial strategy. Prioritization could be initiated through scoring the diseases for different aspects: cost of control, impact on livestock production, zoonotic potential and impact and perceived importance of the control of that condition by communities. In the implementation of a campaign the control should not remain limited to actual control measures such as vaccination but focus on preventive measures such as housing and management and strongly aim to include the local communities in their development. To increase the effect of any control strategy it

should be attempted to synchronize it with the national government and campaigns in planning or implementation in the neighbouring provinces.

7 CONCLUSION

This study has served as a baseline survey to determine the prevalence of diseases considered important in cattle and allows the following conclusions:

Brucellosis is present in the north-eastern area, while likely to be absent from the remainder of the province. The within herd prevalence of this disease varied from low to high.

Leptospirosis was found throughout the province with an increased geographical density of affected diptanks in the south eastern area of the province. The variety of serovars discovered, make it likely that the epidemiology of this disease is multifactorial.

A large proportion of sampled diptanks achieved endemic stability for *Babesia bovis*, and the vector of the disease *Boophilus microplus* can be assumed to be present throughout the province. This is not true for *Babesia bigemina*, for which the serological prevalence was very low and no endemic stability was reached.

Enzootic bovine leucosis is geographically widely spread in the communal areas of Kwa Zulu Natal and the serological prevalence varied in between districts from absent to very high, but the majority of districts showed a low prevalence. The highest prevalence was found in the central area of the province.

Anaplasmosis is present throughout the study and the large majority of diptanks are endemically stable or approaching stability.

Although the questionnaire survey can not be regarded as representative for the rural farming community in Kwa-Zulu Natal, the general impression gained from this survey has many parallels with other studies conducted in this context: Diseases and lack of knowledge do appear to be important management issues for farmers, preventive measures such as vaccination, dipping and deworming are considered important and are practiced. However their efficacy or beneficial effect on for example the impact of tick borne diseases remains questionable and needs to be addressed through further research and knowledge communication. Veterinary services do appear to play an important role and are a frequent point of contact for local farmers. The opinion on veterinary services although possibly biased or influenced by the personnel conducting the interview, appears to be very good which forms a solid basis of trust for further collaboration and development initiatives.

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Appendix

Sampling frame and prevalence for *Anaplasma marginale* at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	14	377	188	51	29 - 0.70	23 818
BABANANGO	12	416	349	89	59 - 96	14 772
BERGVILLE	19	451	336	78	65 - 86	32 105
DANNHAUSER	10	414	307	78	60 - 0.90	11 054
ESHOWE	11	377	281	78	64 - 87	31 978
ESTCOURT	10	377	279	78	62 - 80	25 118
HLABISA	16	482	398	87	71 - 99	52 763
INGWAVUMA	14	418	263	65	53 - 72	79 520
IXOPO	6	298	255	90	75 - 100	29 580
KLIPRIVER	17	415	327	83	70 - 90	47 730
MAHLABATINI	16	499	388	82	71 - 87	60 439
MAPUMULO	13	459	259	58	45 - 66	57 184
MPENDLE	6	300	258	91	72 - 100	24 433
MSINGA	10	355	272	81	68 - 87	61 143
MTONJANENI	9	243	184	80	65 - 89	22 387
MTUNZINI	5	191	152	84	69 - 93	9 462
NDWEDWE	13	399	256	67	54 - 74	20 645
NEW HANOVER	11	451	304	70	58 - 77	
NEWCASTLE	10	401	300	79	64 - 88	12 366
NKANDLA	12	474	288	63	41 - 81	78 520
NONGOMA	16	484	343	74	59 - 84	104 544
NQUTU	27	400	355	94	86 - 100	44 488
PAUL PIETERSBURG	4	179	124	72	40 - 88	15 300
PIETERMARITZBURG	10	408	308	79	68 - 85	17 734
PINETOWN	5	125	53	43	2 - 84	7 000
POLELA	11	446	385	91	81 - 97	38 275
PORT SHEPSTONE	12	446	332	78	63 - 87	12 124
RICHMOND	4	161	132	86	40 - 88	1 336
SIMDLANGENTSHA	6	169	128	80	66 - 89	
UBOMBO	15	449	309	72	60 - 78	64 011
UMBUMBULU	16	459	320	73	57 - 83	14 834
UMZINTO	21	386	278	75	55 - 89	25 505

Sampling frame and prevalence for *Babesia bigemina* at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	14	377	91	11	2 - 27	23 818
BABANANGO	12	416	186	36	21 - 42	14 772
BERGVILLE	19	451	199	36	21 - 48	32 105
DANNHAUSER	10	414	85	7	0 - 22	11 054
ESHOWE	11	377	88	10	0 - 23	31 978
ESTCOURT	10	377	207	49	35 - 61	25 118
HLABISA	16	482	18	0	0 - 0	52 763
INGWAVUMA	14	418	64	0	0 - 15	79 520
IXOPO	6	298	147	42	16 - 68	29 580
KLIPRIVER	17	415	150	26	15 - 37	47 730
MAHLABATINI	16	499	48	0	0 - 14	60 439
MAPUMULO	13	459	118	13	3 - 23	57 184
MPENDLE	6	300	141	39	15 - 63	24 433
MSINGA	10	355	191	47	37- 56	61 143
MTONJANENI	9	243	16	0	0 - 1	22 387
MTUNZINI	5	191	42	9	0 - 35	9 462
NDWEDWE	13	399	81	6	2 - 15	20 645
NEW HANOVER	11	451	190	33	18 - 47	
NEWCASTLE	10	401	35	0	0 - 4	12 366
NKANDLA	12	474	86	4	2 - 17	78 520
NONGOMA	16	484	12	0	0 - 0	104 544
NQUTU	27	400	30	0	0 - 3	44 488
PAUL PIETERSBURG	4	179	29	1	0 - 11	15 300
PIETERMARITZBURG	10	408	105	13	5 - 27	17 734
PINETOWN	5	125	24	5	0 - 35	7 000
POLELA	11	446	222	42	22- 53	38 275
PORT SHEPSTONE	12	446	114	13	2 - 30	12 124
RICHMOND	4	161	33	7	0 - 11	1 336
SIMDLANGENTSHA	6	169	28	2	0 - 16	
UBOMBO	15	449	51	0	0 - 13	64 011
UMBUMBULU	16	459	97	7	2- 23	14 834
UMZINTO	21	386	122	20	5 - 35	25 505

