Point of care diagnosis of bovine trypanosomosis, tick-borne diseases and helminthoses with emphasis on portable anaemia-detection devices and decision support systems

by

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Abstract

Effective diagnosis of major endemic bovine diseases such as trypanosomosis, tick-borne diseases and helminthoses that constrain agricultural production in sub-Saharan Africa is required for their rational treatment. However, this is hampered by the shortage of professional staff and unavailability of simple field-level diagnostic tests. The work presented in this thesis was designed to evaluate simple diagnostic tools and develop guiding decision support tools to facilitate diagnosis and treatment of these diseases in rural areas of Africa.

Portable haemoglobinometers such as the Haemoglobin Colour Scale (HCS), HemoCue (HCU) and DHT-haemoglobinometer (DHT), commonly used in human medicine, were evaluated for their suitability in the detection of anaemia in cattle. Coefficients of variation (CV) were calculated for all methods to determine their precision over a range of 0-17 g/dl. The precision of the HCS (CV 2.9-8.8%) and HCU (CV 3.5-10.6%) was better than that of the DHT (CV 7.7-23%). There was good linearity between the readings of the HCS (R = 0.925), HCU(R = 0.920) and DHT (R = 0.906) and those of the reference method (cyanmethaemoglobin). The diagnostic sensitivity and specificity of all methods for detection of anaemia (haemoglobin (Hb) < 8g/dl) was determined using the cyanmethaemoglobin method as a goldstandard. Sensitivity was defined as the ability of a method to detect true cases of anaemia (Hb < 8g/dl), while specificity was the ability of a method to declare the true non-anaemic status (Hb > 8 g/dl) as negative. The sensitivity of the HCS (94%) and HCU (80.5%) was high, but that of the DHT was low (52.7%). All the haemoglobinometers had high specificity: HCS (93%), HCU (96.5%) and DHT (100%). A high correlation between the packed cell volume (PCV) and Hb measurements was obtained using all methods: HCS (R = 0.974), HCU (R = 0.965) and DHT (R = 0.934). Field veterinarians achieved good precision with the HCS (CV 8 – 13%) and the HCU (CV 1%). The cost of the kit and reagents for analysis of 1000 samples would amount to US$ 22 (€ 19.50) for the HCS, US$ 600 (€ 532) for the DHT and US$ 1100 (€ 975) for the HCU, making the HCS cheapest to run. These haemoglobinometers are potentially useful for penside detection of anaemia, especially in rural areas of Africa.

The influence of time of day and coat colour of Zebu cattle under tropical conditions on rectal temperature was assessed. The time of the day and coat colour had a highly significant influence on rectal temperature (P< 0.001) and thus need to be considered while assessing pyrexia during clinical diagnosis. The period between 13.00 and 17.00 hours was the most suitable time of the day for veterinarians to detect pyrexia, however there is the likelihood of picking healthy cattle (false positives) that have raised rectal temperature. Since veterinarians are usually presented with sick rather than healthy animals by farmers, it is unlikely that picking healthy animals would be a problem.

Diurnal variations of the sensitivity of the common parasitological diagnostic tests for trypanosomosis were investigated. Despite the small sample size (n = 2), the highest detection rate was observed at 21.00 hr and the lowest at 13.00 hr. Neither time of the day nor day-to-day variation had a significant influence

on the sensitivity of the tests, despite occurrence of diurnal variations. Nevertheless, these results suggested optimal detection rate of trypanosomosis with microscopy was achieved when cattle blood was taken and examined between 17.00 and 09.00 hours under tropical conditions.

Epidemiological studies were conducted on endemic diseases in Zebu cattle kept under the mixed crop-livestock production system in South East Uganda. Moderate parasitological prevalences of the diseases: anaplasmosis (23.6%), theileriosis (21.6%), fasciolosis (13.6%), Parasitic gastroenteritis (PGE) (6.7%), trypanosomosis (4.4%) and babesiosis (1.0%) were found.

Morbidity rates were determined based on clinical manifestation of anaemia, weight loss, pallor, lymph node enlargement, staring coat, diarrhoea, lacrymation and fever in addition to presence of aetiological agents. The diseases found had low morbidity rates: anaplasmosis (17.1%), theileriosis (15.6%), fasciolosis (7.0%), PGE (3.3%), trypanosomosis (3.2%) and babesiosis (0.2%). Cattle manifesting clinical signs characteristic of schistosomosis were not found, although a few cattle (0.1%) secreted *Schistosoma* eggs. Cases of cowdriosis were not encountered either, despite the abundance of the tick vector *Amblyomma variegatum*.

Clinical signs manifested by cases and their parasitological findings on sampling visits prior to death were used as the basis for determining the probable disease responsible for death. A low annual crude mortality rate (5.2%) was observed and theileriosis was the most important cause of disease-related mortality, being responsible for 0.8%, followed by anaplasmosis (0.5%). However much of the disease-related mortality (3.7%) was due to multiple diseases. Young animals were most affected, with mortality rates of 12.4% and 11.9% in 0-6 months and 7-12 months old calves, respectively.

Medium to high seroprevalences of 68.9-85.8%, 56.2-85.6% and 54.9-76.9% for *Theileria parva*, *Anaplasma marginale* and *Babesia bigemina* infections, respectively, were found indicating that cattle populations studied were extensively exposed to these infections. A small proportion of cattle experienced more than one seroconversion to *A. marginale* (13%), *B. bigemina* (12.2%) and *T. parva* infections (6%), which was attributed to seasonal variation of abundance of *Rhipicephalus* sp. and *Boophilus* sp. ticks. In some villages over 90% of the cattle were infested with *Rhipicephalus appendiculatus* ticks, while in others fewer animals (10-80%) were infested. Likewise, in some villages 40-80% of the cattle were infested with *Boophilus* spp. ticks, while in others fewer animals (10-40%) were infested. In both instances, there was a significant difference ($P < 0.05$) in the abundance of *Rhipicephalus* sp. and *Boophilus* sp. ticks between the two categories of villages. The villages were hence distinguished into those with a high and low tick challenge.

The seroconversion rates to *A. marginale* infection of cattle of all age groups: 0-6m, 7-12m, 13-24m and >24m under low tick challenge were higher than those of their counterparts under high tick challenge, but not significantly so ($P > 0.05$). Independent of tick challenge, the seroconversion rate decreased with age up to 24 months. For *B. bigemina* infection, seroconversion rates of cattle of different age groups

under high tick challenge were higher than those of their counterparts under low tick challenge, but the reverse was true for 6 months old calves. A significant difference was observed among cattle of >24m old ($\chi^2 = 4.5$, DF = 1, $P < 0.05$). Under low tick challenge, the seroconversion rate decreased with age, but under high tick challenge, the seroconversion rate increased with age up to 24m. For *T. parva* infections, seroconversion rates of cattle older than 6 months under low tick challenge were significantly higher than those of their counterparts under high tick challenge (7-12m, $\chi^2 = 11.7$, DF = 1, $P< 0.05$; 13-24m, $\chi^2 = 11.2$, DF = 1, $P< 0.05$; > 24m, $\chi^2 = 27.2$, DF = 1, $P< 0.001$). Seroconversion rates were similar in all age groups under low tick challenge, but decreased with age under high tick challenge.

Field cases were analysed to identify clinical signs that had significant association to presence of aetiological agents for trypanosomosis, anaplasmosis, theileriosis, babesiosis, fasciolosis, PGE and schistosomosis. Anaemia, pallor, fever, enlarged lymph nodes, lacrymation, staring coat and weight loss had significant association ($P < 0.05$) to trypanosomosis. Anaemia, pallor, fever, weight loss and staring coat had highly significant association ($P < 0.001$) to anaplasmosis. Fever, enlarged lymph nodes, lacrymation, anaemia, pallor, staring coat and diarrhoea had significant association ($P < 0.05$) to theileriosis. Anaemia, pallor and diarrhoea had significant association ($P < 0.05$) to babesiosis. Anaemia, pallor, weight loss, staring coat and diarrhoea had highly significant association ($P < 0.001$) to fasciolosis. Weight loss, staring coat, anaemia and pallor had highly significant association ($P < 0.001$) to PGE. A few cattle secreted *Schistosoma* eggs, but no clinical signs had any significant association to presence of *Schistosoma* eggs.

Important clinical signs and risk factors that could best be used to predict the probability of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infection in cattle were assessed. The level of tick challenge at village level, *Rhipicephalus* spp. and *Boophilus* spp. intensity on individual animals, rectal temperature and PCV were significant for prediction ($P < 0.05$) of seroconversion to *A. marginale* infection. For *B. bigemina* infection, the significant predictors ($P < 0.05$) for seroconversion included the level of tick challenge, *Boophilus* spp. intensity, anaemia, weight loss, staring coat, lacrymation and age. While for *T. parva* infection, significant predictors ($P < 0.05$) included the level of tick challenge, *R. appendiculatus* intensity, lymph node enlargement, rectal temperature and PCV.

A Delphi survey, involving 46 veterinary experts consisting of 32 international and 14 local Ugandan experts, was conducted to elicit quantitative information on important clinical signs and risk factors for clinical diagnosis of endemic bovine diseases under consideration. Experts responded to questions on trypanosomosis (26), theileriosis (21), anaplasmosis (23), babesiosis (23), cowdriosis (20), PGE (23), fasciolosis (22) and schistosomosis (12). Overall scores obtained were used to develop a decision support card that utilizes a combination of pattern-matching and colour-banding scoring systems to execute differential diagnosis. The decision support card was designed for field veterinarians, animal health assistants and community animal health workers for use in the rural areas of Africa. An evaluation under controlled conditions to assess its diagnostic performance in the mixed-crop livestock production system is underway, before it is released for routine use by independent end-users.

Declaration

The research reported in this thesis is my own original work, except where otherwise stated, and has not been submitted for any other degree

Sign.....

Dedication

This thesis is dedicated to my family: wife, Annet, children, Immaculate, Pascal, Winnie and Mildred. I am grateful to them for their invaluable moral support and understanding that contributed to my success.

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Table of Contents

List of Tables

List of Figures

Chapter 1 General introduction

1.1 Economic importance of endemic bovine diseases in sub-Saharan Africa with particular emphasis on East Africa

The major endemic diseases of cattle in sub-Saharan Africa considered in this thesis include trypanosomosis, theileriosis (East Coast fever), anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, schistosomosis and fasciolosis. Endemic refers to the constant occurrence of either overt or subclinical disease at a predictable level in a population, as opposed to epidemic, which is occurrence of often overt and sudden disease in a population at levels in excess of the expected (Thrusfield, 1995).

Major epidemic diseases of cattle such as rinderpest, foot and mouth disease (FMD) and contagious bovine pleuropneumonia (CBPP) usually occur as outbreaks resulting in dramatic, large-scale mortality, morbidity and severe economic losses. They are generally characterised by long inter-epidemic periods during which the disease may either be absent from an area or present at almost undetectable levels, with minimal economic losses. In their control, national governments and international organisations are obliged to act because of the infectiousness and transboundary nature of spread and the associated negative impact on international trade on animals and animal products (OIE, 2003). On the contrary, losses due to major endemic diseases may not be as dramatic as those occurring at the height of epidemics, but their continuous presence and relentless attrition makes them economically important. Unlike epidemic diseases whose control costs are borne by national governments or international organisations, control costs of endemic diseases are borne by individual farmers due to the localised nature of these diseases (OIE, 2003).

Trypanosomosis directly constrains cattle productivity by reducing birth rates, increasing abortion rates, and increasing mortality rates, particularly among young animals (Swallow, 1999). Its existence and severity affects farmers' choices about size and structure of the cattle

herds and the use of the tsetse habitat for grazing (Swallow, 1999). Through the absence of cattle to provide draught power, milk, manure and meat, rural development is severely impaired (Holmes *et al.*, 2000). The most important pathogenic tsetse transmitted trypanosome species affecting cattle in Africa are the vascular trypanosomes: *Trypanosoma congolense* and *T. vivax* (Holmes *et al.*, 2000), whose tsetse vectors are estimated to infest approximately 11 million km^2 of Africa, about 37% of the continent (Trail *et al.*, 1985).

Indirect annual costs of African trypanosomosis to livestock producers and consumers are estimated to be 1340 million US$ (Kristjanson *et al.*, 1999). The incidence of bovine trypanosomosis has led to sub-optimal stocking rate of cattle by 30-50% and reduction in the production of meat and milk by at least 50%, the calving rates by 1-20%, the efficiency of oxen under high risk by 38% and the increase in calf mortality by 10% (Swallow, 1999). Geerts and Holmes (1997) estimated that trypanocidal drugs cost African farmers 35 million dollars per annum.

The impact of trypanosomosis on livestock productivity in terms of reduced draught power, milk, meat and manure at the national scale in Uganda and Kenya is very similar to the continental picture over all sub-Saharan Africa. Tsetse flies are estimated to infest 106,400 km^2, 50% of the entire landmass of Uganda and 2.2 million head of cattle of the estimated national cattle population of 5.4 million are at risk of trypanosomosis (Chizyuka 1998). Tsetse infestation and incidence of trypanosomosis has prevented the keeping of an extra 3.3 million head of cattle in Uganda (Chizyuka, 1998). In Kenya, tsetse flies are estimated to infest 90,300 km^2, which represents 25% of the country, and 1.6 million head of cattle out of the estimated 14 million national cattle population are at risk of trypanosomosis (Chizyuka, 1998). Tsetse infestation is estimated to prevent rearing of an extra 2.7 million head of cattle in Kenya (Chizyuka, 1998).

With 80% of the world's 1,288 million cattle living at risk of tick-borne diseases, the global cost has been estimated to be between US$ 13.9 and 18.7 billion per annum (de Castro, 1997). Among the tick-borne disease, East Coast fever is probably the most important due to the high mortalities caused, the productivity losses on recovery, the cost of control and the exclusion of taurine (*Bos taurus*) and taurine-cross cattle from endemic regions due to their high susceptibility (Young *et al.*, 1988). The economic losses caused by East Coast fever in cattle in 11 countries, where 24 million out of 64 million head of cattle are at risk, has been estimated by Mukhebi *et al.*, (1992) at US$ 168 million per annum.

In Uganda, over 90% of the cattle population are at constant risk of tick-borne diseases and the overall loss of the calf crop in indigenous cattle due to tick-borne diseases is estimated to be 30% over the greater part of the Uganda (Anon., 1997a). The cumulative mortality of 13.5% due to East Coast fever in indigenous calves up to 1 year old has been reported in ranch cattle in Uganda (Okello-Onen, 1996). Of the deaths attributable to tick-borne diseases, East Coast fever is responsible for 79%, anaplasmosis 11%, cowdriosis 5.6% and babesiosis 4.4% (Anon., 1992). The annual cost of imported acaricides in Uganda is estimated at US$ 10 million (Okello-Onen *et al.*, 1998a).

Tick-borne diseases in Kenya are considered a major constraint to dairy farming among the smallholder dairy farms, which account for an estimated 75-90% of all milk produced in the country (Mbogoh, 1984). Actual losses attributable to East Coast fever amount to US$ 95 million (TickCost, 1999). It is also estimated that 70% of the cattle in Kenya are found in areas where anaplasmosis and babesiosis are endemic, and disease incidence is estimated to be 5% in adult cattle. Annual losses due to babesiosis and anaplasmosis combined, amount to US$ 6.9 million (TickCOST, 1999) not including losses due to chronic or subclinical anaplasmosis, which are associated with low production and weight loss. Cowdriosis is estimated to cause mortality losses amounting to US$ 13.3 million (TickCOST, 1999). In the Southern African

Development Commission (SADC) region, the cost of cowdriosis has been estimated at 37-47 million (Minjauw *et al.*, 2000).

Parasitic gastroenteritis is an important cause of production losses in cattle. These losses include poor general performance and even mortality, particularly in young animals. More important are visible subclinical losses, such as decreased weight gain, decreased milk yield and decreased fertility (Eysker and Ploeger, 2000). Helminth infections also cause economic losses through condemnation of whole carcass or specific organs at slaughter. In Kenya, condemnation losses are estimated to constitute 11.8% of the entire economic losses (Githigia *et al.*, 1995).

Fasciolosis is associated with production losses such as liver condemnation, poor growth rates, reduced draught power output, poor feed conversion, lowered milk yield, reduced meat production, infertility and deaths (Wamae and Ihiga, 1991). For example, losses due to liver condemnation attributable to fasciolosis have been estimated to amount to 51%, an equivalent of US$ 228, 244 per annum in Zambia, (Pandey and Ahmadu, 1998) and US$ 10,000 per six months in Nigeria (Olusi, 1996). In Kenya, a combination of liver condemnation and reduction in liveweight gain has been reported to constitute an annual loss value of US$12.11 (4.92%) and US$23.41 (10.34%) for Friesians and Borans cattle respectively (Wamae *et al.*, 1998).

Approximately 530 million head of cattle live in areas in Africa and Asia where schistosomosis is endemic (de Bont and Vercruysse, 1998). Worldwide, overall prevalence ranges between 31 and 81% in cattle populations, hence it is speculated that at least 165 million cattle are infected with schistosomes (de Bont and Vercruysse, 1998). Production losses caused by schistosomosis in cattle include mortality, retarded growth, poor reproductive performance and low milk yield. Mortality of 7% in cattle of six to 30 months and prevalence of over 90% based on faecal egg count surveys has been reported in Sudan (Majid *et al.*, 1980). Females that recover from

severe bovine schistosomosis show poor performance later; many do not conceive until they are five or six years old and have calving intervals greater than two years (McCauley *et al.*, 1983b).