Sampling frame and prevalence for *Babesia bovis* at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	14	377	195	51	38 - 65	23 818
BABANANGO	12	416	324	79	55 - 88	14 772
BERGVILLE	19	451	387	88	83 - 93	32 105
DANNHAUSER	10	414	250	61	44 - 78	11 054
ESHOWE	11	377	225	60	44 - 76	31 978
ESTCOURT	10	377	350	95	90 - 100	25 118
HLABISA	16	482	339	71	60 - 83	52 763
INGWAVUMA	14	418	292	71	59 - 83	79 520
IXOPO	6	298	208	71	50 - 92	29 580
KLIPRIVER	17	415	390	96	87 - 100	47 730
MAHLABATINI	16	499	420	86	79 - 93	60 439
MAPUMULO	13	459	350	77	69 - 86	57 184
MPENDLE	6	300	203	68	54 - 83	24 433
MSINGA	10	355	308	89	84 - 94	61 143
MTONJANENI	9	243	75	29	16 - 42	22 387
MTUNZINI	5	191	131	69	43 - 95	9 462
NDWEDWE	13	399	319	81	75 - 88	20 645
NEW HANOVER	11	451	356	80	73 - 88	
NEWCASTLE	10	401	333	85	72 - 97	12 366
NKANDLA	12	474	292	62	47 - 77	78 520
NONGOMA	16	484	336	70	59 - 82	104 544
NQUTU	27	400	347	89	85 - 96	44 488
PAUL PIETERSBURG	4	179	134	76	54 - 92	15 300
PIETERMARITZBURG	10	408	361	90	84 - 97	17 734
PINETOWN	5	125	41	31	2 - 64	7 000
POLELA	11	446	320	73	62 - 77	38 275
PORT SHEPSTONE	12	446	235	52	41 - 64	12 124
RICHMOND	4	161	140	89	54 - 92	1 336
SIMDLANGENTSHA	6	169	123	74	63 - 81	
UBOMBO	15	449	129	27	12 - 43	64 011
UMBUMBULU	16	459	266	58	48 - 68	14 834
UMZINTO	21	386	183	47	33 - 61	25 505

Sampling frame and prevalence for *Brucella abortus* at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	15	1509	1	0	0 - 0.3	23 818
BABANANGO	15	1703	1	0	0 - 0.3	14 772
BERGVILLE	19	1650	0	NA	NA	32 105
DANNHAUSER	10	1631	0	NA	NA	11 054
ESHOWE	13	1506	2	0.0	0 - 0.6	31 978
ESTCOURT	10	1500	0	NA	NA	25 118
HLABISA	16	1583	27	2.4	0.5 - 5	52 763
INGWAVUMA	14	1370	144	15.6	6 - 28	79 520
IXOPO	15	1500	0	NA	NA	29 580
KLIPRIVER	17	1672	0	NA	NA	47 730
LOWER UMFOLOZI	16	1553	89	8.4	3 - 14	37 883
MAHLABATINI	16	1550	5	0.3	0 - 0.01	60 439
MAPUMULO	20	1531	2	0.0	0 - 0.6	57 184
MPENDLE	13	1300	0	NA	NA	24 433
MSINGA	13	1614	3	0.1	0 - 0.9	61 143
MTONJANENI	9	936	7	1.0	0 - 2	22 387
MTUNZINI	6	809	0	NA	NA	9 462
NDWEDWE	16	1504	0	NA	NA	20 645
NEW HANOVER	11	1742	0	NA	NA	
NEWCASTLE	10	1544	0	NA	NA	12 366
NKANDLA	12	1770	0	NA	NA	78 520
NONGOMA	17	1508	106	10.4	5 - 16	104 544
NQUTU	27	1605	0	NA	NA	44 488
PAUL PIETERSBURG	4	464	0	NA	NA	15 300
PIETERMARITZBURG	10	1565	0	NA	NA	17 734
PINETOWN	5	253	0	NA	NA	7 000
POLELA	19	1897	0	NA	NA	38 275
PORT SHEPSTONE	12	1503	0	NA	NA	12 124
RICHMOND	4	616	0	NA	NA	1 336
SIMDLANGENTSHA	8	528	0	NA	NA	
UBOMBO	15	1509	56	5.4	1 - 11	64 011
UMBUMBULU	17	1503	1	0	0 - 0.3	14 834
UMZINTO	21	1531	4	0.2	0.3 - 0.4	25 505

Sampling frame and prevalence for Enzootic bovine leucosis at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	12	60	0	0	NA	23 818
BABANANGO	12	60	11	16.8	4 - 34	14 772
BERGVILLE	15	52	3	3	0- 11	32 105
DANNHAUSER	10	50	0	0	NA	11 054
ESHOWE	12	70	4	3	0 - 12	31 978
ESTCOURT	10	60	11	16.8	0 - 42	25 118
HLABISA	15	75	8	8.4	3 - 17	52 763
INGWAVUMA	14	56	1	0	0 - 5	79 520
IXOPO	8	61	8	11	0.8 - 26	29 580
KLIPIRIVER	17	61	23	28.1	21 - 59	47 730
LOWER UMFOLOZI	16	80	8	7.7	2 - 17	37 883
MAHLABATINI	15	75	3	1	0 - 10	60 439
MAPUMULO	13	77	22	28.1	15 - 44	57 184
MPENDLE	6	48	4	5.9	0- 27	24 433
MSINGA	12	48	32	70	54 - 88	61 143
MTONJANENI	9	76	3	1	0- 9	22 387
MTUNZINI	5	60	1	0	0 - 3	9 462
NDWEDWE	12	60	1	0	0- 4	20 645
NEW HANOVER	11	66	3	1.7	0 - 11	
NEWCASTLE	10	80	1	0	0 - 3	12 366
NKANDLA	12	60	5	5.9	0 - 18	78 520
NONGOMA	16	80	3	0.8	0 - 12	104 544
NQUTU	27	81	6	4.8	1 - 14	44 488
PAUL PIETERSBURG	4	25	0	0	NA	15 300
PIETERMARITZBURG	10	60	0	0	NA	17 734
PINETOWN	5	50	2	1	0 - 7	7 000
POLELA	10	80	0	0	NA	38 275
PORT SHEPSTONE	6	54	5	6.9	0.2 - 17	12 124
RICHMOND	4	48	0	0	NA	1 336
SIMDLANGENTSHA	6	29	0	0	NA	
UBOMBO	15	60	7	9.5	3 - 20	64 011
UMBUMBULU	9	80	3	0.8	0 - 21	14 834
UMZINTO	15	60	1	0	0 - 5	25 505

Sampling frame and prevalence for Leptospirosis at district level

District	Number of dips sampled	Number of animals sampled	Number of animals positive	True prevalence in percent	90% CI in percent	Cattle population in district
ALFRED	12	60	31	51.8	31.8 - 72	23 818
BABANANGO	12	60	0	0	0 - 0	14 772
BERGVILLE	19	58	6	4.9	0.1 - 21	32 105
DANNHAUSER	10	50	0	0	NA	11 054
ESHOWE	11	60	5	2.6	1 - 14	31 978
ESTCOURT	10	60	3	0	0 - 12	25 118
HLABISA	15	75	1	0	0 - 4	52 763
INGWAVUMA	14	56	2	0	0 - 8	79 520
IXOPO	6	56	18	29.7	14 - 44	29 580
KLIPRIVER	17	61	7	6.2	1 - 20	47 730
LOWER UMFOLOZI	16	80	16	15.9	6 - 32	37 883
MAHLABATINI	16	80	3	0	0 - 11	60 439
MAPUMULO	13	77	25	30	13 - 50	57 184
MPENDLE	6	48	29	61.8	28 - 95	24 433
MSINGA	13	52	10	15	0.1 - 30	61 143
MTONJANENI	8	66	5	1.7	0 - 012	22 387
MTUNZINI	5	60	12	15.9	3 - 28	9 462
NDWEDWE	12	60	27	44.3	21 - 68	20 645
NEW HANOVER	9	66	37	56.8	30 - 87	
NEWCASTLE	10	72	1	0	0 - 3	12 366
NKANDLA	12	60	9	10.2	3 - 25	78 520
NONGOMA	16	80	2	0	0 - 5	104 544
NQUTU	26	78	11	9.2	4 - 24	44 488
PAUL PIETERSBURG	4	25	5	15.9	0 - 36	15 300
PIETERMARITZBURG	10	60	12	15.9	0 - 42	17 734
PINETOWN	5	50	4	2.2	0 - 14	7 000
POLELA	11	72	19	23.1	6 - 32	38 275
PORT SHEPSTONE	7	63	25	38.2	13 - 62	12 124
RICHMOND	4	48	27	57	27 - 87	1 336
SIMDLANGENTSHA	6	25	3	6.8	0.7 - 18	
UBOMBO	15	60	10	12	0.5 - 33	64 011
UMBUMBULU	9	80	12	10	4 - 27	14 834
UMZINTO	15	60	32	53.7	33 - 76	25 505

Survey results at diptank level for Brucellosis

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	100	0	93	119	0
98	100	0	268	43	0
432	100	0	1521	54	0
108	100	1	475	192	1
100	100	0	997	187	0
841	59	0	445	219	0
99	100	0	407	72	0
101	100	0	445	71	0
261	100	0	26	63	0
446	100	0	Witkop	138	0
146	100	0	Ngwebeni A	143	0
425	100	0	Boschoek A	143	0
479	100	0	New Adelaide	124	0
438	150	0	402	127	0
Ridges	100	0	Witklip B	8	0

Bergville			Dannhauser		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
246	102	0	1	165	0
235	6	0	269	244	0
271	100	0	294	171	0
165	16	0	501	169	0
238	102	0	Peachill/Mooi	168	0
135	100	0	Springbok	166	0
2033	101	0	9168	171	0
2005	158	0	9	171	0
166	104	0	Uitkyk	188	0
2010	50	0	Doringkop	18	0
134	48	0			
206	29	0			
543	33	0			
299	102	0			
164	34	0			
236	140	0			
167	105	0			
235	120	0			
2009	200	0			



Hlabisa			Eshowe		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
528	100	0	293	110	0
519	100	2	891	31	0
694	100	0	891	185	0
518	100	2	428	175	0
754	100	2	Ofasimba	100	0
525	200	0	660	100	0
523	100	0	592	153	0
669	90	1	659	186	0
746	36	1	717	100	0
517	100	0	662	157	2
944	100	4	601	116	0
966	85	9	953	81	0
526	100	4	663	12	0
945	100	0			
692	72	0			
527	100	2			

Ixopo			Ingwawuma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
76	100	0	311	117	17
78	100	0	814	89	1
91	100	0	509	115	16
123	100	0	493	100	34
124	100	0	569	100	1
125	100	0	809	90	4
406	100	0	803	100	25
408	100	0	789	100	2
409	100	0	682	115	4
420	100	0	787	100	3
472	100	0	752	100	1
483	100	0	497	100	26
823	100	0	576	100	8
847	100	0	320	44	2
Springvale	100	0			



Klipriver			Lower Umfolozi		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
2027	52	0	756a	69	5
2039	120	0	821	100	8
2037	103	0	756b	100	1
2035	214	0	845	100	3
2036	106	0	785	100	19
2061	79	0	614	100	1
2019	114	0	693	100	8
2062	97	0	501	100	6
8483	100	0	691	100	2
1341	100	0	856	100	15
2176	100	0	852	100	13
1374	74	0	620	84	0
2405	72	0	617	100	3
11697	41	0	619	100	0
1151	100	0	616	100	5
1244	100	0	618	100	0
28	100	0			



Mapumolo			labatini		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
482	64	0	547	100	1
454	90	0	548	100	0
456	17	0	829	100	1
38	125	0	903	100	0
316	135	0	867	100	0
157	61	0	776	100	0
211	157	0	718	150	2
440	62	0	233	100	0
215	85	0	775	100	1
770	43	0	761	100	0
38	9	0	550	100	0
303	44	0	340	100	0
449	16	0	434	100	0
251	187	0	Dunga	100	0
39	100	2	Bhade	100	0
301	72	0			
40	48	0			
405	70	0			
156	75	0			
290	71	0			

Mpendele			New Hanover		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
Essex Farm	100	0	921	150	0
66601	100	0	938	160	0
118	100	0	Mophela	150	0
119	100	0	224	154	0
120	100	0	178	175	0
121	100	0	177	151	0
268	100	0	267	172	0
280	100	0	67B	160	0
351	100	0	279	166	0
430	100	0	880	144	0
439	100	0	53	160	0
931	100	0			
940	100	0			



Msinga			Mogonjaneni		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
18	150	0	318	75	0
298	163	0	608	65	0
228	154	0	670	60	1
929	107	0	Ndundulu	56	0
140	125	0	728	100	1
15	100	3	738	90	0
14	94	0	675	194	1
16	128	0	735	100	0
77701	50	0	611	196	4
8	150	0			
2031	95	0			
2001	109	0			
5	189	0			

Mtunzini			Ndwedwe		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
230	148	0	481	104	0
631	150	0	49	96	0
636	152	0	218	82	0
628	47	0	243	48	0
629	156	0	43	70	0
634	156	0	418	87	0
			242	39	0
			217	145	0
			181	281	0
			50	69	0
			250	173	0
			48	63	0
			461	18	0
			183	145	0
			404	83	0
			184	1	0



Newcastle			Jkandla		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
156	96	0	604	86	0
500	169	0	734B	210	0
84	208	0	604	72	0
334	187	0	578	240	0
Birkenstock	233	0	375	10	0
108	166	0	600	115	0
8883	171	0	734	210	0
108B	170	0	901	86	0
Madadeni	127	0	354	209	0
Madadeni Kraal	17	0	865	177	0
			900	208	0
			579	55	0
			750	52	0
			Nkiwani	40	0



Nqutu			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
740	58	0	536	77	6
Nhlambamasoka	58	0	535	100	18
568	58	0	723	53	0
Kwanjezi	58	0	672	100	0
560	58	0	748	100	9
Mayikana	80	0	541	100	6
555	58	0	981	100	0
553	58	0	536	29	2
Mvuzini	58	0	782	100	12
552	59	0	665	100	4
Ingogo	58	0	723	50	3
556	58	0	909	100	0
Siyakahathala	58	0	758	100	4
Isandluwana	62	0	Oqothweni	99	8
Nyloya	58	0	Esikuthweni	100	12
557	58	0	748B	100	13
916	58	0	541B	100	9
Ntanyandlovu	80	0			
Maceba	58	0			
Ndashana	63	0			
561	58	0			
Magogo	90	0			
Magaga	60	0			
Haladu	58	0			
Skutu	58	0			
558	58	0			
934	9	0			