Control of major endemic bovine diseases in sub-Saharan Africa has until recently been the remit of state veterinary services, which have now dwindled as a result of global trends towards privatisation with the contraction of veterinary services (Armbruster, 1994). With the inception of privatisation of veterinary services, the responsibility of diagnosis of livestock diseases and drug administration is now in the hands of farmers, extension workers and agro-veterinary traders (Machila *et al.*, 2003). Effective diagnosis of these endemic diseases of cattle in sub-Saharan Africa is required for their rational treatment and control, but this is constrained by the unavailability of suitably trained professional staff and field-level diagnostic tests, as well as general lack of knowledge about cattle diseases among livestock owners (Machila *et al.*, 2003).

Appropriate drug use and other disease control strategies depend on correct diagnosis and a working understanding of the suitability of therapeutic options. Existing techniques for diagnosis of these diseases are too costly and unavailable to the groups such as farmers, extension workers and agro-veterinary traders who make treatment decisions (Machila *et al.*, 2003). In the absence of effective diagnosis, premature measures and treatments are applied inappropriately. This may compound the economic losses already associated with the diseases concerned. For instance, indiscriminate use of trypanocides is undesirable because it is uneconomical to treat uninfected animals, and misuse and overuse of trypanocides may be associated with the development of drug resistance (ICPTV, 1999), and occasionally with toxicity (Eisler *et al.*, 1997) or low productivity (Mdachi, 1999). To improve the quality and accessibility of disease diagnosis to resource-poor cattle keepers, there is a need to develop simple, reliable and cheap diagnostic tests and guiding decision support tools to enable simple diagnosis and treatment of endemic bovine diseases by field veterinarians, animal health assistants and community animal health workers in rural areas.

The hypothesis tested in this thesis is:

- Farmer-based interventions utilizing low technology decision support systems including haemoglobin measurements might help improve animal health in the mixed crop-livestock farming systems in South East Uganda.

The main objectives addressed in this thesis include the following:

- To determine the key clinical signs, pathological features, epidemiological features and available definitive diagnostic tests of endemic bovine diseases under consideration through review of literature.
- To compare the performance of portable haemoglobinometers to that of conventional haematological measures of anaemia.
- To examine the effects of diurnal variations on the diagnostic value of rectal temperature and on the sensitivity of parasitological diagnostic tests for trypanosomosis.
- To understand the epidemiology of endemic diseases under consideration in cattle kept under the mixed crop-livestock production system in Uganda.
- To identify clinical signs of diagnostic significance associated with field cases of the individual endemic diseases.
- To develop decision support systems based on clinical signs (risk factors) of high diagnostic value to facilitate field-level diagnosis of the endemic bovine diseases under consideration

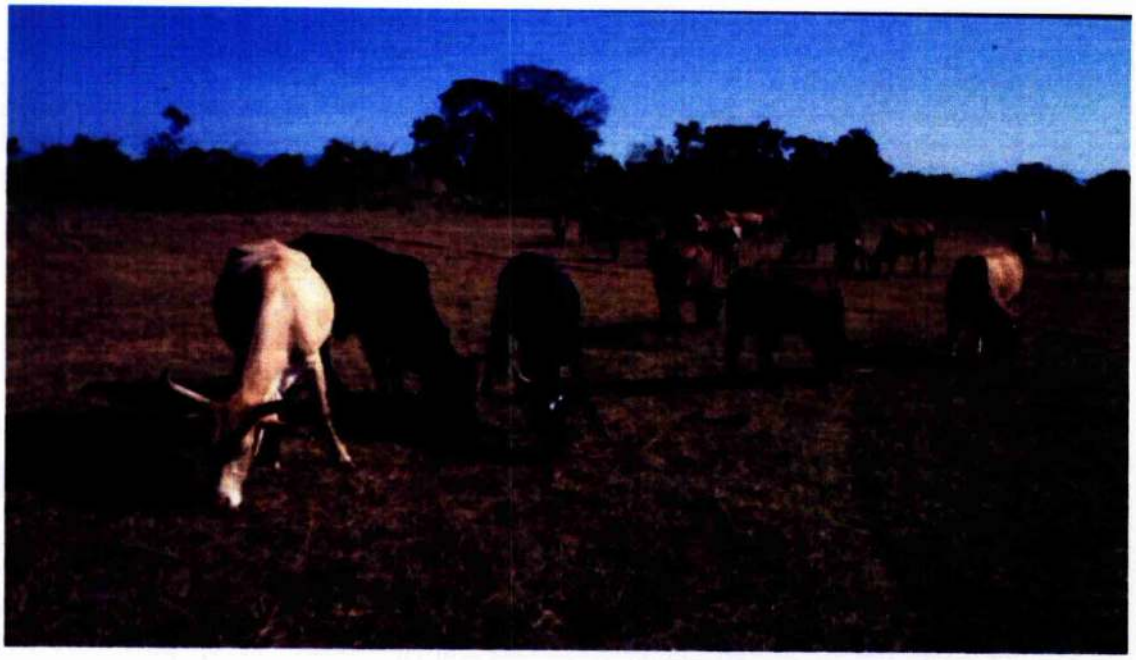


Figure 1.1: A Ugandan village - a characteristic crop-livestock production system in which trypanosomosis, tick-borne diseases and helminthoses are endemic and constrain livestock health and productivity

Chapter 2 Endemic bovine diseases in sub-Saharan Africa and their diagnosis

2.1 Trypanosomosis

2.1.1 Key clinical signs

Bovine trypanosomosis is caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei* transmitted by several tsetse species (*Glossina* spp.), but the most pathogenic trypanosome species affecting cattle in Africa are the vascular trypanosomes: *T. congolense* and *T. vivax*. The disease is characterized by the development of a moderate to severe anaemia, loss of condition and intermittent fever, which is prominent during the early phases of the disease when the waves of parasitaemia are high (Fiennes, 1970, Morrison *et al.*, 1981; Stephen, 1986; Murray and Dexter, 1988; Holmes *et al.*, 2000). Other clinical signs include enlarged superficial lymph nodes, progressive weakness, loss of appetite, loss of weight, stunted growth, epiphora, abortion during the third trimester and decreased fertility (Fiennes, 1970, Stephen, 1986; Holmes *et al.*, 2000). The disease is normally associated with infections that last for weeks or months and a slow and insidious loss of condition resulting in eventual death (Fiennes, 1970; Holmes *et al.*, 2000).

Trypanosomosis due to *T. vivax* manifests as an acute or chronic infection in cattle. The acute form of the disease is characterized by septicaemia and the animal may die within a week (Stephen, 1986; Dirie *et al.*, 1988). Affected animals usually have a fever, with body temperatures rising up to 40-41°C, look dejected, and have inappetence, dyspnoea, diarrhoea and haemorrhages. Loss of weight occurs early in the infection and there is always a heavy parasitaemia. Fatal, haemorrhagic *T. vivax* infection characterized by widespread haemorrhages through the skin, ears and on the tongue, blood-stained diarrhoea, abortion and considerable mortality has been reported in dairy cattle at the Kenya Coast (Mwongela *et al.*, 1981), in local and exotic cattle in Somalia (Dirie *et al.*, 1988) and in Zebu cattle in Eastern Uganda (Magona

et al., 1997). Chronic *T. vivax* infection is less severe. It manifests with progressive anaemia, staring coat, weight loss and swelling of superficial lymph nodes: prescapular and prefemoral lymph nodes. Abortion and stillbirths occur in pregnant cows and milk production is depressed (Stephen, 1986). Chronic trypanosomosis is usually associated with severe wasting of animals, hence called 'thin cow syndrome' as reported at the Kenya Coast (Dowler *et al.*, 1989).

Trypanosoma congolense infection in cattle is usually less acute and less dramatic than haemorrhagic *T. vivax* infection (Fiennes, 1970; Stephen, 1986). Cases have fever; with body temperature rising to 39.4-40 °C and pyrexia parallels the parasitaemia, which is usually lower than in *T. vivax* infection. Other clinical signs include staring coat, loss of weight, reduced fertility, weakness, dyspnoea and almost white mucous membranes. Superficial lymph nodes may or may not be enlarged and loss of appetite often occurs during pyrexia (Stephen, 1986). Central nervous system signs in form of circling have been observed in experimental *T. brucei* infection in cattle (Wellde *et al.*, 1989; Clausen *et al.*, 1999) and in experimental mixed infections of *T. brucei* and *T. congolense* in cattle (Masake *et al.*, 1984).

2.1.2 Pathological features

The dominant feature of trypanosomosis in cattle is anaemia (Murray, 1978; ILRAD, 1990). Though *Babesia*, *Anaplasma* and *Theileria* infections also cause anaemia, the severity is greater in trypanosome infections than in other diseases (Molyneux and Ashford, 1983). There are two phases of anaemia, the acute haemolytic phase during the rising parasitaemia and the chronic phase during declining parasitaemia (Murray, 1978; ILRAD, 1990). A significant drop in packed cell volume (PCV), haemoglobin (Hb) concentration and red blood cell (RBC) counts was observed in cases of bovine trypanosomosis caused by *T. congolense* and *T. vivax* in imported Holstein-Friesian cattle in Nigeria (Ugochukwu, 1986). Other pathological effects include myocarditis and increased vascular permeability (Fiennes, 1970; Murray, 1978; Molyneux and Ashford, 1983). Internal haemorrhages and enlarged spleen have also been

observed at postmortem in animals that died of haemorrhagic *T. vivax* infection in Uganda (Magona *et al.*, 1997).

2.1.3 Epidemiological features

Existing reports in Uganda indicate that the parasitological prevalence of trypanosomosis in cattle is 11.9% under the intensive dairy system and 25% under the communal grazing systems (Okuna *et al.*, 1996). Of all the confirmed cases of trypanosomosis in Uganda, 64% are due to *T. vivax*, 30% due to *T. congolense* and 6% due to *T. brucei* (Anon., 1996). A parasitological prevalence of 26.5-34.8% of *T. vivax* infection has been reported during an outbreak of haemorrhagic *T. vivax* infection in Uganda (Magona *et al.*, 1997). Recent reports on trypanosomosis in Bugoma and Teso districts of Western Kenya infested by *Glossina pallidipes* revealed a parasitological prevalence of 1.5%-8.2% (Anon., 1998). Other studies have reported parasitological prevalences of bovine trypanosomosis in Western Kenya and at the Kenya Coast of up to 40% (Murilla *et al.*, 1998).

The parasitological prevalences of bovine trypanosomosis reported in studies conducted in other African countries include, 1.9% in Malawi (van den Bossche *et al.*, 2000a), 4.2% in Mali (Awan *et al.*, 1988), 7.3-9% in Nigeria (Ahmed *et al.*, 1994; Kalu, 1995), 10% in Zaire (Singh *et al.*, 1988), 16% in Tanzania (Connor and Hallwell, 1987), 17.2% in Ethiopia (Afewerk *et al.*, 2000), 19.1% in Sudan (Hall *et al.*, 1983) and 38.5% in Ghana (Turkson, 1993).

The prevalence and distribution of bovine trypanosomosis is influenced by a number of factors. The prevalence is highest and the associated PCV is lowest where tsetse densities are high, taurine cattle are predominant and herds belong to several owners from rural areas (Hendrickx *et al.*, 2000). Variations in the prevalence of trypanosomosis in cattle depend on variation in tsetse density and tsetse challenge (Leak *et al.*, 1993). The prevalence of trypanosomosis in cattle in tsetse-infested areas increases with age, being highest among old cattle of 5-9 years (Kalu, 1995; Magona *et al.*, 2000). Other factors that influence the prevalence of bovine

trypanosomosis include breed, intensity of management and season (Kalu, 1995). The prevalence of trypanosomosis was observed to be higher in trypanosusceptible cattle, especially the crossbreeds (12.1%) than in the more resistant trypanotolerant breeds such as the N'Dama (5.9%) in Nigeria (Kalu, 1995). The prevalence is higher in extensively managed stock (Kalu, 1995) or in migratory cattle (Hall *et al.*, 1983) and during the wet than the dry season (Kalu and Lawani, 1996).

Adverse effects of trypanosomosis in cattle are frequently exacerbated by undernutrition (Holmes *et al.*, 2000). The rate of anaemia, pyrexia and weight changes are influenced by both protein and energy intakes (Agyemang *et al.*, 1990; Katunguka-Rwakishaya *et al.*, 1995; Holmes *et al.*, 2000). Host nutrition is recognized to have profound effects on many aspects of the immune response such as antibody production, which in turn is known to influence the level of parasitaemia (Holmes *et al.*, 2000).

Trypanosomosis is often confounded by intercurrent infection (Stephen, 1986). Cases presenting with acute fever (up to 40.5°C), lacrymation, salvation, diarrhoea, coughing, severe anaemia, severe emaciation involving over 75% of indigenous Zebu cattle were reported in a village in Eastern Uganda during prolonged drought (Anon., 1997b). Laboratory confirmation of these cases revealed concurrent *T. vivax* and *Theileria parva* infection and postmortem examination further revealed heavy nematode infections and liver fluke infestation.

2.1.4 Definitive diagnosis

Confirmatory diagnosis of bovine trypanosomosis and treatment of sick animals relies on clinical examination and demonstration of the parasites, and evidence of anaemia. Demonstration of parasites relies on microscopic examination of Giemsa stained thin and thick blood smears for detection and identification of trypanosome species and on detection of trypanosomes by examination of the Buffy-coat using dark-ground microscopy (Murray *et al.*, 1977). To ensure good results, care should be taken to ensure that every microscopic field of

the Buffy-coat is examined thoroughly. The packed cell volume is measured using the microhaematocrit centrifugation technique (Woo, 1969).

2.2 Theileriosis

2.2.1 Key clinical signs

Bovine theileriosis (East Coast fever) is caused by *Theileria parva*, which is transmitted by the three-host tick *R. appendiculatus*. Its main clinical signs include fever, enlarged superficial lymph nodes, anorexia, lacrymation, nasal discharge, depression and diarrhoea with bloody faeces (Bruce *et al.*, 1910; Shannon, 1977; Omuse, 1978; Kiptoon *et al.*, 1983; Norval *et al.*, 1992; Kambarage, 1995; Mbassa *et al.*, 1998; Maloo *et al.*, 2001a). Petechial haemorrhages may occur under the tongue and on the vulva (Shannon, 1977; Urquhart *et al.*, 1996; Maloo *et al.*, 2001a). The mortality rate in susceptible cattle can be as high as 100% (Norval *et al.*, 1992; Jain, 1993).

In experimental *T. parva* infection in calves, Mbassa *et al.* (1998) observed pyrexia and enlarged lymph nodes as the major clinical signs. Studies on confirmed field cases of East Coast fever (ECF) in Kenya, found high fever (40.1-40.2°C), enlarged lymph nodes, petechial haemorrhages on mucous membranes and under the tongue, laboured respiration and terminal diarrhoea as the main clinical signs (Omuse, 1978; Kiptoon *et al.*, 1983). It has also been reported that haemorrhages in older animals affected by East Coast fever are numerous pinpoint petechiae, but fewer and ecchymotic ones in calves (Kiptoon *et al.*, 1983).

Shannon (1977) recorded the frequency of individual clinical signs in 61 confirmed field cases of East Coast fever in grade cattle in Uganda. The clinical signs observed in order of frequency included enlarged prescapular lymph nodes (91.8%), enlarged parotid lymph nodes (49.2%), petechial haemorrhages (37.7%), enlarged precrural lymph nodes (29. 5%), soft cough (24.6%), diarrhoea (13.1%), eye lesions (4.9% and circling (1.6%).

Clinical signs of East Coast fever often reported by farmers include extreme depression, anorexia, lacrymation and a drop in milk yield in lactating cows. However, experienced farmers usually observe swollen lymph nodes and in terminal East Coast fever cases, bloody diarrhoea (Omuse, 1978).

2.2.2 Pathological features

Post-mortem findings in field cases usually include congestion and oedema of the lungs with variable amounts of frothy discharge in respiratory airways, liver friability, small grey-white nodules in the renal cortex, petechiation of serous membranes and congestion of the spleen (Bruce *et al.*, 1910; Kambarage, 1995).

Unlike anaplasmosis and babesiosis, that mainly affect erythrocytes, theileriosis normally affects mainly leucocytes. But anaemia is a variable finding with theileriosis, being commonly observed in cattle with multiple haemotropic parasite infections (Kiptoon *et al.*, 1983) and chronic cases (Omuse, 1978). Low packed cell volume (28 ± 5%), haemoglobin concentration (9.93 ± 1.20 g/dl) and red cells counts (6.05 ± 1.16 /mm^3 x 10^6) have been reported in cases of ECF (Omuse, 1978), though they fell within the normal ranges of PCV (24-46 %), RBC counts (5-10 x10^6/ mm^3) and haemoglobin concentration (8-15 g/dl) as described by Schalm (1975). A high degree of leucopenia with a neutropenia and lymphopenia has also been observed (Omuse, 1978). In experimental infections of *T. parva* in Ankole and crossbred cattle, an average 30% reduction of the PCV of infected cattle was reported to occur; though classical *T. parva* infection did not affect the red blood cell picture (Paling *et al.*, 1991).