Pinetown		
Diptank	Number tested	Number positive
272	114	0
222	41	0
481	15	0
32	64	0
411	19	0

Pietermaritzburg		
Diptank	Number tested	Number positive
926	150	0
170	150	0
169	150	0
57	159	0
264	159	0
58	150	0
56	152	0
937	164	0
171	168	0
945	163	0

Paul Pietersburg		
Diptank	Number tested	Number positive
250	72	0
253	76	0
252	84	0
248	116	0



Polela		
Diptank	Number tested	Number positive
111	100	0
112	100	0
113	100	0
114	100	0
115	100	0
161	100	0
163	100	0
204	100	0
236	100	0
257	100	0
258	100	0
270	100	0
288	100	0
306	100	0
394	100	0
398	98	0
943	99	0
123	100	0
124	100	0

Port Shepstone		
Diptank	Number tested	Number positive
208	112	0
774	136	0
389	137	0
210	134	0
942	137	0
319	136	0
247	137	0
260	63	0
19	142	0
363	113	0
83	117	0
88	139	0

Richmond		
Diptank	Number tested	Number positive
308	145	0
466	161	0
405	150	0
403	160	0

Ubombo		
Diptank	Number tested	Number positive
743	109	6
766	100	0
813	97	12
513	90	19
510	100	8
939	100	6
701	100	1
898	97	0
936	100	4
514	100	0
597	100	0
820	109	0
500	100	0
678	100	0
937	107	0

Umbumbulu		
Diptank	Number tested	Number positive
248	19	0
467	110	0
248	36	0
63	127	1
240	123	0
159	138	0
227	111	0
160	86	0
191	5	0
470	142	0
941	130	0
469	79	0
470	1	0
60	112	0
248	54	0
281	200	0
64	30	0

Umzinto		
Diptank	Number tested	Number positive
231	77	4
10	22	0
198	75	0
12	78	0
962	47	0
200	72	0
72	72	0
139	72	0
230	77	0
70	70	0
414	75	0
477	75	0
822	76	0
17	60	0
920	72	0
231	22	0
269	100	0
196	36	0
935	137	0
276	115	0
948	101	0

Estcourt		
Diptank	Number tested	Number positive
144	150	0
365	100	0
5550	200	0
205	150	0
Wembezi C	150	0
Sobalili	150	0
Good Home	150	0
Dungamanzi	150	0
Ripley	150	0
Ganna-Hoek	150	0

Simdlangentsha		
Diptank	Number tested	Number positive
33	133	0
212	56	0
37	70	0
132	55	0
145	58	0
21	147	0
36	64	0
888801	127	0

Survey results at diptank level for Enzootic bovine leucosis

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	5	0	93	5	1
98	5	0	268	5	2
432	5	0	1521	5	1
108	5	0	475	5	0
100	5	0	997	5	0
841	5	0	445	5	0
99	5	0	407	5	0
101	5	0	445	5	0
261	5	0	26	5	1
446	5	0	Witkop	5	0
146	5	0	Ngwebeni A	5	3
425	5	0	New Adelaide	5	3



Ixopo			Klip River		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
78	10	0	2027	4	1
91	8	2	2039	4	0
123	5	0	2037	4	0
124	8	0	2035	4	0
420	8	0	2036	4	0
472	8	2	2061	4	2
483	5	3	2019	3	1
823	9	1	2062	3	0
			8483	4	3
			1341	4	3
			2176	4	2
			1374	4	2
			2405	3	2
			11697	3	3
			1151	3	2
			1244	3	0
			28	3	2

Lower Umfolozi			Mapumolo		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
756a	5	0	482	9	5
821	5	2	454	9	2
756b	5	1	456	9	1
845	5	2	38	5	0
785	5	1	316	5	0
614	5	0	157	5	2
693	5	0	211	5	1
501	5	0	440	5	0
691	5	0	215	5	2
856	5	1	770	5	1
852	5	0	303	5	3
620	5	0	449	5	2
617	5	0	251	5	3
619	5	1			
616	5	0			
618	5	0			



Ndwedwe			Newcastle		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
481	5	0	156	8	0
49	5	0	500	8	1
218	5	0	84	8	0
243	5	1	334	8	0
43	5	0	Birkenstock	8	0
418	5	0	108	8	0
242	5	0	8883	8	0
217	5	0	108B	8	0
181	5	0	Madadeni	8	0
250	5	0	Madadeni Kraal	8	0
48	5	0			
183	5	0			

Nkandla		
Diptank	Number tested	Number positive
734B	5	0
604	5	0
578	5	0
600	5	1
734	5	0
901	5	0
354	5	2
865	5	0
900	5	0
579	5	0
750	5	0
Nkiwani	5	2



Nqutu			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
740	3	0	536	5	0
Nhlambamasoka	3	1	535	5	0
568	3	0	723	5	0
Kwanjezi	3	0	672	5	0
560	3	1	748	5	0
Mayikana	3	0	541	5	0
555	3	0	981	5	0
553	3	0	536	5	0
Mvuzini	3	0	782	5	0
552	3	0	665	5	0
Ingogo	3	0	909	5	0
556	3	0	758	5	0
Siyakahathala	3	0	oqothweni	5	3
Isandluwana	3	1	Esikuthweni	5	0
Nyloya	3	2	748B	5	0
557	3	0	541B	5	0
916	3	0			
Ntanyandlovu	3	0			
Maceba	3	1			
Ndashana	3	0			
561	3	0			
Magogo	3	0			
Magaga	3	0			
Haladu	3	0			
Skutu	3	0			
558	3	0			
934	3	0			

Paul Pietersburg		
Diptank	Number tested	Number positive
250	5	0
253	5	0
252	5	0
248	5	0

Pietermaritzburg			Pinetown		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
926	6	0	272	10	0
170	6	0	222	10	0
169	6	0	481	10	0
57	6	0	32	10	1
264	6	0	411	10	1
58	6	0			
56	6	0			
937	6	0			
171	6	0			
945	6	0			



Polela		
Diptank	Number tested	Number positive
111	8	0
113	8	0
114	8	0
161	8	0
163	8	0
270	8	0
306	8	0
394	8	0
124	8	0
398	8	0

Port Shepstone		
Diptank	Number tested	Number positive
112	9	0
136	9	1
134	9	2
137	9	2
136	9	0
142	9	0

Richmond		
Diptank	Number tested	Number positive
308	12	0
466	12	0
405	12	0
403	12	0

Simdlangentsha		
Diptank	Number tested	Number positive
33	5	0
212	5	0
37	5	0
132	4	0
145	5	0
21	5	0