2.2.3 Epidemiological features

Several epidemiological studies have been conducted in East and Central African countries on East Coast fever. In Central Kenya, ECF has been reported to account for 70.1% of the total cases (Mulei and Rege, 1989), while an incidence of 22% in Zebu calves was found on Rusinga Island in Western Kenya (Latif *et al.*, 1995). Seroprevalences of *T. parva* infection of 22-85%

have been reported across a number of agroecological zones in Kenya (Deem *et al.*, 1993). In Uganda, a parasitological prevalence of ECF of 48% has been reported (Anon., 1996), and in Zambia, ECF is reported to cause over 50% mortality of cattle, even in endemic areas (Billiouw *et al.*, 1999). East Coast fever has been reported to accounts for 73% of the cases of tick-borne diseases in Tanzania (Kambarage, 1995) and has been reported to cause 14% mortality in crossbred calves of 10 months old in Zanzibar (Jacobsen, 1983).

The distribution of East Coast fever is associated with the distribution of its vector. In Kenya, ECF is known to be confined to the cool wet climate of the high altitude areas where *R. appendiculatus* is found (Omuse, 1978; Mulei and Rege, 1989). Other studies have revealed that the activity of *R. appendiculatus* instars and *T. parva* transmission are restricted by the unimodal rainfall pattern (Norval *et al.*, 1992; Billiouw *et al.*, 1999). Adult tick activity invariably peaks during the wet seasons and is associated with the highest ECF incidence (Norval *et al.*, 1992). Fluctuations in tick abundance and intensity as well as temporal patterns of *T. parva* transmission are influenced by the year-to-year variation in rainfall (Norval *et al.*, 1992; Billiouw *et al.*, 1999).

Indigenous cattle such as the Small East African Zebu reared in ECF endemic areas of tropical Africa are known to show a high degree of resistance and exhibit some disease tolerance to *T. parva* infection (Norval *et al.*, 1992; Latif *et al.*, 1995). Infection is often mild in Zebu calves and mortality is low, with an average ECF-case fatality rate of only 21% (Latif *et al.*, 1995) as compared to fatality rates of 100% in exotic breeds of cattle (Jain, 1993). However, indigenous cattle often remain as carriers and act as reservoirs of infection for ticks (Norval *et al.*, 1992; Latif *et al.*, 1995; Perry and Young, 1995). Susceptible cattle introduced in endemic areas often suffer high mortality, irrespective of age or breed, unless rigid precautions are observed (Jain, 1993). Indigenous cattle may also suffer from East Coast fever during prolonged rains in marginal areas when the immunity declines as a result of low tick survival and challenge (Deem *et al.*, 1993). Intermingling of cattle with wild African buffalo (carriers) in the presence

of *R. appendiculatus* often leads to outbreaks of *T. parva lawrenci* in cattle and a high mortality rate (Norval *et al.*, 1985; Urquhart *et al.*, 1996).

High innate susceptibility of cattle, virulence of the parasite and the climate are the main factors reported to limit progress towards endemic stability (see Section 2.9.4). In cases where endemic stability does not exist, cattle mortality due to *T. parva* infection is high (Norval *et al.*, 1992; Perry and Young, 1995; Billiouw *et al.*, 1999; Gitau *et al.*, 2000).

Age-related susceptibility of cattle to ECF in endemic areas occurs. Calves are affected most before they sero-convert, especially at the age of 4-10 months (see Section 6.1). Thus, ECF is considered to be a serious calf disease, with reported mortality rates of 21% and 13.5% in indigenous calves up to 1 year old in Kenya (Latif *et al.*, 1995) and in Uganda (Okello-Onen, 1996), respectively.

Breed differences in susceptibility influence incidence, morbidity and mortality of ECF in cattle. For example in Zanzibar, Jacobsen (1983) found a higher ECF-induced mortality rate (14%) in crossbred calves than in Zebu calves (4.2%) and a lower recovery rate (57%) in crossbred calves than in Zebu calves (90%). In another study, mortality following experimental infection with *T. parva* was 64% in the crossbreds and 32% in Ankole cattle (Paling *et al.*, 1991). The difference in susceptibility was attributed to the genetic ability of the Ankole to limit the explosive multiplication of macroschizonts, resulting in less severe damage of the lymphoid tissue during the acute phase of the disease (Paling *et al.*, 1991).

The occurrence of the ECF has been associated with grazing susceptible cattle on tick-infested pastures and unsatisfactory tick control due to improper dipping of the herds (Norval *et al.*, 1992; Kambarage, 1995; Gitau *et al.*, 2000). Furthermore, it has been observed that use of pastures by pastoral cattle, which are rarely dipped, increases tick infestation, and mixing susceptible cattle from ECF-free areas with carrier cattle from infested areas, especially during

periods of drought, introduces ECF into previously unaffected areas (Kambarage, 1995; Billiouw *et al.*, 1999). Open-grazing systems allow for more characteristic cycles of *T. parva* transmission to occur, both from carrier and clinically affected cattle and hence lead to higher sero-conversion rates of *T. parva* infection than zero grazing systems (Norval *et al.*, 1992; Gitau *et al.*, 2000). However, the level of challenge in the open grazing system depends on climatic suitability, grazing practice and range (and thus mixing with other potentially infected cattle), tick control practice and cattle breed (Norval *et al.*, 1992; Gitau *et al.*, 2000). Increased exposure of calves to *T. parva*-infected ticks found in higher numbers in open-grazing systems is known to be responsible for high ECF incidence (Gitau *et al.*, 1999).

2.2.4 Definitive diagnosis

Confirmatory diagnosis of ECF relies on finding *T. parva* macroschizonts in Giemsa stained smears of lymph node biopsies or on finding piroplasms on Giemsa stained ear-vein blood smears in the red cells (Urquhart *et al.*, 1996). However, animals that show *Theileria* piroplasms in blood smears before the schizont stage is detectable in lymph node biopsies are often not considered as clinical cases of ECF (Latif *et al.*, 1995). Body temperature monitoring, which involves monitoring of animals whose body temperature is above 39.5°C and preparing of lymph node biopsies and blood smears, has been recommended as a useful method for early detection of ECF infection and prompt treatment of cases in an outbreak situation (Minjauw *et al.*, 1998).

2.3 Anaplasmosis

2.3.1 Key clinical signs

Bovine anaplasmosis is a rickettsial disease caused by *Anaplasma marginale* and transmitted by over 20 tick species including the one-host tick genus, *Boophilus*. It is also transmitted mechanically by biting flies of the genus *Tabanus*, *Chrysops* and *Siphora* in areas which are free of ticks (Ristic, 1968; Potgieter and Stoltz, 1994) and by blood contaminated instruments during castration, dehorning and eartagging operations and through needle passage during

vaccinations (Dikmans, 1950). The major clinical signs of anaplasmosis include pyrexia, anaemia, jaundice, anorexia, dehydration, laboured breathing, and a severe drop in milk yield or abortion in cows (Theiler, 1910; Ajayi *et al.*, 1987; Potgieter and Stoltz, 1994; Egbe-Nwiyi *et al.*, 1997).

In an abattoir study conducted in Nigeria on cattle destined for slaughter, *Anaplasma*-positive cattle were observed to have pyrexia, anorexia, pale mucous membranes, weight loss, weakness, dehydration (of 5-7%) and laboured respiration as the main clinical signs (Egbe-Nwiyi *et al.*, 1997). In Kenya, the common clinical signs observed in field cases of anaplasmosis include a drop in milk yield, anorexia, dullness and passage of scanty hard faeces (Omuse, 1978). In experimental infections, Ajayi and others (1987) observed severe anaemia and pyrexia as the main clinical signs, in addition to other signs such as general weakness, loss of appetite, depression, accelerated respiratory rate and muscle tremors.

2.3.2 Pathological findings

The main postmortem features of anaplasmosis include enlargement of the liver, gall bladder, spleen and lymph nodes (especially the superficial ones). The liver and spleen may be moderately congested, the carcass is jaundiced and the gall bladder is grossly enlarged. The liver is suffused with bile and petechial haemorrhages appear on the heart muscles (Losos, 1986; Egbe-Nwiyi *et al.*, 1997).

The major haematological feature of anaplasmosis is anaemia, with mean PCV, Hb and RBC decreasing below normal (Potgieter and Stoltz, 1994). *Anaplasma*-infected cattle were observed to have lower mean PCV (24.8 ± 3.2 %), mean Hb (6.7 ± 1.8 g/dl) and mean RBC counts (3.73 ± 1.6 x 10^6/mm^3) compared to non-infected cattle (mean PCV=32.6 ± 4.2, mean Hb = 10.9 ± 3.8 and mean RBC count = 6.21 ± 2.7 x 10^6/ mm^3) in a study conducted in Nigeria (Egbe-Nwiyi *et al.*, 1997). Confirmed cases of bovine anaplasmosis were observed to show typical

signs of haemolytic anaemia, with a mean PCV of 14 ± 4.25, mean RBC counts of 2.54 ± 1.16 x 10^6/ mm^3 and a mean haemoglobin content of 4.6 ± 1.36 in Kenya (Omuse, 1978).

2.3.3 Epidemiological features

Parasitological prevalence of bovine anaplasmosis in tropical African countries reported in literature includes 29-36% in Uganda (Ssenyonga *et al.*, 1991; Anon., 1996), 36-38.2% in Kenya (Mulei and Rege, 1989; Latif *et al.*, 1995) and 37.7% in Nigeria (Egbe-Nwiyi, 1997). A seroprevalence of bovine anaplasmosis ranging from 81 to 94% was reported across different agroecological zones in Kenya (Deem *et al.*, 1993) and from 14.7 to 38.6% in Zambia (Jongejan *et al.*, 1988).

The distribution of anaplasmosis is recognised to be determined by the distribution of its vectors: *Boophilus* species and *Rhipicephalus evertsi evertsi* (Jongejan *et al.*, 1988; Omuse, 1978) and detection of *A. marginale* in Zebu calves has been observed to coincide with the peak of *Boophilus decoloratus* infestations (Latif *et al.*, 1995). However, other factors such as anaplasmosis being transmitted by a variety of one-, two- and three-host ticks including *Boophilus* spp., *Rhipicephalus evertsi evertsi* and biting flies, life-long carrier status of recovered animals and presence of wild and domestic reservoirs of infection are known to determine the distribution of anaplasmosis (Norval *et al.*, 1984; Potgieter and Stoltz, 1994; Urquhart *et al.*, 1996).

In animals that survive acute anaplasmosis, parasitaemia levels fluctuate for several months before dropping below microscopically detectable levels (Potgieter and Stoltz, 1994). Such animals are usually protected against clinical disease, but can serve as reservoirs and infections may recrudesce following stress (Egbe-Nwiyi *et al.*, 1997). Stress due to concurrent trypanosomosis or babesiosis with anaplasmosis may lead to occurrence of clinical anaplasmosis in immune carrier animals (Fox *et al.*, 1993).

Anaplasmosis has an age-related susceptibility. It is mild in calves up to 1 year, but animals of 2-3 years old develop typical, often fatal anaplasmosis and cattle over 3 years develop a peracute and frequently fatal anaplasmosis (Jain, 1993; Potgieter and Stoltz, 1994; Urquhart *et al.*, 1996). Age-specific seroprevalence of bovine anaplasmosis has also been reported to decline with increasing age (Jongejan *et al.*, 1988). Instances have been reported where up to 80% of adult cattle have been found affected by anaplasmosis (Egbe-Nwiyi *et al.*, 1997). Adult cattle introduced into endemic areas are susceptible and a mortality rate of up to 80% occurs (Ajayi *et al.*, 1982).

Breed susceptibility to bovine anaplasmosis occurs, with imported breeds being more susceptible to anaplasmosis than indigenous cattle found in endemic areas in Africa (Ajayi *et al.*, 1982). Clinical signs of anaplasmosis are mild in Zebu calves (Latif *et al.*, 1995).

Hypomagnesaemic cattle are reported to be resistant to experimental clinical infection of *A. marginale* (Jain, 1993). However, the influence of this factor on the occurrence of natural *Anaplasma* infections is not yet well documented.

2.3.4 Definitive diagnosis

Definitive diagnosis of bovine anaplasmosis relies on history, pathological and clinical signs, coupled with estimation of PCV and postmortem findings. Confirmation of the diagnosis depends on the microscopic examination of stained thin blood smears in order to demonstrate *Anaplasma* parasites in the erythrocytes of the affected animals, especially for acute cases (Potgieter and Stoltz, 1994). It is recommended that blood smears be taken from the extremities especially from capillary blood by tail or ear prick with a razor blade or scalpel blade or sharp needle (Anon., 1997c). Thin and thick blood smears become unsuitable for diagnosis when exposed to formalin, contaminated with bacteria, too thick and when jugular blood is used for making smears (Anon., 1997c). Serological tests are not suitable for detection of clinical disease, but are useful as research tools (Anon., 1997c).

2.4 Babesiosis

2.4.1 Key clinical signs

Babesiosis is an important tick-borne disease of cattle that is widespread in tropical Africa. Babesiosis, caused by *Babesia bovis* and *B. bigemina,* is transmitted by ticks of the genus *Boophilus*. *Babesia bovis* is transmitted only by *Boophilus microplus* whereas *B. bigemina* is readily transmitted by both *Boophilus microplus* and *Boophilus decoloratus* (De Vos, 1979; Norval *et al.*, 1983; Jongejan *et al.*, 1988). There is a wide variation in the clinical signs of babesiosis because some species are not pathogenic and within species, there are strain differences with respect to virulence (Losos, 1986). Generally, babesiosis manifests in two forms: acute and chronic syndrome (Ristic, 1981; Losos, 1986). The chronic syndrome is poorly defined clinically, but is associated with anaemia and weight loss. The acute syndrome is characterized by high fever (41-45.5°C), anaemia, haemoglobinuria (redwater), jaundice, abortion and variable mortality (Ristic, 1981; Losos, 1986). If death does not occur, there is loss of weight, reduced milk yield, diarrhoea followed by constipation (Losos, 1986; Urquhart *et al.*, 1996).

The main clinical signs observed in field cases of bovine babesiosis are: anaemia, pyrexia, haemogloburia and jaundice. Such cases present with pale mucous membranes that may be yellow-tinged and owners frequently report animals passing red urine (Omuse, 1978). Field cases of bovine babesiosis and anaplasmosis usually present with very similar clinical signs, but the passage of red urine is only observed in cases of babesiosis (Omuse, 1978). In cases of cerebral babesiosis of cattle caused by *B. bovis*, terminal central nervous signs involving paddling of limbs, ataxia and circling occur (Losos, 1986).

2.4.2 Pathological features

The major pathological features in babesiosis are anaemic anoxia and red cell destruction (Hildebrandt, 1981). Anoxia causes degeneration and swelling of endothelial cells, which impede blood flow and further complicate the anoxic state of the organs. Damaged endothelium and hypoproteinaemia resulting from hepatic dysfunction lead to oedema (Hildebrandt, 1981). At necropsy, subcutaneous tissue and mucous membranes are pale, the liver is enlarged, gall bladder and urinary bladder are distended, the kidneys are congested, the lungs are oedematous and there is massive splenomegaly (Losos, 1986). In case of cerebral babesiosis, marked packed parasitized erythrocytes are seen in the capillaries of the gray matter of the brain and in the kidneys (Losos, 1986).

Intravascular haemolysis is the most distinctive haematological feature in bovine babesiosis. This is manifested by a decrease in PCV, red blood cell counts and haemoglobin values by over 50% (Losos, 1986). Omuse (1978) found that confirmed cases of bovine babesiosis in Kenya had lower mean PCV (16.3 ± 6.8 %), mean RBC count (3.24 ± 1.7 x 10^6/ mm^3) and mean haemoglobin content (5.8 ± 2.5 g/dl) than normal ranges of PCV (24-46 %), RBC counts (5-10 10^6/ mm^3) and haemoglobin content (8-15 g/dl) as described by Schalm (1975).

2.4.3 Epidemiological features

Reported prevalences of bovine babesiosis as detected by presence of piroplasms in Giemsa stained smears in tropical African countries cited in the literature include 7.2% in Nigeria (Ajayi, 1978), 16% in Uganda (Anon., 1996), 41.4% in Central Kenya (Mulei and Rege, 1989) and 47% on Rusinga Island, Western Kenya (Latif *et al.*, 1995).

The reported seroprevalences of bovine babesiosis include, 52% (*B. bigemina*) and 32% (*B. bovis*) in Zimbabwe (Katsande *et al.*, 1999), 10.3-78.6% (*B. bigemina*) and 7.1-38.1% (*B. bovis*) in Mali (Miller *et al.*, 1984), 42-74% (*B. bigemina*) and 44.7-68% (*B. bovis*) in Zambia (Jongejan *et al.*, 1988), 78% (*B. bigemina*) in Botswana (Mehlitz and Ehret, 1974), 79-94% (*B.*

bigemina) in Kenya (Deem *et al.*, 1993), and 88% (*B. bigemina*) and 96% (*B. bovis*) on Pemba Island, Tanzania (Woodford *et al.*, 1990).

The occurrence and severity of bovine babesiosis is influenced by vector, parasite and host factors. A high level of *Boophilus* tick challenge in endemic areas is known to maintain the immunity of animals to babesiosis at a high level (Urquhart *et al.*, 1996). Jongejan and others (1988) found that cattle throughout Zambia had antibodies against *B. bigemina* according to the distribution of *B. decoloratus*, whereas those with antibodies to *B. bovis* were restricted according to the distribution of *B. microplus*. A similar pattern of association between the distribution of *B. microplus* and seropositivity to *B. bovis* was observed in Zimbabwe (Norval *et al.*, 1983). In Kenya, no clinical signs were detected in Zebu calves, in which *B. bigemina* parasites were detected during peak infestation of *B. decoloratus* ticks (Latif *et al.*, 1995).

Babesiosis is particularly severe in naïve animals introduced into endemic areas and is a considerable constraint on livestock development in many parts of the world (De Vos, 1979; Norval *et al.*, 1983; Urquhart *et al.*, 1996). However, the severity also depends on the virulence of the particular species of *Babesia* parasite. *Babesia bovis* is relatively more pathogenic than *B. bigemina* in cattle (Losos, 1986; Urquhart *et al.*, 1996). Studies conducted in Zambia revealed that outbreaks of babesiosis caused by *B. bigemina* are rare in traditionally managed herds of Sanga and Zebu cattle, but babesiosis due to *B. bovis*, though rare, was fatal when introduced into traditional or commercial farms, especially outside the distributional areas of *B. microplus* ticks (Jongejan *et al.*, 1988).

The severity of babesiosis also depends on the breed of cattle involved. Acute babesiosis due to *B. bigemina* often occurs in imported and crossbreed cattle, while *B. bovis* infection is acute in very young calves of imported breeds (Losos, 1986).

The severity of bovine babesiosis is related to the age of animal, with higher fever and more morbidity and mortality occurring among the aged cows than the yearlings and steer, while calves have innate resistance (Losos, 1986). For example, Zebu calves were observed to have mild reactions to babesiosis in Kenya (Latif *et al.*, 1995). *Babesia bigemina* was detected in Zebu calves of 6-8 months old, but there were no associated clinical signs manifested.

The severity of babesiosis depends on the immune status of the host. Young animals acquire passive immunity through colostrum and subsequently active immunity is stimulated as they get exposed to infection, at which time they become premune carriers (De Vos, 1979; Hildebrandt, 1981; Urquhart *et al.*, 1996).

Animals that survive babesiosis become carriers and harbour a subclinical infection, which is maintained by premunition (Hildebrandt, 1981). However, this balance may be disturbed by stresses associated with intercurrent diseases, transport, age and immunodeficiency (Hildebrandt, 1981). Environmental stress often triggers outbreaks of clinical babesiosis in adult animals in endemic areas (Ajayi *et al.*, 1982; Losos, 1986).

2.4.4 Definitive diagnosis

Diagnosis of babesiosis, especially in chronic cases, is difficult because detection of *Babesia* parasites by examination of Giemsa stained blood smears is limited for very few parasites can be demonstrated usually after a long search (Omuse, 1978; Böse *et al.*, 1995). Non-detection of parasites often accounts for the low number of cases of babesiosis confirmed in the laboratory, but taking blood for smears during the cool periods of the day from the ear vein can increase chances of microscopic detection of *Babesia* parasites (Omuse, 1978). Confirmation of diagnosis depends on the history, clinical signs and examination of blood films stained with Giemsa. However, once the acute febrile phase has subsided parasites are often difficult to detect since they are rapidly removed from the circulation (Urquhart *et al.*, 1996). Microscopic detection using thin and thick blood smears is the most recommended method for on-site

diagnosis of acute disease (Böse *et al.*, 1995). Better results are obtained in diagnosis of *B. bovis* and other species, when capillary blood from the tail prick is used (Böse *et al.*, 1995).

2.5 Cowdriosis

2.5.1 Key clinical signs

Bovine cowdriosis is a fatal tick-borne disease caused by *Cowdria ruminantium* transmitted by ticks of the genus *Amblyomma*. Cowdriosis is common in susceptible populations of cattle introduced into endemic areas, particularly in imported exotic breeds, in which the severe form of the disease occurs (Losos, 1986). The disease in cattle is characterised by pyrexia, with a temperature of 40-42°C lasting one week before dropping to subnormal before death. Occasionally there are nervous symptoms such as muscular twitching, tetanic seizures, squinting of the eyes, excessive salivation and galloping movement after the animal has fallen to the ground (Cowdry, 1926). Mild or subacute syndromes usually occur in very young animals with high innate resistance (Cowdry, 1926; Losos, 1986). Subacute and chronic forms are mainly characterised by diarrhoea, while the mild form is characterised by elevation of temperature. The peracute disease is characterised by high fever, collapse, convulsions and sudden death. In the acute form, pyrexia is followed by depression, loss of appetite, appearance of nervous signs such as high stepping, unsteady gait and progressive signs of encephalitis such as chewing movements, twitching of eyelids, walking in circles and aggressive attitude (Cowdry, 1926; Losos, 1986). A mortality rate of 50-90% is often associated with the typical nervous signs of cowdriosis, especially when the severe disease occurs in susceptible animals imported into endemic areas (Losos, 1986).

2.5.2 Pathological features

Anaemia, leucopenia and thrombocytopenia are occasionally reported. Less severe forms of cowdriosis are associated with ascites, hydrothorax, hydropericardium, reddening of mucous membranes of the respiratory tract and gastrointestinal tract, oedema of the lungs, haemorrhages on serosal surfaces, enlarged lymph nodes and spleen, and signs of encephalitis

usually observed at post mortem (Bezuidenhout *et al.*, 1994). Haematological changes often seen in affected cattle include a drop in eosinophils and PCV and decrease in plasma volume (Losos, 1986).

2.5.3 Epidemiological features

Cowdriosis is reported to be responsible for 5.6% of the cattle mortalities in Uganda (Anon., 1996). In Kenya, cowdriosis has been reported in several areas, but the cases are sporadic (Wamukoya, 1992) and mortality, especially in indigenous Zebu cattle, is low (Latif *et al.*, 1995) because of a high seroprevalence (73-80%) in cattle and widespread endemic stability across different agroecological zones (Deem *et al.*, 1993). In Senegal, low mortality rate of cattle due to cowdriosis and high seroprevalence (33–90%) of cowdriosis have been observed to coincide with the distribution and high infection rate of *C. ruminantium* in the *Amblyomma* ticks (Gueye *et al.*, 1993). In Zimbabwe, seroprevalence of cowdriosis of 11-52% has been observed in the *Amblyomma*-infested and *Amblyomma*-free areas (Vries *et al.*, 1993). Cowdriosis-related mortality on large-scale farms in Zimbabwe is reported to be only 2.3% in calves (0-12 months) and 0.6% in older cattle (over 13 months), despite being responsible for 51% of the mortalities on affected farms (Meltzer *et al.*, 1996). In South Africa, the annual mortality rate of cattle due to cowdriosis is reported to be 1.3% (du Plessis *et al.*, 1994).

The distribution of cowdriosis depends on the distribution of the vector ticks, *Amblyomma* spp., which in turn depends on the climatic suitability (TickCost, 1999). Moreover, the incidence of cowdriosis depends on the prevalence and abundance of the *Amblyomma* spp. ticks, especially of *A. variegatum* (Losos, 1986; Gueye *et al.*, 1993).

Cowdriosis is an important disease affecting imported breeds of cattle in sub-Saharan Africa and indigenous cattle in endemic areas rarely suffer clinical disease (Uilenberg, 1981; Losos, 1986) due to endemic stability. The incidence and mortality of indigenous Zebu calves due to cowdriosis were observed to be negligible on Rusinga Island in Western Kenya (Latif *et al.*,

1995). Susceptibility to cowdriosis is enhanced by lack of previous exposure to the disease, even with indigenous breeds of cattle (Ilemobade, 1977). An age-related resistance to cowdriosis, with young cattle having a higher resistance (innate) and consequently lower case fatalities than older cattle occurs (Norval *et al.*, 1995).

2.5.4 Definitive diagnosis

Definitive diagnosis of cowdriosis depends on demonstration of the *C. ruminantium* parasites in blood or endothelial cells at post mortem by examination of brain squash preparation stained with Giemsa (Purchase, 1945). *Cowdria ruminantium* can easily be recognised in squash smears prepared from the grey matter of the cerebrum. Preparations from the spinal cord are usually as good as those from cerebral grey matter (Purchase, 1945). The hammer and nail method is preferred to syringe and needle method for easy of extraction of brain material (Malika *et al.*, 1992). For effective treatment of cowdriosis early in the course of febrile reactions before clinical signs appears, provisional diagnosis may be made on the basis of history, clinical signs and postmortem lesions of previous cases (Losos, 1986).

2.6 Parasitic gastroenteritis

2.6.1 Key clinical signs

Parasitic gastroenteritis is usually observed in young animals and is a herd problem. The major nematode species that cause PGE in cattle in tropical Africa include *Haemonchus placei*, *Trichostrongylus axei*, *Bunostomum phlebotomum*, *Oesophagostomum radiatum*, *Cooperia pectinata* and *Cooperia punctata* (Mango *et al.*, 1974; Sauvage *et al.*, 1974; Omara-Opyene, 1985; Kaufmann *et al.*, 1989; Waruiru *et al.*, 1993; Waruiru *et al.*, 1998). Of the nematodes reported above, *H. placei* and *O. radiatum* are recognised as the most pathogenic and economically important parasites of cattle in the tropics, but *T. axei* and *Cooperia* spp. are of lower pathogenicity and limited significance (Waruiru *et al.*, 1998). The significance of *H. placei* and *O. radiatum* is due to the severe trauma and blood loss caused by their migrating and feeding stages. Egg counts of up to 1000 *H. placei* per gram of faeces are accompanied by

serious clinical signs and counts of 500-700 *H. placei* eggs per gram indicate a dangerous infection if combined with 300 epg of *Oesophagostomum* or *Bunostomum* species (Waruiru *et al.*, 1998).

The major clinical signs of nematode infections in cattle include diarrhoea, staring hair coat, 'pot belly' in calves, submandibular oedema, anaemia, poor body condition, stunted growth, weakness and progressive emaciation, usually with a good appetite persisting until shortly before death (Kaufmann *et al.*, 1989; Waruiru *et al.*, 1993; Eysker and Ploeger, 2000). In haemonchosis, anaemia and submandibular oedema are the major clinical signs (Eysker and Ploeger, 2000).

2.6.2 Pathological features

The key pathological features of nematode infections in cattle include, haemorrhagic gastritis, generalized oedema, anaemia and fat degeneration. At necropsy, a large number of *Haemonchus, Trichostrongylus, Bunostomum* and *Oesophagostomum* worms are recovered from the lumen of the gastrointestinal tract (Kaufmann *et al.*, 1989; Waruiru *et al.*, 1993).

2.6.3 Epidemiological features

The prevalences of nematode infections in cattle revealed by various abattoir and faecal examination surveys conducted in tropical African countries include, 48.8% in Zimbabwe (Vassilev, 1999), 22-61% in Uganda (Sauvage *et al.*, 1974; Magona and Musisi, 1998), 43-85.5% in Kenya (Mango *et al.*, 1974; Waruiru *et al.*, 2000) and 97% in The Gambia (Kaufmann and Pfister, 1990).

The intensity of the nematode infection found in cattle is often moderate. Nematode egg counts of 400 have been reported in cattle in Kenya, with *H. placei, Cooperia* species and *O. radiatum* accounting for 52.3%, 28.5% and 6.9% respectively, of the total burden (Waruiru *et al.*, 1998). Other studies have revealed 28% of Zebu calves having over 1000 nematode eggs per gram of

faeces in Western Kenya (Latif *et al.*, 1995), and 50% and 16% of Ankole calves having over 200 and 500 nematode eggs per gram of faeces, respectively, in Western Uganda (Sauvage *et al.*, 1974).

The prevalence of nematode infections in cattle is influenced by season. The level of strongyle infection as well as the relative abundance of *Haemonchus placei, Cooperia* species, *Oesophagostomum radiatum and Trichostrongylus axei* have been observed to increase during the rainy season and decrease during the dry season (Waruiru *et al.*, 2000).

Nematode infections are a more serious problem in cattle in subhumid rather than in arid areas in sub-Saharan Africa as evidenced in a study conducted in Northern Kenya (Omara-opyene, 1985). Parasites are checked by the arid climate, but the subhumid climate provides the favourable warm, wet conditions required for rapid development of eggs to the infective larval stage (L_3) (Reineckie, 1960). In addition to a hot and humid climate, predisposing causes for haemonchosis include overcrowding and lush pastures (Waruiru *et al.*, 1993).

Farm-related factors such as grazing management, helminth control and local microclimatic conditions also influence the level of nematode infection and occurrence of PGE (Kaufmann and Pfister, 1990; Waruiru *et al.*, 2000). Increased stocking rate associated with communal and intensive grazing management systems often leads to increased contamination of pastures and hence the greater risk of nematode infections.

Nematode infections are a serious problem in young stock of the post-weaning age (6months – 2 years), especially those in the subhumid areas of Africa (Omara-Opyene, 1985; Waruiru *et al.*, 1998; Rubaire-Akiiki *et al.*, 1999). The heaviest burden is reported to occur in 1.5-3-year-old animals. Dairy calves are the commonly affected group among cattle but steers and other young cattle up to 3 years of age may also be affected by haemonchosis (Waruiru *et al.*, 1993; Rubaire-Akiiki *et al.*, 1999). The prevalence of nematode infections and worm burden is often

highest in young cattle (6months - 2 years) followed by adults and calves less than 6 months, which are least affected (Omara-Opyene, 1985; Rubaire-Akiiki *et al.*, 1999). This is attributed to calves acquiring nematode infections as they start grazing and the infections increasing with both age and pasture contamination, reaching a climax in young cattle when stock become entirely reliant on grazing. Surviving young cattle after this stage get rid of the worm burden and develop resistance so that only a low level of infection exists in adults (Omara-opyene, 1985). Sex of the host seems not influence the prevalence or intensity of infection with nematode worms (Waruiru *et al.*, 2000).

Release of highly susceptible calves onto a highly contaminated pasture during the wet season causes PGE outbreaks (Kaufmann and Pfister, 1990; Waruiru *et al.*, 1993; Eysker and Ploeger, 2000). When young animals grazing with adult animals or when susceptible animals are put on dairy calf lots, infections are influenced by the intensity of worm larvae available to infect or re-infect the animals (Waruiru *et al.*, 1993).

Development of clinical illness is also favoured by a fall in the plane of nutrition, particularly in calves. Under adequate nutritional levels cattle may develop a subclinical infestation but when the nutritional level subsequently declines the diseases develops (Waruiru *et al.*, 1993). Malnutrition and concurrent disease may impair host resistance against helminths, resulting in higher worm burdens (Kaufmann and Pfister, 1990; Kaufmann *et al.*, 1992; Waruiru *et al.*, 1998).

2.6.4 Definitive diagnosis

Clinical diagnosis of parasitic gastroenteritis relies on a combination of clinical signs and history of the animal (Eysker and Ploeger, 2000). History should consider the age (young animals), season, grazing history and anthelmintic usage (Eysker and Ploeger, 2000).

Confirmatory diagnosis relies on faecal egg counts using the McMaster method as described by Hansen and Perry (1994). Finding nematode egg counts of 200 in adults and 500 in calves is indicative of PGE (Sauvage *et al.*, 1974), and anthelmintic treatment is recommended when a minimum of 500 eggs per gram of faeces is found (Hansen and Perry, 1994).

Pathological examination of dead animals or those killed *in extremis* can demonstrate parasitic gastroenteritis, quantify the burden and identify which species of gastrointestinal nematodes are involved, hence providing a basis for treatment of the entire herd (Eysker and Ploeger, 2000). For infections with blood sucking nematodes like *H. contortus*, pallor of the mucous membrane and red blood cell values (packed cell volume) provide useful tools for diagnosis.

2.7 Fasciolosis

2.7.1 Key clinical signs

Bovine fasciolosis in tropical Africa is mainly caused by *Fasciola gigantica* whose transmission is associated with *Lymnea natalensis* snail intermediate hosts. It is characterised by gradual wasting, progressive weakness, anaemia, dehydration and oedema in the intermandibular space and over the abdomen (Egbe-Nwiyi and Chaudrai, 1996: Bowman and Lynn, 1999). Most liver fluke infections in cattle are subclinical and often lead to retarded growth, reduced productivity and make affected animals more susceptible to other infections and to continuously contaminate pastures (Waruiru *et al.*, 2000).

2.7.2 Pathological features

Key pathological features include anaemia, hypoproteinemia, and an enlarged and necrotic liver at necropsy (Boray, 1985; Bowman and Lynn, 1999). Anaemia is the most characteristic symptom of subacute and chronic fasciolosis. Death of infected animals is usually a result of profound anaemia due to blood loss and failure of liver functions (Boray, 1985). In experimental *F. gigantica* infection in calves, marked reductions in PCV, Hb and RBC were

not observed, the PCV, Hb and RBC remained within the normal range (Mugambi *et al.*, 1990). Other pathological features observed include proliferation of lymphocytes, macrophages and plasma cells in the spleen and hepatic lymph nodes (Egbe-Nwiyi and Chaudrai, 1996).

2.7.3 Epidemiological features

The reported prevalence of bovine fasciolosis in African countries cited in the literature include 17-34% in Kenya (Mango *et al.*, 1974; Waruiru *et al.*, 2000), 29-36% in Uganda (Magona *et al.*, 1999), 11- 41% in Nigeria (Olusi, 1996; Egbe-Nwiyi and Chaudrai, 1996), 27% in Senegal (Diaw *et al.*, 1998) and 33% in Zimbabwe (Vassilev, 1999).