Ubombo		
Diptank	Number tested	Number positive
743	4	1
766	4	1
813	4	0
513	4	1
510	4	1
939	4	0
701	4	0
898	4	0
936	4	1
514	4	2
597	4	0
820	4	0
500	4	0
678	4	0
937	4	0

Umbumbulu		
Diptank	Number tested	Number positive
467	5	0
248	5	0
63	5	0
240	5	0
159	5	0
227	5	3
941	5	0
60	5	0
248	5	0



Umzinto		
Diptank	Number tested	Number positive
231	4	0
10	4	0
198	4	1
12	4	0
962	4	0
200	4	0
72	4	0
139	4	0
230	4	0
70	4	0
414	4	0
477	4	0
822	4	0
17	4	0
920	4	0

Survey results at diptank level for Leptospirosis

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	5	2	93	5	0
98	5	2	268	5	0
432	5	4	1521	5	0
108	5	2	475	5	0
100	5	1	997	5	0
841	5	1	445	5	0
99	5	3	407	5	0
101	5	3	445	5	0
261	5	5	26	5	0
446	5	4	Witkop	5	0
146	5	1	Ngwebeni A	5	0
425	5	3	New Adelaide	5	0



Ingwawuma		
Diptank	Number tested	Number positive
311	4	0
814	4	0
509	4	0
493	4	0
569	4	1
809	4	1
803	4	0
789	4	0
682	4	0
787	4	0
752	4	0
497	4	0
320	4	0
576	4	0

Klip river		
Diptank	Number tested	Number positive
25	4	0
25	4	1
25	4	0
18	4	1
12	3	0
25	3	0
25	3	0
25	3	1
25	3	0
25	3	2
25	3	0
15	4	0
40	4	1
26	4	1
30	4	0
27	4	0
22	4	0

Lower Umfolozi		
Diptank	Number tested	Number positive
756a	5	1
821	5	0
756b	5	0
845	5	2
785	5	0
614	5	0
693	5	0
501	5	0
691	5	1
856	5	1
852	5	3
620	5	3
617	5	0
619	5	2
616	5	1
618	5	2

Ixopo		
Diptank	Number tested	Number positive
78	10	2
91	10	3
420	8	4
472	10	3
483	8	4
823	10	2

Mapumolo		
Diptank	Number tested	Number positive
482	9	4
454	9	2
456	9	0
38	5	0
316	5	2
157	5	2
211	5	1
440	5	1
215	5	0
770	5	3
303	5	3
449	5	5
251	5	2



Mahlabatini		
Diptank	Number tested	Number positive
547	5	0
548	5	0
829	5	0
903	5	0
867	5	0
776	5	0
718	5	0
233	5	0
775	5	0
761	5	0
550	5	0
340	5	0
434	5	0
dunga	5	3
bhade	5	0
718	5	0

Mpendele		
Diptank	Number tested	Number positive
119	8	2
120	8	8
268	8	5
351	8	3
430	8	8
940	8	3

New Hanover		
Diptank	Number tested	Number positive
921	6	1
938	6	6
Mophela	6	3
224	6	6
178	6	0
177	6	0
267	6	5
67B	6	5
279	6	6
880	6	3
53	6	2

Msinga		
Diptank	Number tested	Number positive
18	4	1
298	4	1
228	4	3
929	4	0
140	4	1
15	4	0
14	4	0
16	4	2
77701	4	2
8	4	0
2031	4	0
2001	4	0
5	4	0

Mtonanjeni		
Diptank	Number tested	Number positive
318	6	0
608	10	2
670	10	0
Ndundulu	10	1
728	10	1
738	5	1
675	5	0
611	10	0

Mthunzini		
Diptank	Number tested	Number positive
230	12	2
631	12	3
636	12	1
629	12	4
634	12	2



Ndwedwe			Newcastle		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
481	5	1	156	8	0
49	5	5	500	8	0
218	5	5	84	8	0
243	5	2	334	8	0
43	5	2	Birkenstock	8	1.04
418	5	3	108	8	0
242	5	0	8883	8	0
217	5	1	108B	8	0
181	5	1	Madadeni	8	0
250	5	4			
48	5	2			
183	5	1			

Nkandla			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
604	5	0	536	5	0
578	5	1	535	5	0
600	5	1	723	5	0
734	5	2	672	5	0
901	5	0	748	5	0
354	5	2	541	5	0
865	5	1	981	5	1
900	5	0	536	5	0
579	5	0	782	5	0
750	5	0	665	5	0
734 b	5	2	723	5	0
Nkiwani	5	0	909	5	0
			Oqothweni	5	0
			Esikuthweni	5	0
			748B	5	1
			541B	5	0



Nqutu		
Diptank	Number tested	Number positive
740	3	0
Nhlambamasoka	3	0
568	3	0
Kwanjezi	3	0
560	3	0
Mayikana	3	0
555	3	1
553	3	0
Mvuzini	3	1
552	3	0
Ingogo	3	0
556	3	0
Siyakahathala	3	1
Isandluwana	3	0
Nyloya	3	2
557	3	0
916	3	0
Ntanyandlovu	3	0
Maceba	3	0
Ndashana	3	0
561	3	1
Magogo	3	2
Magaga	3	1
Haladu	3	0
Skutu	3	2
558	3	0

Paul Pietersburg		
Diptank	Number tested	Number positive
250	5	1
253	5	0
252	5	2
248	5	1

Pietermaritzburg		
Diptank	Number tested	Number positive
926	6	2
170	6	1
169	6	0
57	6	1
264	6	0
58	6	0
56	6	6
937	6	0
171	6	2
945	6	0

Pinetown		
Diptank	Number tested	Number positive
272	10	0
222	10	2
481	10	1
32	10	1
411	10	0

Polela		
Diptank	Number tested	Number positive
111	8	0
113	8	1
114	8	2
161	8	2
163	8	1
270	8	1
306	8	2
394	8	4
398	8	1
123	5	3
124	8	2

Port Shepstone		
Diptank	Number tested	Number positive
208	9	3
774	9	1
389	9	3
210	9	2
942	9	5
319	9	3
19	9	8



Simdlangentsha		
Diptank	Number tested	Number positive
33	5	0
212	5	1
37	5	1
132	5	0
145	5	0
21	5	1

Richmond		
Diptank	Number tested	Number positive
308	12	11
466	12	5
405	12	5
403	12	6

Ubombo		
Diptank	Number tested	Number positive
743	4	1
766	4	4
813	4	2
513	4	0
510	4	0
939	4	2
701	4	0
898	4	1
936	4	0
514	4	0
597	4	0
820	4	0
500	4	0
678	4	0
937	4	0

Umbumbulu		
Diptank	Number tested	Number positive
467	5	0
248	5	1
63	5	0
240	5	2
159	5	0
227	5	0
160	5	1
191	5	1
470	5	0
941	5	0
60	5	2
487	5	3
64	5	1
469	5	1