The greatest risk of bovine fasciolosis occurs in areas of extended high annual rainfall associated with high soil moisture and surplus water, but it diminishes in areas of shorter wet season and/or low temperatures (Malone *et al.*, 1998). Bovine fasciolosis acquisition is favoured in areas where animals graze on naturally or artificially flooded areas or around permanent water channels or dams normally inhabited by *Lymnea* spp. snails (Urquhart *et al.*, 1996; Olusi, 1996; Malone *et al.*, 1998). Outbreaks of fasciolosis often occur when there is increased congregation of animals utilizing metacercariae-infested pastures in marshy areas during dry season grazing (Urquhart *et al.*, 1996; Waruiru *et al.*, 2000). Thus, communal grazing management systems are more likely to expose animals to bovine fasciolosis than enclosed open grazing systems. Most liver fluke infections in cattle are subclinical and often lead to retarded growth, reduced productivity and make affected animals more susceptible to other infections and to continuously contaminate pastures (Waruiru *et al.*, 2000). Indigenous small Zebu cattle in tropical Africa have been observed to have a lower prevalence of fasciolosis than European exotic and large Zebu breeds, a fact that has been attributed to genetic resistance (Bitakaramire, 1973).

Waruiru and others (2000) reported occurrence of concurrent strongyle and liver fluke infections in cattle of all age groups, which accounted for 25.9% and 10.2% of the mixed

helminth infections during the dry and wet periods, respectively. They further observed the prevalence of liver fluke eggs to be significantly higher in adult cattle (68.3%) compared to either the immatures (24.1%) or young calves (9.7%). Thus the prevalence of liver fluke infections increased with age because of the long period for which animals are exposed to metacercariae-infested pastures (Waruiru *et al.*, 2000). Season has a significant influence on the prevalence of liver fluke infection in the different age groups of cattle, but farm effects and its interactions with season do influence the prevalence of liver fluke infections as well (Waruiru *et al.*, 2000).

2.7.4 Definitive diagnosis

The diagnosis of bovine fasciolosis depends on the observation of clinical symptoms, demonstration of parasite eggs and parasites at necropsy. Definitive diagnosis of fasciolosis in cattle often relies on the detection of *Fasciola* eggs in the faeces of infected animals using the sedimentation technique and examination by microscopy; trematode eggs are readily visible against the pale blue background of Methyl blue (Boray, 1985; Hansen and Perry, 1994).

2.8 Schistosomosis

2.8.1 Key clinical signs

Bovine schistosomosis in Africa is caused by *Schistosoma bovis*, *S. mattheei* and *S. curassoni* whose transmission is associated with the *Bulinus* spp. snail intermediate hosts. *Schistosoma bovis* is dominant in Eastern, Central and Northern Africa, whereas *Schistosoma mattheei* is dominant in Zambia southwards to South Africa and *S. curassoni* in West Africa (de Bont and Vercruysse, 1998).

Bovine schistosomosis generally occurs as a mild disease even when a high prevalence of detected parasites in slaughter cattle is encountered and most schistosome infections in endemic areas occur at a subclinical level (de Bont and Vercruysse, 1998). Generally, clinical signs of the diseases are rare, but the chronic disease is characterized by retardation of growth of

animals on a herd basis and the acute disease, which occurs where intensive transmission is present, is characterized by severe bloody or mucoid diarrhoea, marked anorexia, thirst, anaemia and emaciation (McCauley *et al.*, 1983a; de Bont and Vercruysse, 1998).

Schistosoma bovis is the main cause of bovine schistosomosis in Uganda and Kenya (de Bont and Vercruysse, 1998). Acute *Schistosoma bovis* infection in cattle is characterized by intermittent haemorrhagic diarrhoea lasting several days, reduced appetite or sometimes inappetance, weakness, staring coat followed in a few days with severely sunken-eyed appearance, emaciation and deteriorating condition (McCauley *et al.*, 1983a; de Bont and Vercruysse, 1998). In severe cases of bovine schistosomosis in Sudan, sunken-eyed appearance due to severe dehydration has been reported to be prominent (McCauley *et al.*, 1983a). Affected animals that survive have poor body condition for several months resulting in delayed growth and later have poor reproductive and milking performance (McCauley *et al.*, 1983a; de Bont and Vercruysse, 1998).

Presence of haemorrhagic diarrhoea, anorexia with retardation of growth rate or loss of body weight and mortality are sufficient to suggest clinical visceral schistosomosis in endemic areas. However, the chronic form rather than clinical visceral schistosomosis is common. Clinical signs in the majority of chronically infected animals are insufficient to distinguish the illness from other debilitating diseases (de Bont and Vercruysse, 1998).

Concurrent infection of *S. bovis* and *F. gigantica* is common in cattle (Majid *et al.*, 1980; McCauley *et al.*, 1983a). However, *F. gigantica* infection is associated with more severe anaemia, more consistent presence of inappetance, chronic wasting, depression to the point of frequent recumbency, absence of diarrhoea and lack of the sunken-eyed appearance (McCauley *et al.*, 1983a).

2.8.2 *Pathological features*

The key pathological features of *S. bovis* infection in cattle include anaemia, lymphopenia, hypoalbuminaemia, severe eosinophilia, mucosal and submucosal granulomas and inflammatory cell infiltrations. Haemoglobin level, packed cell volume and red blood cell counts fall and a normocytic, normochromic anaemia develops. The neutrophil counts fluctuate, lymphocyte counts decrease sharply and albumin level falls (de Bont and Vercruysse, 1998). In natural infections in cattle, haemoglobin concentrations of *S. bovis* cases are reported to fall below 8g/dl, but in experimental infections, haemoglobin concentrations range between 7.3 to 10.5g/dl (McCauley *et al.*, 1983a). Pathological lesions often seen at postmortem include portal fibrosis in the liver and greyish, thickened and oedematous haemorrhagic lesions in the mucosa of the intestines (Mango *et al.*, 1974; Urquhart *et al.*, 1996).

2.8.3 *Epidemiological features*

The reported prevalence of bovine schistosomosis in African countries cited in literature ranges from 0.1% in Uganda (Magona *et al.*, 1999a), 2.77% in Nigeria (Olusi, 1996), 4.9% in Zimbabwe (Vassilev, 1999), 25.6% in Kenya (Mango *et al.*, 1974), 30% in Senegal (Diaw *et al.*, 1998), 30.8% in Tanzania (Kassuku *et al.*, 1986), 51% in Zambia (de Bont *et al.*, 1994) to 90% in Sudan (Majid *et al.*, 1980).

The epidemiology of bovine schistosomosis is influenced by an interaction of ecological factors, depending on the geographical, climatic and management-related conditions (Kassuku *et al.*, 1986).

Presence of the right intermediate host (*Bulinus* species snails) is a prerequisite for schistosomes' development, and snail population and activity vary depending on the temperature (Urquhart *et al.*, 1996). The prepatent period of schistosomes in the molluscan host is highly dependent upon environmental temperature, the size and age of snail, the number of

miracidia penetrating, host factors such as nutrition and crowding, and the compatibility between schistosomes and the snails (de Bont and Vercruysse, 1998; Urquhart *et al.*, 1996).

Schistosomes' transmission is entirely dependent on availability of a water medium. Cattle movement and grazing management strategies that expose cattle to dangerous stretches of water during the maximum snail activity predispose them to schistosomosis (Urquhart *et al.*, 1996). In Sudan, McCauley and others (1983b) observed that the highest rate of new infections was during the dry season when cattle grazed near the water canal, while the lowest incidence was during the wet season because of lowest exposure to *S. bovis* cercariae since cattle grazed away from the dried up canals.

Intensity and seasonality of *S. bovis* transmission is affected by geographical and seasonal distribution of the host snail *B. africanus* (Kassuku *et al.*, 1986). Transmission around water canals is limited mainly to the dry season when the water current is sufficiently slow to allow the establishment of the *B. africanus* snail population and for the temporary ponds to the end of the rainy seasons, but transmission in permanent ponds occurs intermittently throughout the year (Kassuku *et al.*, 1986). Under appropriate management when cattle watering is done at *B. africanus*-free ponds, there is less transmission of *S. bovis* and the risk of transmission is limited to water contact with secondary sites (Kassuku *et al.*, 1986). The traditional management system in Africa with a large number of cattle utilizing a limited water resource highly suitable for sustaining populations of the snail host *B. africanus*, results in intensive transmission in which calves take in massive infections and dairy cows develop resistance to *S. bovis* (Kassuku *et al.* 1986). *Schistosoma bovis* infections are common in cattle in areas where there is either canal or pump irrigation because of potentially high infection rates due to high prevalence of the intermediate hosts and a conducive husbandry system that enables frequent livestock contact with snail-infested water (McCauley *et al.*, 1983b).

Cattle in the ages 6-30 months are most susceptible (Majid *et al.*, 1980; McCauley *et al.*, 1983b). New cases of bovine schistosomosis are rarely seen in calves prior to weaning at about six months of age or in cattle over about 30 months of age (Majid *et al.*, 1980; McCauley *et al.*, 1983b). Occasional outbreaks of clinical intestinal schistosomosis occur in young livestock or adult animals carrying heavy primary infections on exposure to intensive transmission: worm burdens as high as 7100 (de Bont and Vercruysse, 1998).

Occurrence of bovine schistosomosis is highly focalised in endemic areas because of the underlying aggregated distribution of intermediate snail hosts and the restricted stock movement from on farm to another (de Bont and Vercruysse, 1998). Since cattle usually move as a herd on the same farm, they are exposed to similar cercarial challenge (de Bont and Vercruysse, 1998).

2.8.4 Definitive diagnosis

Diagnosis of bovine schistosomosis relies on presence of clinical signs such as diarrhoea (blood stained), wasting and anaemia coupled with a history of access to natural water sources. Confirmatory diagnosis depends on detection of *Schistosoma* eggs in faeces and an egg count above 40 suggests heavy *S. bovis* infection (Urquhart *et al.*, 1996). Haemorrhagic lesions on the mucosa of the intestines, portal fibrosis and presence of numerous schistosomes in the veins are important postmortem features (Mango *et al.*, 1974; McCauley *et al.*, 1983a).

Table 2.1 summarises the important clinical signs and haematological features of the different endemic bovine diseases as documented in literature.

Table 2.1: Summary of clinical signs and haematological features of selected diseases

Disease	Clinical signs	HB/PCV/RBC of confirmed cases	Nature of infection	Reference
Trypanosomosis	Anaemia, pyrexia, weight loss, stunted growth, staring coat, enlarged lymph nodes, progressive weakness, anorexia, lacrymation and abortion.			
Theileriosis	Pyrexia, enlarged lymph nodes, anorexia, lacrymation, dyspnoea, petechial haemorrhages, nasal discharge, depression and diarrhoea	PCV = 28 ± 5% Hb = 9.93 ± 1.2 g/dl Rbc = 6.05 ± 1.16/mm^3 x 10^6	Natural	Omuse, 1978
Anaplasmosis	Pyrexia, anaemia, icterus, anorexia, dehydration, dyspnoea, weight loss, weakness, dullness, constipation and muscle tremors	PCV = 14 ± 4.25% Hb = 4.6 ± 1.36 g/dl Rbc = 2.54 ± 1.16/mm^3 x 10^6	Natural	Omuse, 1978
Babesiosis	Anaemia, haemogloburia, icterus, pyrexia, weight loss, abortion, reduced milk yield, diarrhoea, constipation and nervous signs	PCV = 16.3 ± 6.8% Hb = 5.8 ± 2.5 g/dl Rbc = 3.24 ± 1.7/mm^3 x 10^6	Natural	Omuse, 1978
Cowdriosis	Pyrexia, nervous signs, diarrhoea, depression and anorexia	PCV = 19% Hb = 6.83g/dl Rbc – 9 /mm^3 x 10^6	Experimental (goats)	Abdel Rahim and Shommein, 1979
PGE	Diarrhoea, staring coat, pot belly in calves, submandibular oedema, anaemia, weight loss, stunted growth and weakness			
Fasciolosis	Gradual weight loss, weakness, anaemia, dehydration and submandibular/ventral oedema		Expérimental	Mugambi *et al.*, 1990
Schistosomosis	Haemorrhagic diarrhoea, anorexia, stunted growth, weight loss, sunken-eyed appearance (dehydration), staring hair coat, anaemia and weakness	Hb = 8 g/dl Hb = 7.3 – 10.5 g/dl	Natural Experimental	McCauley *et al.*, 1983

2.9 Factors that influence the distribution and transmission of bovine tick-borne diseases

2.9.1 Tick species of importance

The ticks of economic importance (and the diseases they transmit) include *R. appendiculatus* (East Coast fever), *B. decoloratus* (anaplasmosis and babesiosis) and *A. variegatum* (heartwater). The commonest tick species in order of abundance observed in Uganda are: *R. appendiculatus*, *R. evertsi evertsi*, *A. variegatum* and *B. decoloratus* (Okello-Onen *et al.*, 1998b). Reports from elsewhere in Africa indicate *B. decoloratus* is the commonest tick species in South Africa (De Vos, 1979) and Cameroun (Ndi *et al.*, 1991). While *A. variegatum* is the predominant tick species in Senegal (Gueye *et al.*, 1993).

2.9.2 Factors influencing distribution of ticks

Tick population on cattle usually vary from year to year in many places, depending largely on climatic factors (Norval *et al.*, 1992; Perry and Young, 1995; Lawrence *et al.*, 1996; Billiouw *et al.*, 1999). A dry season adversely affects ticks and leads to low tick numbers on animals. Tick population on cattle also depend on the intensity of acaricides use and resistance of cattle to ticks when there is continuous exposure (Kaiser *et al.*, 1982; Mattioli *et al.*, 1995; Lawrence *et al.*, 1996; Okello-Onen *et al.*, 2003).

The most important factor limiting the distribution of *B. decoloratus* is decreasing humidity. It is absent in areas with an average annual rainfall of less than 380 mm in South Africa and where the annual rainfall is below 500 mm in Tanzania (De Vos, 1979). It was observed in Cameroun that the three stages (larva, nymph and adults) of *Boophilus* ticks infest cattle throughout the year, but tick numbers on each animal varied greatly during the course of the year; 50% of the ticks occurred during the dry season (Ndi *et al.*, 1991).

Rhipicephalus appendiculatus, R. evertsi and *A. variegatum* have been reported to occur in low numbers on cattle in a pastoral farming system in Mbarara Uganda, despite the abundance of alternative wild hosts, which was attributed to resistance of the cattle to tick infestation (Okello-Onen *et al.*, 1998a).

2.9.3 Factors influencing infection rates

Tick infection rates are influenced by parasites effects, tick effects, level of tick resistance in the host population and environment effects. The level of infection within the tick population depends on the abundance of feeding stages of ticks on infected cattle and their vectorial capacity (Norval *et al.*, 1992; Okello-Onen, 1995). The sex ratio of the tick population influences the infection rates in ticks (Irvin *et al.*, 1981). Female ticks have a higher infection rate than males, both in the percentage of ticks infected and the mean number of infected acini per tick (Irvin *et al.*, 1981), since female ticks have more 'e' cells and type III acini in their salivary glands, which are target sites for *Theileria* kinetes infection (Young *et al.*, 1981a).

Infection of ticks depends on the tick resistance in the host population. A highly resistant host population will produce lower infection in the tick, by reducing the size of the blood meal and success of tick feeding, than a population highly susceptible to ticks (Fivaz *et al.*, 1989). Taurine cattle populations produce higher piroplasm parasitaemia than cattle from endemic areas, resulting in higher infection rates in ticks (Young *et al.*, 1981b; Perry and Young, 1995). In endemic areas, about 1-2% of the field ticks are infected with *T. parva* and the majority of the infected ticks contain only one infected salivary gland acinus (Young *et al.*, 1981b). Infection rates of 7.8% and 1.1% of *A. variegatum* nymphs and adults, respectively, with *C. ruminantium* parasites have also been reported in endemic areas in Senegal (Gueye *et al.*, 1993).

In Murang'a district, Kenya, Gitau and others (2000) found the prevalence of *T. parva* infections in ticks of 0-25%. The highest prevalence of infections in ticks was observed on a

farm that had reported recent outbreak of East Coast fever prior to tick collection. The majority of ticks appeared to have acquired infection by feeding on carrier rather than clinically affected cattle (Gitau *et al.*, 2000).

Duration of parasite infection in ticks is affected by environmental factors. It is known to be temperature-dependent and the developments of *Theilerial* infections is reduced or stopped if the temperatures are too low or too high (Young and Leitch, 1981a). The success of *theilerial* parasites depends on the level of tick infestations, the tick infection rates and the success of tick feeding as well as external influences such as climate (Norval *et al.*, 1992; Perry and Young, 1995). Decreasing calf-infestation rates with *R. appendiculatus* were observed across agro-ecological zones in the Coast Province of Kenya by Deem and others (1993), which was suggestive of the declining suitability of this tick across agroecological zones. This influenced the seroprevalences in cattle across agroecological zones due to low *T. parva* challenge that might have been insufficient to induce herd immunity in the calf population (Deem *et al.*, 1993).

The pathogenicity of *Theileria* depends on the stock of the parasite, the dose of parasite and type of mammalian host (Norval *et al.*, 1992). The rate of transmission of *T. parva* depends on the stage of development of the parasite in salivary glands of *R. appendiculatus*, which depends on the ambient temperature that the adults ticks are exposed and strain of parasites (Ochanda *et al.*, 1988). *T. parva* is transmitted transtadially by the three-host tick *R. appendiculatus*. A low level of exposure may imply a reduced *T. parva* challenge that may results from less favourable conditions for *R. appendiculatus* (Maloo *et al.*, 2001a).