Umzinto		
Diptank	Number tested	Number positive
231	4	3
10	4	2
198	4	4
12	4	3
962	4	3
200	4	3
72	4	1
139	4	3
230	4	0
70	4	0
414	4	1
477	4	1
822	4	3
17	4	4
920	4	1

Survey results at diptank level for Anaplasmosis

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	27	24	93	40	32
98	27	21	268	22	16
432	27	25	1521	19	14
108	27	0	475	40	35
100	27	22	997	80	65
841	27	10	445	40	35
99	27	19	407	20	15
101	27	17	445	20	16
261	26	19	26	10	9
446	27	15	Witkop	35	32
146	27	12	Boschoek A	50	45
425	27	0	New Adelaide	40	35
479	27	1			
438	27	3			

Bergville			Dannhauser		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
246	32	24	1	44	36
335	6	4	269	44	25
271	33	23	294	45	37
165	6	3	501	44	19
238	34	12	Peachill/Mooi	44	22
135	26	21	Springbok	43	42
2033	33	27	9168	44	40
2005	25	23	9	44	34
166	32	30	Uitkyk	44	38
2010	12	9	Doringkop	18	14
134	10	9			
206	11	7			
543	10	8			
299	25	22			
164	10	8			
236	45	35			
167	25	12			
235	26	23			
2009	50	36			



Eshowe		
Diptank	Number tested	Number positive
293	27	19
891	52	46
428	43	27
Ofasimba	25	18
660	18	18
592	39	18
659	64	45
662	37	28
601	29	27
953	23	21
663	20	14

Estcourt		
Diptank	Number tested	Number positive
144	39	22
365	40	23
5550	35	24
205	37	31
Wembezi C	38	33
Sobalili	35	31
Good Home	37	33
Dungamanzi	38	35
Ripley	39	18
Ganna-Hoek	39	29

Hlabisa		
Diptank	Number tested	Number positive
528	30	25
519	31	30
694	30	0
518	30	24
754	30	29
525	60	50
523	30	26
669	30	29
746	25	23
517	34	31
944	30	30
966	30	27
526	32	26
945	30	22
692	30	26

Ingwawuma		
Diptank	Number tested	Number positive
311	30	14
814	30	19
509	29	20
493	30	19
569	30	16
320	29	16
809	30	15
803	30	19
576	30	21
789	30	26
Mpophmi	30	27
787	30	18
752	30	19
497	30	14

Ixopo		
Diptank	Number tested	Number positive
78	50	43
91	50	48
420	50	31
472	50	48
483	50	45
823	48	40



Klipriver			Mhlabatini		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
8483	25	18	547	30	24
1341	25	25	548	30	20
2176	25	17	829	30	25
1374	18	15	903	30	21
2405	12	9	867	30	24
11697	25	12	776	30	24
1151	25	20	718	30	19
1244	25	20	233	30	23
28	25	19	775	30	29
2062	25	18	761	30	30
2019	25	18	550	30	25
2027	15	11	340	30	22
2039	40	25	434	30	27
2037	26	26	dunga	30	21
2035	30	28	bhade	30	19
2036	27	24	718	49	35
2061	22	22			

Msinga			Mpendele		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
18	36	26	119	50	50
298	37	33	120	50	33
228	36	28	268	50	44
929	26	23	351	50	50
140	28	15	430	50	33
15	25	19	940	50	48
14	25	24			
16	25	14			
8	37	30			
2031	30	24			
2001	25	15			
5	25	21			



Mtonanjeni		
Diptank	Number tested	Number positive
318	6	4
608	15	12
670	64	56
Ndundulu	14	13
728	50	36
738	22	18
675	47	32
735	25	13

Mthuzini		
Diptank	Number tested	Number positive
230	38	35
631	37	32
636	38	31
629	39	26
634	39	28

Ndwedwe		
Diptank	Number tested	Number positive
481	63	29
49	63	41
218	38	28
243	36	24
43	36	27
418	30	24
242	19	14
217	40	24
181	11	8
250	16	8
48	22	11
183	25	18

Nkandla		
Diptank	Number tested	Number positive
604	16	10
734b	43	36
578	60	49
600	28	23
734	50	36
901	23	16
354	50	38
865	18	16
900	79	44
579	55	10
750	12	10
Nkiwani	40	0

New Hanover		
Diptank	Number tested	Number positive
921	45	33
938	40	27
Mophela	42	33
224	40	22
178	44	35
177	40	31
267	40	23
67B	40	28
279	40	31
880	40	20
53	40	21

Newcastle		
Diptank	Number tested	Number positive
156	44	35
500	45	34
84	44	32
334	45	37
Birkenstock	44	41
108	44	37
8883	44	19
108B	44	36
Madadeni	30	17
Madadeni Kraal	17	12



Nqutu			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
740	15	9	536	30	22
Nhlambamasoka	15	15	535	30	25
568	15	15	723	30	25
Kwanjezi	15	15	672	34	27
560	15	15	748	30	20
Mayikana	15	14	541	30	24
555	15	12	981	30	23
553	15	12	536	30	2
Mvuzini	15	12	782	30	29
552	15	15	665	30	19
Ingogo	15	12	723	30	23
556	15	15	909	30	17
Siyakahathala	15	14	Oqothweni	30	23
Isandluwana	16	16	Esikuthweni	30	21
Nyloya	15	15	748B	30	21
557	15	14	541B	30	22
916	15	15			
Ntanyandlovu	15	15			
Maceba	15	15			
Ndashana	16	15			
561	15	12			
Magogo	15	14			
Magaga	15	15			
Haladu	15	10			
Skutu	15	12			
558	15	12			

Paul Pietersburg		
Diptank	Number tested	Number positive
250	30	17
253	40	18
252	29	19
248	40	35

Pietermaritzburg			Pinetown		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
926	41	33	222	41	0
170	41	30	481	15	9
169	42	32	32	50	34
57	41	31	411	19	10
264	40	34			
58	40	21			
56	43	36			
937	40	32			
171	40	33			
945	40	26			



Polela		
Diptank	Number tested	Number positive
111	50	42
113	48	47
114	50	50
161	50	42
163	50	35
270	50	40
306	50	40
394	48	41
398	50	48
123	50	44
124	50	44

Port Shepstone		
Diptank	Number tested	Number positive
208	37	37
774	37	32
389	37	33
210	37	28
942	37	19
319	37	20
247	37	27
260	37	29
88	39	19
19	37	32
363	37	26
83	37	30

Simdlangentsha		
Diptank	Number tested	Number positive
33	40	34
212	20	10
37	19	15
132	10	8
145	20	13
21	70	56

Richmond		
Diptank	Number tested	Number positive
308	40	29
466	40	33
405	41	33
403	40	38

Ubombo		
Diptank	Number tested	Number positive
743	30	16
766	30	20
813	30	24
513	30	23
510	30	20
939	30	19
701	30	17
898	30	21
936	29	23
514	30	15
597	30	24
820	30	18
500	30	17
678	30	27
937	30	25