Infection of the ticks of the *Boophilus* species with *B. bigemina* occurs as they ingest infected blood during the late feeding stage of the adult female (De Vos, 1979). After transovarial transmission infectivity starts midway through the nymphal stage. Thereafter, transmission of *B. bigemina* appears to be continuous through the remainder of the nymphal stage and then by

both female and male adult ticks. Examination of tick salivary glands indicates that females, and males up to 35 days old may be infective. *B.bigemina* in the infected adults is reported to be capable of uninterrupted development and even transovarial passage to further generations in the absence of reinfection (De Vos, 1979).

2.9.4 Endemic stability of tick-borne diseases

The term "endemic stability" has been defined as a "climax relationship between host, vector and environment in which all coexist with the virtual absence of clinical disease, while endemic instability refers to an incomplete relationship (between host, vector and environment) in which clinical disease occurs" (Norval *et al.*, 1992). According to Norval and others (1992) an endemically stable state is characterized by high antibody prevalence, low disease incidence and case-fatality proportion and a rapid acquisition of infection in young calves. An endemically unstable state is characterized by the opposite: low antibody prevalence, high disease incidence and case-fatality proportion and primary infections that occur in all age groups. Classic areas of endemic stability are characterized by very low ECF-fatality proportions and close to 100% seroconversion by 6 months of age and a high prevalence (over 96%) of maternal antibodies (Gitau *et al.*, 2000). Under endemically stable conditions, clinical theileriosis is uncommon although mortality risks are very variable (0-50%) often limited to young animals (Norval *et al.*, 1992; Okello-Onen *et al.*, 1998a).

Endemic stability with regards to bovine babesiosis is defined by Callow, 1977 (quoted by De Vos, 1979) as the condition where there is frequent transmission of the parasites and infection of all animals occurs during the period that young animals are protected by passively acquired and non-specific factors i.e. within the first 6 to 9 months of life. Acquired immunity develops without the host becoming obviously sick and local animals are therefore generally immune to the *Babesia* species involved and suffer minimally from this disease (De Vos, 1979). A stable situation for one *Babesia* species does not imply a similar situation for the other species. Endemic instability on the other hand, defines the situation in which some animals in the herd

fail to become infected for a considerable period after birth i.e. a host-parasite imbalance exists resulting from infrequent transmission. Disease is then seen when susceptible animals in a herd encounter infected ticks (De Vos, 1979).

Evidence of widespread exposure to ECF, babesiosis and anaplasmosis of cattle indicates that a large proportion of the population either developed clinical or mild disease and recovered spontaneously following treatment, or were in a state of endemic stability to tick-borne diseases (Maloo *et al.*, 2001b).

Indigenous cattle have a degree of genetic resistance to both ticks and tick-borne diseases (Norval *et al.*, 1992; Lawrence *et al.*, 1996), and it is possible for them to attain a state of endemic stability for most TBD without any control measures. Infection is maintained in the population by continuous exposure to ticks; cattle become infected as calves, when they have a degree of age-related resistance or colostral immunity, which minimises the effect of diseases, and are thereafter immune (Norval *et al.*, 1992; Lawrence *et al.*, 1996). However, cattle of European breeds (*Bos taurus*) or their crosses do not enjoy the same degree of resistance to ticks and TBD as indigenous cattle (Lawrence *et al.*, 1996).

An endemically unstable states for *T. mutans*, *A. marginale* and *B. bigemina* infections in Ankole cattle was reported in a pastoral farming system in Mbarara district in Uganda (Okello-Onen *et al.* 1998a). However, the prevalence of serum antibodies to *T. parva* averaging 70% in calves older than 6 months was an indication of an endemically stable state to ECF (Okello-Onen *et al.*, 1998a). A direct relationship occurred between antibody prevalence rate to *T. parva* piroplasm prevalence, tick challenge and the age of calves. The true mortality rate due to East Coast fever was 5.8%, which was strongly age-related. However, an inverse relationship was observed between calf mortality and sero-positivity (Okello-Onen *et al.*, 1998a). The highest mortality of calves occurred at the age of 3 months when the antibody prevalence to *T. parva* was lowest (29%), due to disappearance of protective maternal antibody and second

lower peak occurred at 9 months, which is attributed to stress experienced by calves at weaning, in addition to heavy tick-borne parasites experienced during extensive grazing (Okello-Onen *et al.*, 1998a).

In the Coastal Kenya, Deem and others (1993) found high *T. parva* antibody prevalence in Zebu calves suggestive of a state of endemic stability. Such high prevalences in young animals are associated with little or no clinical ECF. Seroconversion to *T. parva* infection without undergoing severe clinical disease may be due to existence of *T. parva* strains causing mild clinical disease due to low infection challenge and possible population immunity (Maloo *et al.*, 2001b). For this to occur the majority of the calf population must be exposed to sufficient tick challenge during early 4-6 months of age (Deem *et al.*, 1993).

2.9.5 Serology of tickborne diseases

In a study conducted in Murang'a district in Kenya (Gitau *et al.*, 2001), *T. parva* seroconversion status implied increase in per cent positivity value of >15 and maintained for at least 2 visits or if above the cut-off level, a rise of ≥ 15 PP except where a calf died soon after. Calf morbidity was defined as any calf sickness that had a recognisable clinical manifestation. East coast fever morbidity was defined on the basis of clinical diagnosis. *T. parva* infection was defined as a rise in antibody titre of >15 PP and not the cut-off level of 15 PP (calves without maternal antibodies) or any rise of ≥ 15 PP (for calves with declining maternal antibodies). Calves were judged to have seroconverted if: they were initially antibody negative and became antibody positive for at least one visit or if they had maternal antibody that declined to negative levels and then subsequently became positive for at least one visit, or they had initial maternal antibody that had not yet declined to negative but subsequently increased by at least 15 PP units for at least one visit (Gitau *et al.*, 2000). High PP values in young calves (< 40 days) are almost exclusively maternal antibody and high PP values in older calves (> 100 days) were of antibodies produced by the calf in response to *T. parva* infection (Gitau

et al., 2000). Mean antibody levels declined from birth to reach their lowest point at approximately 94 days of age, then the antibody rose (Gitau *et al.*, 2000).

Calves less than 6 months of age under herded grazing system in the Coast Province of Kenya were observed to have high seroprevalence of 87%, which increased according to age (Maloo *et al.*, 2001b). In dairy and Zebu breeds, seroprevalence of over 75% of *T. parva* was found, which indicated a state of endemic stability (Maloo *et al.*, 2001b). In another study in the Coast Province of Kenya, mean prevalence rates to *T. parva* of 22-49% in Zebu calves were found by Deem and others (1993). This was indication of endemic instability and probably meant a significant proportion of the adult population was susceptible to clinical ECF (Deem *et al.*, 1993).

Seroprevalences of *A. marginale* of over 80% in all age groups of cattle have been reported by Maloo and others (2001b) in Coast Province of Kenya. Seroprevalences increased with age and were 81%, 91% and 97% in animals of <6, 6-18 and >18 months of age, respectively (Maloo *et al.*, 2001b).

Seroprevalences of *B. bigemina* of 40.9-73% for dairy cattle were observed in Coast Province of Kenya (Maloo *et al.*, 2001b). The mean antibody prevalence increased with age and was higher in cattle over 18 months of age (71%) than in those 18 months of age or younger (44%).

2.10 The role of anaemia in the pathogenesis of endemic bovine diseases

2.10.1 Anaemia

Anaemia is indicated when one or more of the red blood cell parameters: PCV, haemoglobin content and red blood cell count are below the normal level for the age, sex and breed of species concerned (Jain, 1993). Clinical signs of anaemia include exertional dyspnoea, reduced exercise tolerance, pallor of mucous membranes, increased heart rate, haemic murmurs, increased inappropriate respiratory rate, depression and dementia (Jain, 1993).

Anaemia occurs because of excessive blood loss from haemorrhage or cell destruction and decreased erythrocyte production (Meyer *et al.*, 1992). In anaemia due to acute blood loss, major clinical signs include tachycardia and dyspnoea, and terminally shock and death. In contrast, in anaemia due to acute haemolysis, the major clinical signs include icterus, haemoglobinemia, haemoglobinuria and fever (Jain, 1993).

Anaemia can be classified as normocytic, microcytic and macrocytic depending on whether the red blood cell size is normal, smaller than normal or larger than normal respectively. It can be classified according to the haemoglobin content as normochromic or hypochromic depending on whether the average haemoglobin content in a given volume of packed erythrocytes in circulation is normal or less than normal respectively (Meyer *et al.*, 1992).

2.10.2 Forms of anaemia associated with endemic bovine diseases

Parasitic diseases of cattle such as fasciolosis, schistosomosis and nematode infections, especially haemonchosis, cause blood loss anaemia. Other parasitic bovine diseases such as trypanosomosis, anaplasmosis, babesiosis and theileriosis cause haemolytic anaemia. Haemolytic anaemia primarily results from increased erthyrocyte destruction, whether intravascular or extravascular (Jain, 1993).

2.10.3 Diseases associated with blood loss anaemia and bone marrow suppression

Nematode infections due to *Haemonchus* spp. cause anaemia due to blood loss, but non-blood-sucking parasites such as *Trichostrongylus* spp., *Ostertagia* spp. and *Cooperia* spp. cause anaemia due to impaired erythropoiesis (depression anaemia). This depression anaemia is attributed to dysfunctional erythropoiesis, copper deficiency and specific amino acid deficiency as a result of disturbed protein metabolism and toxic marrow suppression caused by chronic parasitism (Jain 1986). Chronic parasitism interferes with iron utilization through increased plasma iron and excessive iron stores in the liver (Jain, 1986). Shortened red cell life span associated with helminthoses due to blood sucking and non-blood sucking nematodes may also contribute to both blood loss anaemia and depression anaemia. In haemonchosis, anaemia is attributed to iron deficiency, which gradually develops because of increased iron utilization accompanied by increased faecal iron loss and diminished iron absorption (Jain, 1986). Both chronic haemonchosis and trichostrongyloidosis cause normocytic-normochromic type of anaemia (Jain, 1993). Poor nutrition, especially low protein intake, is known to have a profound impact on the pathophysiology of gastrointestinal nematode infections (Holmes *et al.*, 2000) and hence leads to more severe anaemia.

Schistosomosis in cattle causes blood loss anaemia (Jain, 1993). The development of anaemia and hypoalbuminaemia in *S. bovis* infection, an intestinal syndrome associated with heavy primary infection in cattle, is reported to arise primarily from blood loss due to intestinal haemorrhage and leakage of plasma from intestinal lesions (de Bont and Vercruysse, 1998). Occurrence of anaemia and hypoalbuminaemia has been observed to coincide with increased faecal egg excretion. During infection, haemoglobin levels, packed cell volume and red cell counts start falling after 3 weeks post-infection and reach lowest levels 13 weeks post-infection. In most cases, a normocytic, normochromic anaemia develops. The severe fall in haemoglobin, PCV and red blood cell counts usually coincides with the early acute stage of

clinical schistosomosis and the period of greatest blood loss in the faeces (de Bont and Vercruysse, 1998).

Anaemia in liver fluke infections in cattle is due to blood loss as a result of simple mechanical lesions that cause extensive haemorrhages when there are large numbers of mature flukes in the liver or when immature stages migrate (Jain, 1986). Anaemia thus develops when there is blood loss into the gut through bile. However, shortening of the erthyrocyte survival time and an early reduction of plasma iron concentration, which results from malfunction of the mononuclear phagocytic system due to ascorbic acid deficiency, contribute to the occurrence of anaemia (Jain 1986).

2.10.4 Diseases associated with haemolytic anaemia

Trypanosomosis in cattle causes marked haemolytic anaemia and impairment of erythrocyte regeneration (Murray, 1978; Doxey, 1983). The severity of anaemia, which follows trypanosome infection, is influenced by factors such as virulence of species or strain of trypanosome involved, age of the host, nutritional status and breed (Murray and Dexter, 1988). Normocytic-normochromic anaemia develops in trypanosomosis due to haemodilution from the expansion of plasma volume, but in terminal stages, it is due to impaired erythropoiesis (ILRAD, 1990). If there is a concomitant iron deficiency with trypanosomosis, microcytic–hypochromic anaemia results (Jain, 1993).

During the acute phase of trypanosomosis, the PCV of the infected cattle commonly falls to 20% or may continue to drop, resulting in the death of the animal. During the chronic stage, the PCV of the animal fluctuates around 20% and does not improve (Murray, 1978). Normally at this stage, the anaemia is normocytic and normochromic or microcytic and normochromic and is non-regenerative (ILRAD, 1990). Anaemia that occurs during the acute phase of trypanosomosis is primarily due to the removal of large numbers of red cells by macrophages. This process is thought to be triggered by the physical alteration of the surface membrane of

red blood cells as a result of increased osmotic fragility, decreased plasticity and increased permeability due to the febrile responses caused by the disease. Furthermore, disseminated intravascular coagulation that occurs due to a high parasitaemia during *T. vivax* infections causes capillaries to be partially blocked by thrombi and aggregation of platelets and hence damage red blood cells, which are then phagocytosed by macrophages (ILRAD, 1990). Fragments of disrupted trypanosomes may bind to red cells, causing them to be identified as foreign and thus to be ruptured by antibody and complement or to be phagocytosed by macrophages (ILRAD, 1990). Antibodies to normal erythrocytes produced during haemorrhagic *T. vivax* infections have been reported to contribute to this severe anaemia too (Assoku and Gardiner, 1989). During the acute phase of trypanosome infection, the bone marrow of the infected animals is responsive, but is overwhelmed later by the accelerated red cell destruction by macrophages such that during the chronic phase the bone marrow becomes unresponsive. Trypanosomes are reported to produce factors that induce bone marrow unresponsiveness (ILRAD, 1990).

In areas where trypanosomosis is endemic, the disease is exacerbated when there is obvious undernutrition of the livestock. The severity of anaemia in bovine trypanosomosis is affected by the levels of energy intake, with lower levels being associated with more severe and rapidly developing anaemia (Agyemang *et al.*, 1990; Katunguka-Rwakishaya *et al.*, 1995; Holmes *et al.*, 2000).

Anaemia in East Coast fever has been reported to occur in a mild, normocytic, normochromic unresponsive form as a result of bone marrow suppression (Omuse, 1978; Doxey, 1983; Losos, 1986). However, Jain (1993) clarified that anaemia can result from immune-mediated erythrophagocytosis.

Anaplasmosis causes marked haemolytic anaemia in cattle (Doxey, 1983). The anaemia is macrocytic, normochromic and responsive, but sometimes it is normocytic and normochromic

(Egbe-Nwiyi et al, 1997). Anaemia in anaplasmosis results largely from the extravascular destruction of parasitized erythrocytes in the spleen and bone marrow by macrophages through erythrophagocytosis initiated by parasitic damage to erythrocytes and antieryothrocytic antibody (Ajayi *et al.*, 1987). The degree of anaemia is disproportionate to the prevailing parasitaemia because of immune-mediated destruction (autoimmunization) of both non-parasitized and parasitized erythrocytes (Ajayi, 1987; Jain, 1993). The mechanism involves *Anaplasma* antigen-antibody complexes in erythrocytes of infected cattle, autohaemogglutinins and opsonins, which are associated with erythrophagocytosis of both infected and non-infected erythrocytes thus leading to anaemia. Maximal anaemia in *Anaplasma* infection occurs normally 21 days post-infection and is recorded two days after the maximal level of parasitaemia has occurred. In chronic anaplasmosis, continuous haemolysis causes progressive anaemia (Ajayi, 1987).

Babesiosis in cattle is associated with a reduction in PCV, red blood cell count and haemoglobin concentration over 50% (Doxey, 1983; Losos, 1986). Babesiosis causes a regenerative macrocytic-hypochromic anaemia. As with anaplasmosis, the degree of severity of anaemia is disproportionate because of the haemolysis of both parasitized and non-parasitized erythrocytes (Jain, 1993). Anaemia in babesiosis is usually due to complexes formed by reaction of circulating antigen, antibody and complement. This reaction depletes complement levels, releases anaphylatoxin, and contributes to shock. The red blood cell destruction usually parallels increasing parasitaemia, but phagocytosis of noninfected cells also occurs, probably as a result of alterations in the shape of the erythrocytes, osmotic fragility and increased reticulo-endothelial activity (Losos, 1986).

Anaemia occurs in cowdriosis and it is microcytic hyperchromic (Losos, 1986). However, normocytic normochromic type of anaemia, which is associated with bone marrow depression, has been reported in experimentally induced heartwater infections in cattle. Changes in the haemoglobin content and haematocrit were observed to coincide with the onset of the febrile

reaction (van Amstel *et al.*, 1988). In goats, microcytic hypochromic form of anaemia has been observed in experimental infections of *C. ruminantium* (Abdel Rahim and Shommein, 1977). The mean haemoglobin content, PCV and RBC decreased from 9.69 g/dl, 31% and 10 x 10^6 before infection to 6.83 g/dl, 19% and 9 x 10^6 post infection, respectively. Reduction in Hb, PCV and RBC coincided with the beginning of anaemia and was attributed to deficiency in iron or to failure in the proper utilization of iron for formation of haemoglobin or to increased output of iron from the body (Abdel Rahim and Shommein, 1978).