Umbumbulu		
Diptank	Number tested	Number positive
248	19	15
467	50	40
248	36	23
63	30	25
240	39	34
159	31	28
227	30	21
160	30	23
191	5	4
470	33	25
941	31	23
469	32	18
470	33	4
60	29	20
487	31	17



Umzinto		
Diptank	Number tested	Number positive
231	19	17
10	22	22
198	20	19
12	30	30
962	19	19
200	20	19
72	20	19
139	20	15
230	20	17
70	20	0
414	20	16
477	20	3
822	20	3
17	30	20
920	20	10
231	11	7
269	11	6
196	11	9
935	11	6
276	11	7
948	11	9

Survey results at diptank level for *Babesia bovis*

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	27	3	93	40	23
98	27	6	268	22	19
432	27	7	1521	19	18
108	27	19	475	40	30
100	27	11	997	80	59
841	27	10	445	40	33
99	27	20	407	20	16
101	27	8	445	20	16
261	26	16	26	10	10
446	27	19	Witkop	35	31
146	27	21	Boschoek A	50	37
425	27	18	New Adelaide	40	32
479	27	18			
438	27	19			



Bergville		
Diptank	Number tested	Number positive
246	32	30
335	6	5
271	33	31
165	6	5
238	34	27
135	26	21
2033	33	28
2005	25	22
166	32	28
2010	12	11
134	10	10
206	11	9
543	10	9
299	25	24
164	10	10
236	45	39
167	25	18
235	26	20
2009	50	40

Dannhauser		
Diptank	Number tested	Number positive
1	44	23
269	44	26
294	45	25
501	44	32
Peachill/Mooi	44	33
Springbok	43	35
9168	44	26
9	44	1
Uitkyk	44	35
Doringkop	18	14

Estcourt		
Diptank	Number tested	Number positive
144	39	36
365	40	36
5550	35	31
205	37	33
Wembezi C	38	38
Sobalili	35	31
Good Home	37	36
Dungamanzi	38	33
Ripley	39	37
Ganna-Hoek	39	39

Eshowe		
Diptank	Number tested	Number positive
293	27	9
891	52	12
428	43	28
Ofasimba	25	16
660	18	13
592	39	36
659	64	40
662	37	27
601	29	18
953	23	10
663	20	16

Hlabisa		
Diptank	Number tested	Number positive
528	30	13
519	31	14
694	30	10
518	30	20
754	30	20
525	60	46
523	30	23
669	30	24
746	25	12
517	34	23
944	30	30
966	30	30
526	32	26
945	30	21
692	30	27



Ingwawuma			Ixopo		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
311	30	30	78	50	40
814	30	24	91	50	49
509	29	24	420	50	40
493	30	26	472	50	21
569	30	26	483	50	39
320	29	25	823	48	19
809	30	23			
803	30	17			
576	30	14			
789	30	13			
Mpophmi	30	21			
787	30	10			
752	30	19			
497	30	20			

Klipriver			Mapumolo		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
8483	25	20	482	42	35
1341	25	24	454	47	39
2176	25	25	456	10	7
1374	18	24	38	69	56
2405	12	16	316	76	50
11697	25	12	157	23	18
1151	25	25	211	81	69
1244	25	23	440	21	20
28	25	23	215	49	31
2062	25	23	770	9	5
2019	25	23	303	13	10
2027	15	13	449	9	3
2039	40	40	251	10	7
2037	26	26			
2035	30	28			
2036	27	23			
2061	22	22			



Ndwedwe			New Hanover		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
481	63	44	921	45	31
49	63	52	938	40	31
218	38	30	Mophela	42	38
243	36	30	224	40	31
43	36	31	178	44	36
418	30	24	177	40	32
242	19	12	267	40	33
217	40	37	67B	40	34
181	11	8	279	40	37
250	16	13	880	40	31
48	22	18	53	40	22
183	25	20			

Newcastle			Nkandla		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
156	44	42	604	16	6
500	45	44	734b	43	30
84	44	43	578	60	42
334	45	41	600	28	22
Birkenstock	44	37	734	50	34
108	44	36	901	23	19
8883	44	37	354	50	41
108B	44	24	865	18	9
Madadeni	30	14	900	79	55
Madadeni Kraal	17	15	579	55	22
			750	12	10
			Nkiwani	40	2



Nqutu			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
740	15	14	536	30	20
Nhlambamasoka	15	14	535	30	26
568	15	11	723	30	29
Kwanjezi	15	15	672	34	32
560	15	12	748	30	28
Mayikana	15	11	541	30	21
555	15	14	981	30	19
553	15	10	536	30	20
Mvuzini	15	14	782	30	18
552	15	15	665	30	6
Ingogo	15	12	723	30	22
556	15	13	909	30	17
Siyakahathala	15	15	oqothweni	30	25
Isandluwana	16	14	Esikuthweni	30	22
Nyloya	15	14	748B	30	18
557	15	12	541B	30	13
916	15	13			
Ntanyandlovu	15	13			
Maceba	15	14			
Ndashana	16	14			
561	15	14			
Magogo	15	15			
Magaga	15	15			
Haladu	15	13			
Skutu	15	12			
558	15	14			

Pinetown		
Diptank	Number tested	Number positive
222	41	0
481	15	5
32	50	21
411	19	15

Pietermaritzburg		
Diptank	Number tested	Number positive
926	41	39
170	41	38
169	42	42
57	41	36
264	40	39
58	40	33
56	43	33
937	40	37
171	40	33
945	40	31

Paul Pietersburg		
Diptank	Number tested	Number positive
250	30	13
253	40	32
252	29	21
248	40	34



Polela			epstone		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
111	50	33	208	37	17
113	48	35	774	37	8
114	50	37	389	37	15
161	50	38	210	37	17
163	50	37	942	37	22
270	50	41	319	37	32
306	50	38	247	37	28
394	48	33	260	37	18
398	50	28	88	39	17
123	50	31	19	37	24
124	50	23	363	37	18
			83	37	19

Simdlangentsha		
Diptank	Number tested	Number positive
33	40	33
212	20	16
37	19	14
132	10	5
145	20	13
21	70	47

Richmond		
Diptank	Number tested	Number positive
308	40	33
466	40	36
405	41	35
403	40	36

Ubombo		
Diptank	Number tested	Number positive
743	30	14
766	30	13
813	30	14
513	30	11
510	30	3
939	30	5
701	30	2
898	30	0
936	29	0
514	30	1
597	30	3
820	30	28
500	30	22
678	30	8
937	30	5

Umbumbulu		
Diptank	Number tested	Number positive
248	19	15
467	50	28
248	36	19
63	30	19
240	39	27
159	31	9
227	30	20
160	30	17
191	5	4
470	33	12
941	31	15
469	32	25
470	33	12
60	29	25
487	31	19