Under field conditions in tropical Africa, cattle are frequently affected by a combination of intestinal and blood parasites, which mutually aggravate each other's pathogenic effects (Dwinger *et al.*, 1994). Trypanosomes are reported to cause immunosuppression in cattle thus increasing susceptibility to helminth infection (Dwinger *et al.*, 1994). Concurrent trypanosome and gastrointestinal infections in cattle have been observed to have significant negative effects on the packed cell volume (Dwinger *et al.*, 1994). In an outbreak of ECF in cattle in Tanzania, concurrent infections of *T. parva*, *B. bigemina*, *T. congolense* and *T. vivax* were observed in cattle. Animals infected with *T. parva* and *T. vivax* had low PCV and anaemia (Kambarage, 1985). In an outbreak of acute trypanosomosis in sedentary herds of Friesian cattle in Nigeria, which manifested anaemia, concurrent infections of *T. vivax*, *A. marginale* and gastrointestinal nematode infections were found (Kalu, 1996). On Mkwaja ranch in Tanzania, in concurrent infections of trypanosomosis and anaplasmosis in cattle, it was recognised that stress resulting from chronic subclinical trypanosomosis due to *T. vivax* or *T. congolense* caused patent parasitaemia and clinical anaplasmosis to emerge in premune carrier animals which lead to increased mortality and reduced PCV of cattle (Fox *et al.*, 1993).

2.11 Diagnosis of endemic bovine diseases

2.11.1 General approach to clinical diagnosis

A disease is defined as "the inability of the living organism to perform physiological function at normal levels even though nutritional and other environmental requirements are provided at an adequate level" (Radostits *et al.*, 1994). Presence of a disease is revealed by qualitative or quantitative clinical signs associated with changes in the structure of organs or tissues and their functions and the behaviour of the whole living organism. Distinctive individual clinical signs pathognomonic for specific diseases can be an accurate basis for diagnosis of a disease (Kelly, 1984). A clinical diagnosis is "the identification of the disease affecting the patient with the aim of recognising the nature of the ailment so that effective treatment and control measures can be undertaken" (Radostits *et al.*, 1994). Clinical diagnosis involves clinical examination i.e. examination of the animal, history and the environment as well as laboratory confirmation (Radostits *et al.*, 1994).

2.11.2 Diagnostic process and therapeutic decision-making

Recognized methods for making a diagnosis include the syndrome or pattern recognition method, the hypothetico-deductive reasoning method, the arborisation or algorithm method, the key abnormality method and the database method (Radostits *et al.*, 1994).

In syndrome or pattern recognition method, the subject case is compared to previous cases in the clinician's memory whereby one is recognized as a replica of the other (Radostits *et al.*, 1994). The hypothetico-deductive reasoning method involves the clinician drawing up a short list of diagnostic possibilities as soon as the client commences to present signs, starting with the key clinical signs. If hard primary data or ancillary data is lacking, the clinician may decide to give treatment against two or more diseases (polypharmacy). However, the disadvantages of the polypharmacy approach are additional expenses, contamination of food products of animal origin by medication (Radostits *et al.*, 1994) and drug misuse and likely emergence of drug resistance (Stevenson *et al.*, 1993; Eisler *et al.*, 1997; Geerts and Holmes, 1998; ICPTV, 1999).

The arborisation or algorithm method involves the clinician listing all possible disease diagnoses, and then each diagnosis is examined in turn with supporting or disproving questions. Suitability of algorithms for computerization, simplicity of this method for inexperienced clinicians and non-reliance of clinicians on the impressionistic human memory are the major advantages of this method (Radostits *et al.*, 1994).

In the key abnormality method clinicians have to rely on their knowledge of normal structure and function to select the key abnormality or clinical signs (Radostits *et al.*, 1994). For the database method or problem-oriented method, the clinician has to conduct a complete clinical and clinicopathological examination of the patient in order to acquire a comprehensive patient database. Best-fit diagnosis with the patient's data is then selected by matching the patient's problem or key signs with the reference diagnoses in the diagnostic database (Radostits *et al.*, 1994). Because, this method is time demanding on clinicians and laboratory resources, it is not suitable for food animal medicine where speed is vital (Radostits *et al.*, 1994).

Experienced practitioners tend to make most of their decisions by intuitive judgement using accumulated information generated through experience, which usually involves an intellectual reflexive response (Blood and Brightling, 1988). However, inexperienced clinicians have to follow a more systematic and rational process to make a diagnosis.

There are four criteria for making a disease diagnosis, namely, clinical diagnosis that uses clinical signs and symptoms, aetiological diagnosis that relies on detection of specific agents, functional diagnosis where a diagnosis is based on the outcome of a diagnostic test and pathological diagnosis that depends on the identification of lesions (Thrusfield, 1995) Each of the criteria can be combined with another to provide a more robust diagnosis depending on the purpose of the diagnosis.

2.11.3 Diagnostic failures

During the diagnostic process, problems may occur at various levels. The majority of incorrect diagnoses are associated with the omission of one or more parts of a clinical diagnosis: general inspection of the animal and its surroundings, history of the case and thorough physical examination (Kelly, 1984). A failure to generate and consider relevant differential diagnoses or premature closure of the differential diagnosis is recognised as the most common cause of incorrect diagnosis (Morley, 1991; Cockcroft, 1999a). Other common causes of misdiagnosis include inattention to and misinterpretation of key clinical signs, overinterpretation of findings in light of suspected disease, inexperience and error in judgement due to oversimplification of complex diagnostic problems (Morley, 1991). Involving unqualified and non-professional animal health auxiliaries to make disease diagnosis also enhances misdiagnosis.

2.11.4 Existing diagnostic tests for endemic bovine diseases

Most recent work on diagnosis of endemic bovine diseases in Africa, such as trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, schistosomosis and fasciolosis has focussed on the development of diagnostic tests to detect or isolate specific agents from animals that may or may not have evidence of clinical disease.

2.11.4.1 Diagnostic test for trypanosomosis

Diagnosis of trypanosomosis relies on parasitological diagnosis using thin and thick blood smears, haematocrit centrifuge technique (Woo, 1969), phase contrast buffy-coat technique (Murray *et al.*, 1977) and serological diagnosis using the antigen-detection enzyme linked immunosorbent assay (Nantulya and Lindqvist, 1989), the antibody ELISA (Luckins, 1977; Hopkins *et al.*, 1998; Rebeski *et al.*, 1999) and the immunofluorescent antibody test (Katende *et al.*, 1987). More recently polymerase chain reaction-based techniques have been used in detecting trypanosomes in bovine blood (Solano *et al.*, 1999; Picozzi *et al*, 2002).

Direct parasitological techniques such as phase-contrast buffy-coat technique and thick and thin films have poor to fair diagnostic sensitivity under field conditions. Unlike the thick and thin films, recent findings revealed the phase-contrast buffy-coat technique has good diagnostic sensitivity (67-96% for *T. congolense* infection and 60-76% for *T. vivax* infection) under optimised conditions (Eisler *et al.*, 1998). Despite their low sensitivity, direct parasitological tests are suitable for field application and for diagnosis of individual cases, especially in rural areas in Africa.

On the other hand, indirect serological diagnostic methods have the advantages of testing under controlled laboratory conditions, provision of quality assurance, allowance for repeat testing of samples and investigation of other important diseases using the same samples (Eisler *et al.*, 1998; Hopkins *et al.*, 1998; Rebeski *et al.*, 1999). The ELISA tests have the further potential advantage of rapid sample throughput suitable for large-scale epidemiological investigations, automated data processing and analysis using widely available information technology, and use of either conventional serum samples or whole-blood as dried blood spots on filter paper (Eisler *et al.*, 1998; Hopkins *et al.*, 1998; Rebeski *et al.*, 1999). Use of dried spots on filter paper further simplifies the tasks of sample collection and management, and greatly reduces the expense involved, particularly because of the reduced requirement for centrifugation and cold-chain facilities in remote areas (Hopkins *et al.*, 1998). The antibody-detection ELISA has high sensitivity and specificity (Hopkins *et al.*, 1998; Rebeski *et al.*, 1999). However, it does not differentiate between past and present infections, nor does it distinguish between species of trypanosomes. Hence, it cannot be used as a basis for chemotherapy of individual cases.

Serological tests for trypanosomosis may only be useful in providing information for targeting tsetse and trypanosomosis control (Hopkins *et al.*, 1998) and for epidemiological studies. Unlike the antibody-detection ELISA, the antigen-detection ELISAs for trypanosomosis have been shown to lack specificity and also sensitivity (Desquesness, 1996; Eisler *et al.*, 1998).

PCR-based techniques are highly sensitive and specific experimental tests for detecting trypanosomal DNA in either the vector or the host. PCR has been shown to be useful for the detection of trypanosome infections in cattle with a PCV below 25% (Solano *et al.*, 1999). Nevertheless, PCR has been scarcely used to assess the prevalence of trypanosomosis on field samples, due to its time-consuming and prohibitive cost aspects, and requirement for technical expertise (Solano *et al.*, 1999). Furthermore, PCR requires specialised equipment and is not suitable for use as a basis for treatment of individual animals, especially in remote rural areas. Attempts have been made to make it less labour intensive and time-consuming while reducing the cost per test by reducing the cost of reagents used (Solano *et al.*, 1999).

2.11.4.2 Diagnostic tests for theileriosis

Theileria parva parasites have two recognizable stages within the bovine host, a schizont stage within lymphoid cells and a piroplasm stage within erythrocytes. Diagnosis of *T. parva* infections commonly relies on the detection of thelerial piroplasms and schizonts by examination of stained thin blood smears and needle biopsies of enlarged lymph nodes (Urquhart *et al.*, 1996). For epidemiological work, serological tests such as IFAT, the indirect haemoglutination assay, capillary agglutination, immunodiffusion and complement fixation test (Duffus and Wagner, 1980; Billiouw *et al.*, 1999) and ELISA (Katende *et al.*, 1998) have been used in the diagnosis of *T. parva* infection in cattle. Performance evaluation studies on the ELISA test for detection of antibodies against *T. parva* have revealed a sensitivity of over 99% and a specificity of 97% (Katende *et al.*, 1998). Other evaluation studies have revealed specificities of 87%, 92%, 93% and 95% for the CF, CA, IFAT and IHA, respectively (Duffus and Wagner, 1980). Positive CF titre indicates recent exposure to *T. parva*, but a positive IFAT and IHA titres indicate either a recent or longstanding exposure. The PCR technique has been used in characterisation and quantification of *T. parva* in blood and tick vectors for epidemiological diagnosis (Stiller, 1992; Watt *et al.*, 1998).

2.11.4.3 Diagnostic tests for anaplasmosis

Diagnosis of individual cases of anaplasmosis often relies on the detection of rickettsial infections in the bovine erythrocytes by examination of Giemsa stained blood smears (Urquhart *et al.*, 1996). For sero-epidemiology studies, the complement-fixation test (CFT), capillary-agglutination (CA), ELISA and card agglutination technique have been used (Jones and Brock, 1966; Jongejan *et al.*, 1988). The CFT and the IFAT are useful in diagnosing anaplasmosis in suspected carrier cattle (Stiller, 1992). The DOT-ELISA has been found especially suitable for large-scale screening of dry blood spots on filter paper for sero-epidemiology of anaplasmosis (Ssenyonga *et al.*, 1992). PCR-based techniques have been used to detect *A. marginale* parasites in blood for detecting carrier infection. The method is very sensitive and specific and is suitable for evaluating a large number of samples in a single day and thus is well suited for large-scale epidemiological studies (Stiller, 1992).

2.11.4.4 Diagnostic tests for babesiosis

Detection of babesial piroplasms relies on examination of stained thin smears (Urquhart *et al.*, 1996). IFAT is the most widely accepted method for serological diagnosis of *B. bovis* infection and has been used in many epidemiological studies (Jongejan *et al.*, 1988; Molloy *et al.*, 1998). However, its major disadvantages are that it is subjective in interpretation, labour intensive and has limited throughput (Molloy *et al.*, 1998). The ELISA has been demonstrated to perform similarly to the IFAT, but is more robust, simple to perform and more suitable for testing a large number of sera than the IFAT. The ELISA is useful for epidemiological studies on *B. bovis* and has sensitivity and specificity estimates of 100% and 99.7% in Africa (Molloy *et al.*, 1998). DNA probes for detection of *Babesia* organisms in host and in the tick vector have been developed (Stiller, 1992).

2.11.4.5 Diagnostic tests for cowdriosis

Diagnosis of cowdriosis usually takes place at post mortem and relies mainly on detection of *C. ruminantium* parasites in endothelial cells of stained brain smears and on post mortem lesions such as hydropericardium. IFAT is the commonest serological tests used in epidemiological studies of cowdriosis (Martinez *et al.*, 1990; Gueye *et al.*, 1993). DNA probes are now available for detection of *C. ruminantium* parasites in the tick vectors (Waghela *et al.*, 1991).

2.11.4.6 Diagnostic tests for parasitic gastroenteritis

Diagnosis of gastrointestinal nematode infections in livestock usually relies on faecal examination for presence of worm eggs or larvae (Hansen and Perry, 1994; Urquhart *et al.*, 1996; Eysker and Ploeger, 2000). The major limitation that affects sensitivity of faecal egg count is the inability to reflect nematode infection levels, thus affecting its performance in estimating clinical disease. However, with the predominance of *Haemonchus placei*, a high egg producer in cattle in tropical environments, faecal egg counts may reflect the intensity of infection (Eysker and Ploeger, 2000). Pathological examination of animals that die or are killed *in extremis* is an alternative useful diagnostic aid for parasitic gastroenteritis. However, post-mortem examination may be too expensive at farm level (Eysker and Ploeger, 2000), and is obviously unsuitable for the clinical management of individual animals.

In the diagnosis of infection of blood sucking nematodes such as *Haemonchus* spp., the pallor of the mucous membranes and the packed cell volume are useful tools (Eysker and Ploeger, 2000), as utilised in the FAMACHA colour chart for diagnosis of haemonchosis (van Wyk *et al.*, 1997).

Elevated blood pepsinogen values are a useful diagnostic tool, especially for ostertagiosis, when used in conjunction with clinical and parasitological findings. Blood pepsinogen level is known to correlate with infection levels of abomasal nematodes. The major limitations of this

method, however, are that it is very labour intensive and expensive (Eysker and Ploeger, 2000), and is more suitable for herd health monitoring rather than for diagnosis of individual cases. Furthermore, this method may be more suitable for diagnosis of ostertagiosis in cattle, a temperate problem, rather than the abomasal nematode infections such as haemonchosis that are predominant in tropical environments.

An antibody-detection ELISA using crude *O. ostertagi* and *C. oncophora* proteins as antigens has been used for diagnosis of gastrointestinal nematode infections. This assay is able to demonstrate differences between infection levels on different farms and in animals of different age classes (Eysker and Ploeger, 2000). However, the disadvantage with crude worm ELISA is that antigenic epitopes are shared by many worms and cross reactions with other nematodes and even with *F. hepatica* do occur. Moreover, it cannot be used for individual animal diagnosis since a wide within-host variation exists in the ability to develop antibody responses against gastrointestinal nematode antigens (Eysker and Ploeger, 2000). An antibody ELISA based on recombinant *C. oncophora* has been developed (Poot *et al.*, 1997) and has proved to be highly specific for *Cooperia* spp. The assay utilising recombinant nematode protein is reported to be more specific than the one based on crude worm antigen and is suitable for monitoring herd health monitoring programmes (Eysker and Ploeger, 2000), but it is not suitable for diagnosis of individual cases of parasitic gastroenteritis.

More recently PCR-based techniques have been developed for detection of gastrointestinal nematode infections in ruminants (Eysker and Ploeger, 2000). Availability of DNA technology such as PCR allows the development of highly sensitive and specific tests for detecting infectious agents and for measuring of within-species variation in populations of infectious agents. However, these specific molecular tests are not suitable for diagnosis of gastrointestinal nematode infections, for they only indicate whether the parasite is present or not, which generates no additional information since grazing ruminants normally acquire infection during grazing (Eysker and Ploeger, 2000). Moreover, PCR-based techniques require laboratories with

specialised equipment. Though diagnostic tests have a role in confirming the clinical diagnosis of parasitic gastroenteritis, they are more important for herd health monitoring of nematode infections in cattle (Eysker and Ploeger, 2000).

2.11.4.7 Diagnostic tests for fasciolosis

Diagnosis of infections of *Fasciola* spp. in ruminants relies on the detection of *Fasciola* eggs in the faeces of infected animals (Boray, 1985), but the difficulty is that a relatively small number of eggs have to be detected in a relatively large amount of faeces (Anderson *et al.*, 1999). Furthermore, early diagnosis is not possible because eggs are not found in faeces until flukes reach maturity, usually between 10 to 14 weeks after infection (Anderson *et al.*, 1999).

Deficiencies of parasitological diagnosis can be overcome by detection of serum antibodies to specific antigens of *Fasciola* parasites. Serodiagnosis is generally performed using ELISA and Western immunoblot methods based on antigens derived from adult fluke extracts or ES materials (Anderson *et al.*, 1999). Evaluation studies have revealed sensitivities and specificities of 66.7% and 100% for the egg counting method, and 86.1% and 70% for the ELISA, respectively (Anderson *et al.*, 1999). Since both tests have similar performance, if estimates are to made on a group rather than on a single animal, the less costly shaking sieve method for estimating egg counts would be preferred to the ELISA method. However, for detection of the degree of fluke infection, for purposes of warranting treatment, the ELISA has been suggested since it is the only test that directly measures the amount of antigen released into faeces or serum of infected animals (Anderson *et al.*, 1999).