Umzinto		
Diptank	Number tested	Number positive
231	19	4
10	22	10
198	20	8
12	30	7
962	19	3
200	20	0
72	20	3
139	20	15
230	20	18
70	20	17
414	20	8
477	20	11
822	20	14
17	30	7
920	20	11
231	11	10
269	11	10
196	11	8
935	11	6
276	11	7
948	11	6

Survey results at diptank level for *Babesia bigemina*

Alfred			Babanango		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
96	27	4	93	40	18
98	27	1	268	22	9
432	27	0	1521	19	8
108	27	16	475	40	21
100	27	5	997	80	36
841	27	5	445	40	16
99	27	15	407	20	12
101	27	2	445	20	7
261	26	5	26	10	4
446	27	4	Witkop	35	16
146	27	15	Boschoek A	50	17
425	27	6	New Adelaide	40	22
479	27	4			
438	27	9			



Bergville		
Diptank	Number tested	Number positive
246	32	17
335	6	1
271	33	12
165	6	2
238	34	8
135	26	10
2033	33	6
2005	25	10
166	32	13
2010	12	6
134	10	7
206	11	4
543	10	7
299	25	11
164	10	6
236	45	20
167	25	7
235	26	15
2009	50	37

Dannhauser		
Diptank	Number tested	Number positive
1	44	3
269	44	2
294	45	3
501	44	8
Peachill/Mooi	44	14
Springbok	43	23
9168	44	14
9	44	6
Uitkyk	44	7
Doringkop	18	5

Eshowe		
Diptank	Number tested	Number positive
293	27	0
891	52	0
428	43	7
Ofasimba	25	4
660	18	11
592	39	3
659	64	2
662	37	16
601	29	0
953	23	0
645	20	16

Hlabisa		
Diptank	Number tested	Number positive
528	30	2
519	31	1
694	30	1
518	30	1
754	30	3
525	60	6
523	30	1
669	30	1
746	25	1
517	34	1
944	30	0
966	30	0
526	32	0
945	30	0
692	30	0

Estcourt		
Diptank	Number tested	Number positive
144	39	29
365	40	21
5550	35	24
205	37	20
Wembezi C	38	22
Sobalili	35	13
Good Home	37	14
Dungamanzi	38	16
Ripley	39	19
Ganna-Hoek	39	29

Ingwawuma			Ixopo		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
311	30	6	78	50	38
814	30	3	91	50	38
509	29	4	420	50	17
493	30	4	472	50	7
569	30	8	483	50	29
320	29	8	823	48	18
809	30	1			
803	30	12			
576	30	1			
789	30	0			
Mpophmi	30	13			
787	30	0			
752	30	0			
497	30	4			

Klipriver			Mapumolo		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
8483	25	4	482	42	18
1341	25	15	454	47	19
2176	25	9	456	10	4
1374	18	9	38	69	20
2405	12	5	316	76	18
11697	25	2	157	23	4
1151	25	8	211	81	13
1244	25	16	440	21	3
28	25	13	215	49	10
2062	25	8	770	9	2
2019	25	11	303	13	4
2027	15	6	449	9	2
2039	40	13	251	10	1
2037	26	11			
2035	30	10			
2036	27	6			
2061	22	4			



Ndwedwe			New Hanover		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
481	63	6	921	45	21
49	63	14	938	40	7
218	38	5	Mophela	42	18
243	36	6	224	40	11
43	36	8	178	44	22
418	30	8	177	40	23
242	19	3	267	40	19
217	40	11	67B	40	29
181	11	4	279	40	19
250	16	4	880	40	14
48	22	3	53	40	7
183	25	9			

Nkandla		
Diptank	Number tested	Number positive
604	16	1
734b	43	4
578	60	14
600	28	8
734	50	2
901	23	5
354	50	16
865	18	1
900	79	11
579	55	18
750	12	6
Nkiwani	40	0

Newcastle		
Diptank	Number tested	Number positive
156	44	3
500	45	2
84	44	1
334	45	3
Birkenstock	44	5
108	44	3
8883	44	4
108B	44	8
Madadeni	30	1
Madadeni Kraal	17	5



Nqutu			Nongoma		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
740	15	0	536	30	0
Nhlambamasoka	15	1	535	30	2
568	15	0	723	30	1
Kwanjezi	15	1	672	34	0
560	15	0	748	30	0
Mayikana	15	0	541	30	0
555	15	0	981	30	0
553	15	0	536	30	0
Mvuzini	15	1	782	30	2
552	15	2	665	30	0
Ingogo	15	2	723	30	2
556	15	0	909	30	0
Siyakahathala	15	2	oqothweni	30	2
Isandluwana	16	1	Esikuthweni	30	3
Nyloya	15	0	748B	30	0
557	15	1	541B	30	0
916	15	2			
Ntanyandlovu	15	1			
Maceba	15	2			
Ndashana	16	1			
561	15	1			
Magogo	15	2			
Magaga	15	3			
Haladu	15	0			
Skutu	15	3			
558	15	4			

Paul Pietersburg		
Diptank	Number tested	Number positive
250	30	3
253	40	6
252	29	2
248	40	9

Pietermaritzburg		
Diptank	Number tested	Number positive
926	41	5
170	41	4
169	42	11
57	41	13
264	40	18
58	40	15
56	43	19
937	40	12
171	40	3
945	40	5

Pinetown		
Diptank	Number tested	Number positive
222	41	0
481	15	4
32	50	20
411	19	0



Polela			Port Shepstone		
Diptank	Number tested	Number positive	Diptank	Number tested	Number positive
111	50	20	208	37	14
113	48	28	774	37	4
114	50	24	389	37	3
161	50	32	210	37	10
163	50	13	942	37	15
270	50	36	319	37	20
306	50	25	247	37	4
394	48	31	260	37	3
398	50	13	88	39	4
123	50	22	19	37	26
124	50	7	363	37	6
			83	37	5

Simdlangentsha		
Diptank	Number tested	Number positive
33	40	5
212	20	6
37	19	8
132	10	1
145	20	1
21	70	8

RICHMOND		
Diptank	Number tested	Number positive
308	40	8
466	40	5
405	41	12
403	40	8

Ubombo		
Diptank	Number tested	Number positive
743	30	4
766	30	2
813	30	4
513	30	11
510	30	0
939	30	0
701	30	0
898	30	0
936	29	0
514	30	0
597	30	0
820	30	13
500	30	10
678	30	5
937	30	2

Umbumbulu		
Diptank	Number tested	Number positive
248	19	6
467	50	22
248	36	19
63	30	2
240	39	7
159	31	3
227	30	8
160	30	8
191	5	0
470	33	0
941	31	3
469	32	7
470	33	0
60	29	4
487	31	8



Umzinto		
Diptank	Number tested	Number positive
231	19	1
10	22	10
198	20	3
12	30	4
962	19	4
200	20	2
72	20	3
139	20	12
230	20	19
70	20	17
414	20	3
477	20	4
822	20	11
17	30	5
920	20	4
231	11	3
269	11	1
196	11	3
935	11	2
276	11	5
948	11	0