2.11.4.8 Diagnostic tests for schistosomosis

Diagnosis of schistosome infections is commonly achieved through counting and identification of eggs in faeces (de Bont and Vercruysse, 1998) especially for field veterinarian dealing with groups of animals at risk where severe morbidity in cattle is generally associated with high egg excretion. Moreover, routine diagnostic work demands more of convenience and less of

accuracy, which further favours the use of faecal examination (Olaecha *et al.*, 1990). Faecal egg counting techniques such as the filtration techniques are highly sensitive, but their field applicability is limited by their being time-consuming, difficult to perform and requiring specialized equipment (Olaecha *et al.*, 1990). The syringe filtration and the Teesdale smear technique are recommended for field diagnosis of schistosomosis in domestic stock since they are less demanding techniques on time, resources and expertise (Olaecha *et al.*, 1990).

Miracidial hatching technique is useful for the diagnosis and quantification of schistosome infections in cattle. The advantage is that much larger faecal samples can be used, which improves the sensitivity and facilitates the detection of light or old infections. Pooled samples from several animals may be examined to detect infection at the herd level (de Bont and Vercruysse, 1998).

Serological tests such as IFAT, IHA and CFT have been used in the diagnosis of bovine schistosomosis (de Bont and Vercruysse, 1998). IHA has been used in detection of *S. bovis* and found to be more reliable than faecal egg examination. However, IFAT titres have been found not to correlate with worm burden, unless it is heavy infection, especially due to *S. mattheei*. The disadvantage with CFT is its strong cross-reactivity between *S. mattheei* and either *F. gigantica* or *P. microbothrium*. Since clinical bovine schistosomosis is associated with heavy worm burden, IFAT is of great value in the diagnosis of the clinical disease (de Bont and Vercruysse, 1998). However, the major disadvantage of antibody tests is their inability to distinguish between active and past infection, and antibody levels generally do not correlate with worm burden. Thus, antibody test may be used only for screening acquired infections (de Bont and Vercruysse, 1998).

Antigen detection in serum is now a useful diagnostic test for schistosomosis. The circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are two antigens produced in the guts of schistosomes. These two types of antigen are released in the circulation of the hosts by

the regular vomiting of the parasites. Antigen levels in serum / or in urine are thought to reflect the worm burden more accurately than egg counts (de Bont and Vercruysse, 1998). Although antigen detection has excellent sensitivity (95-100%) and may provide an indication of worm burden, possible variations of the antigen clearance rate with the physiological condition of the host e.g. nutritional stress, may complicate the interpretation of the results (de Bont and Vercruysse, 1998).

Most of the aforementioned diagnostic methods are unavailable to groups such farmers, extension workers and agro-veterinary traders that are involved in the diagnosis and treatment of endemic bovine diseases in Africa in the modern scenario (Machila *et al.*, 2003). Detection of clinical disease may be facilitated by use of a combination of clinical signs, simple field-level diagnostic tests and guiding decision support tools.

2.12 Diagnostic Decision Support Tools

Diagnostic Decision Support Tools (DDST) are commonly applied to disease diagnosis where they incorporate a set of rules for solving problems, details of clinical signs, lesion, laboratory results and opinions of experts (Thrusfield, 1995). DDST used in veterinary medicine may either be computerized or manual.

2.12.1 Computer-based Decision Support Tools

Computer-aided diagnosis (CADx) is utilisation of computers to aid human diagnostic reasoning. Essentially utilising the advantages of machine computation to provide improved outcomes for veterinary patients (Steward, 1996). Current CADx tools provide statements as part of their output suggesting laboratory tests useful to rule in or out any active disease hypothesis (Steward, 1996). CADx are based on diagnostic algorithms, probabilistic reasoning, interpretive rules and pattern matching analyses which have been programmed in many common languages meant to provide intelligent expert analysis and recommendation reliably and faster than any human beings (Kassirer, 1994; Steward, 1996; McKendrick *et al.*, 2000).

Probabilistic reasoning is based on Bayes' rules, a mathematical means of combining the prior probability of a disease (proxy of disease prevalence) with a conditional probability based on clinical findings and test results for all diseases under consideration to produce a revised or posterior probability (Kassirer, 1994).

Bayes' theorem states that

$$P(H_i|E) = \frac{P(E|H_i)\ P(H_i)}{\sum_{n=1}^{k} P(E|H_n).\ P(H_n)}$$

This expression stands for the probability of hypothesis H given that we have observed evidence E (Rich and Knight, 1991).

The components of this theorem stand for the following:

P (Hi|E) – the probability that hypothesis H_i is true given evidence E (posterior probability).

P (E|Hi) – the probability that we will observe evidence E given that hypothesis i is true.

P (Hi) – the *priori* probability that hypothesis i is true in the absence of any specific evidence.

k = the number of the possible hypotheses.

Bayesian probabilistic reasoning has been employed in designing CaDDiS, an expert system developed to aid differential diagnosis of tropical bovine diseases (McKendrick *et al.*, 2000). Given that similar clinical signs can arise in animals with different diseases and severity of the same disease and clinical signs can vary in different animals within a population, diagnosis of diseases is better analysed using the Bayesian probabilistic system. This system utilises conditional probability in tackling conjunction occurrence of various disease signs in the same animal rather than using production rule-based expert system that follow binary logic (McKendrick *et al.*, 2000).

CaDDiS is a computerized expert system that consists of possible clinical signs, signs believed to be present and those absent, the likely diagnosis and the beliefs (probabilities). The output of CaDDiS gives both the possible diagnoses and the differential diagnoses. This programme has been developed in such a way that disease probabilities are updated dynamically as each new piece of evidence is added and those with a score below a minimal threshold are removed from the diagnosis window (McKendrick *et al.*, 2000)

The structure of information in production rule-based CADx is in the form of rules composed of two major parts: a premise or 'if ' clause and a conclusion or ' then' clause. The premise may be a conjugation of conditions and the conclusion may be simple or compound. This helps the machine to search all the rules and 'fire' or reckon as true all conclusions of rules for which the premise is known to be true. A subsequent pass through the rules database or knowledge base allows the programme to 'fire' any rules with premises now known to be true because of the first round of conclusions. The second round of conclusions may be once again used to search the knowledge base (Steward, 1996).

EQWISE is an expert system designed to provide tailored advice to veterinarians on diagnosis and treatment of equine diseases (Revie *et al.*, 1994). EQWISE consists of an Equine Cough Consultation System (EQCCS), a hypertext-based information retrieval system and a simple link to demographic and epidemiological data. EQCCS is a production rule system that makes use of an appropriate link into the hypertextual information as a means of providing an explanation. A screenshot of the EQCCS displays possible conditions, answers and rejected conditions.

Fuzzy systems are based on fuzzy logic and have been developed into computer-assisted diagnostic systems in veterinary medicine (Bellamy, 1997). The systems have been designed to select a diagnosis from among a number of disease categories. They represent complex, non-linear and imprecise relationships with a minimum of rules and often provide more accurate knowledge representation than traditional expert systems. Fuzzy rules are based on sets rather than points. The advantage for fuzzy systems is that they are more efficient to design, often provide a more accurate diagnosis and selection of input variables is critically important to diagnostic accuracy for any computer-based diagnostic system (Bellamy, 1997).

2.12.2 Manual Decision Support Tools

A computer-independent pattern matching decision support model for use in veterinary diagnosis that identifies diseases with the best-fit profile according to the summation of the disease sign frequencies for clinical observations recorded has been described by Cockcroft (1999a) for six tropical diseases; anaplasmosis, babesiosis, fasciolosis, parasitic gastroenteritis, seneciosis and trypanosomosis. In pattern matching, the clinical signs observed are compared with profiles or descriptions of diseases. The differential diagnosis list is constructed and ranked according to which of the disease profiles most closely match the clinical signs (Cockcroft, 1999a).

Edge-punched and feature cards that have the same function as slide rule for identifying differential diagnoses for various combination of signs and symptoms have been described by Thrusfield (1995).

FAMACHA© method is a chart against which the colour of the mucous membranes of all sheep in a flock can be regularly checked to identify sheep with pale membranes, a clinical identification of anaemia, for treatment against haemonchosis (Van Wyk *et al.*, 1997). The chart consists of a series of 5 identical drawings of a sheep's eye, but with each conjunctiva coloured a different shade of red or pink, respectively corresponding to PCV of 30, 25, 20, 15 and 10%. Each drawing is matched with the predominant band of colour of the mucous membrane on the inside of the lower eyelid. The predictability of 77% has been obtained for sheep with haematocrit values of 20% or below using clinical evaluation according to the FAMACHA© (Van Wyk *et al.*, 1997).

A mechanical device resembling a slide rule has been used in human medicine to identify differential diagnoses for various combinations of signs and symptoms of diseases (Nash, 1954). The mechanical device consisted of a table with hundreds of removable columns, which allow the manipulation at will of any chosen groups of prefabricated groups of data for the solution of classificatory problems in medicine and other subjects. The device was meant to supplement books and to aid logical thinking (Nash, 1954) by providing fact manipulation.

An algorithm in the form of a branching tree with decision nodes has been constructed to identify diseases that match a series of observations. Essex (1977) devised and evaluated algorithms with short pathways and simple decision nodes for non-experts in the domain of tropical human medicine.

2.12.3 Merits and limitation of decision support tools

DDST have a number of merits. A reliable effect of CADx on diagnostic processes is its expansion of lists of disease to be considered and the diagnostic pathways. Current CADx programmes also provide suggestions for laboratory tests useful to rule in or out any active disease hypothesis (Steward, 1996). Algorithms have the advantage of increasing the user's diagnostic accuracy and prompting the user for the next observation in the clinical examination (Essex, 1977; Cockcroft, 1999a). However, there are a number of limitations associated with DDST. No solution is guaranteed with CADx since expert systems only apply the knowledge contained in their representation scheme as given by the inference engine with which they are endowed. None of the currently available CADx programmes are powerful enough in their inference technology to offer a specific diagnosis. CADx demand a great deal of maintenance of the knowledge or rule base. CADx's initial investment is costly regarding initial capital expense, motivation of supporting specialists and user patrons for a large paradigm shift and for a critical mass of veterinarians to foster the research and development of such tools (Steward, 1996).

There is limited advancement in the development of veterinary CADx due to scarcity of theoretical publication and curtailment of collaboration due to difficulties in conducting comparative clinical performance trials as a result of scarcity of research funds for veterinary CADx. This has been due to more emphasis being put on commercial rather academic research in veterinary and artificial intelligence fields (Steward, 1996). Other limitations of DDST include differences of opinions between experts (specialists) and the CADx, technology advancement and CADx development being dictated by the user population's willingness to adopt it, and lack of reliability of CADx if inaccurate input is being fed into the computer (Steward, 1996).

Methods for the determination of anaemia under field conditions are further investigated in Chapter 4. Diurnal variations of rectal temperature and sensitivity of diagnostic tests for

trypanosomosis are described in Chapter 5. In Chapter 6 the epidemiology of endemic diseases in cattle kept under a mixed crop-livestock farming system in Uganda is explored. The findings of field studies on clinical signs associated with bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis are described in Chapter 7. Finally, the development of a decision support card for the differential diagnosis of endemic diseases is elaborated in Chapter 8.

Figure 3.1: The author and a team collecting blood, lymph node biopsy and faecal samples, and clinical data in Uganda during the longitudinal study – Eight field sites were visited every 4 weeks for 12 months

Chapter 3 General Materials and Methods

3.1 Introduction

Cross-sectional and longitudinal studies were conducted in South East Uganda, in succession, to assess the occurrence of the common endemic diseases of cattle kept under a mixed-crop-livestock production system.

3.2 The cross-sectional study area

The cross-sectional study was conducted in May 2000 in Kamuli, Tororo, Busia and Soroti districts of Uganda. The main vegetation type in the study area is savannah grassland. The study area receives 1200-1500 mm of rainfall annually, which is bimodal in distribution. There are two wet seasons (March to May and September to November) and two dry seasons (December to February and June to August). The daily mean minimum temperature is 15°C and mean maximum is 27°C. Small seasonal variations occur in rainfall and temperature between the districts (Ford and Katondo, 1976).

The districts visited are infested by the tsetse species, *Glossina fuscipes fuscipes* and to a limited extent *G. pallidipes* (Lancien *et al.*, 1990; Okoth, *et al.*, 1991; Magona *et al.*, 1997; Okuna *et al.*, 1999) that transmit trypanosomosis. *Rhipicephalus appendiculatus, R. evertsi evertsi, B. decoloratus* and *A. variegatum* are the major tick species of economic importance that occur in the study area (Okello-Onen *et al.*, 1999), which transmit *T. parva, A. marginale, B. bigemina* and *C. ruminantium*, respectively. Climatic conditions and the presence of swamps and marshland in the study area are conducive for the survival of snail, *Lymnea natalensis* (Ogamba-Ongoma, 1972) the intermediate host of *F. gigantica* that is prevalent in cattle in the study area (Magona *et al.*, 1999). Climatic conditions in the area also favour continuous survival of other species of helminth larvae on pasture, while contamination of communal

pastures is maintained the year round by sharing of pastures by cattle of all ages belonging to different herds. Cattle were examined in Bugondha, Bukafuga and Kinu villages of Kamuli district, Bugwera and Mulanga villages of Tororo district, Manakor village of Busia district, and Akoroi, Obar and Obur villages of Soroti district. In Figure 3.2 red dots represent the villages visited. Selection of villages was purposive based on reports on tsetse infestation and presence of animal trypanosomosis (Lancien *et al.*, 1990; Okuna *et al.*, 1996). In addition, these villages were conducive for transmission of other parasites looked for such as *Fasciola*, gastrointestinal nematodes, *T. parva*, *A. marginale*, *B. bigemina* and *C. ruminantium*.

3.3 The longitudinal study area

A longitudinal study was conducted in Tororo district from July 2001 to July 2002 and in Busia district from October 2001 to September 2002. Selection of villages was done based on the criterion described in Section 3.2. Four villages were chosen in Tororo and another four in Busia district. The 8 villages included Bunghaji, Hitunga, Magoje, Ojilai, Kubo, Buyimini, Nanjeho and Sitengo (Figure 3.3).

3.4 Description of the production system in the study sites

Agricultural production is primarily undertaken by smallholder farmers who produce a number of different food and cash crops and integrate crop production with livestock keeping (Okello-Onen *et al.*, 2003). Zebu and Sanga breeds constitute over 95% of the cattle of population in Uganda (Magona and Mayende, 2002). Cattle of the Zebu breed are predominantly kept under traditional communal grazing management and are either tethered or grazed on communal pastures during the day and tied up around homesteads or kept in bomas at night (Magona *et al.*, 2000; Okiria *et al.* 2002). Calves below 6 months of age are always tied up around homesteads (Magona *et al.*, 2000). Under traditional management, the majority of the cattle owners neither undertake tick control nor organised regular treatments for trypanosomosis and

helminthoses. Cases of very sick animals are reported to the government field veterinarians. Trypanosomosis, tick-borne diseases and helminthoses are responsible for the bulk of the reported cases (Anon. 1992; Magona and Mayende, 2002).

Mixed farming is practised with cotton being the major cash crop and maize, millet, cassava, beans and sweet potatoes being the major food crops (Okiria *et al.*, 2002). The vegetation cover is mainly composed of savannah grassland interspersed with *Lantana camara* shrubs (Magona *et al.*, 2000; Okiria *et al.*, 2002; Magona and Mayende, 2002). The average monthly rainfall and ambient temperature during the longitudinal study is shown in Figure 3.4.

3.5 Selection of cattle

In the cross-sectional study, 50 cattle of all ages and both sexes were randomly selected from all herds brought by owners at the designated collection centres in each village. Selection of individual animals for examination was carried out using the systematic sampling method as described by Thrusfield (1995), following the order in which cattle were presented by the owners. Nine villages in total (see Section 3.2) were visited. Clinical examination of cattle was conducted as described in Section 3.7.1. In addition, blood, faeces and lymph node aspirates were collected from cattle as described in Sections 3.7.3, 3.7.4 and 3.7.5.

In the longitudinal study, cattle census, including collection of owner information, was conducted with the help of local village administrators to guide the subsequent recruitment of animals for the study. The objective of the study was explained to cattle owners and their permission was obtained to use the animals in the study. Selection of cattle took into consideration representation of all herds in the villages. However 80 Zebu cattle of all ages and both sexes were selected purposively in each of the 8 villages depending on the owner's willingness to participate in the study. For purposes of ensuring availability of a satisfactory

sample size of younger animals to assess seroconversion events of major tick-borne diseases amongst other diseases, selection of animals was age-weighed to compensate for higher rates of drop out in younger animals. Older animals are already serologically positive but stay longer in the herd than younger animals most of which are serologically negative (O'Callaghan, 1998). On the day of ear-tagging, the estimated date of birth obtained from the cattle owner, breed, colour and sex were recorded.

3.6 Longitudinal study design

Village visits were made once every 4 weeks by the author and a team from LIRI, Tororo, Uganda. Ear-tagged cattle were either sampled within owners' homesteads or at designated collection centres where owners gathered their animals. To help mobilise the cattle owners, a contact person was chosen by the community in each village. People preferably already working as local administrators were chosen to be the contact persons, to ensure smooth operation. Prior notice about the cattle-sampling programme was passed on to cattle owners through the contact persons in each village before the monthly sampling visit. Blood, faeces and lymph node aspirates were collected from ear-tagged cattle presented by owners during the visits (see Sections 3.7.3, 3.7.4 and 3.7.5). Collection of samples and clinical examination of individual animals took place in the villages between 8 a.m and 1 p.m. Table 3.1 gives the mean, maximum and minimum number of cattle presented during sampling visits in different villages and the overall total.

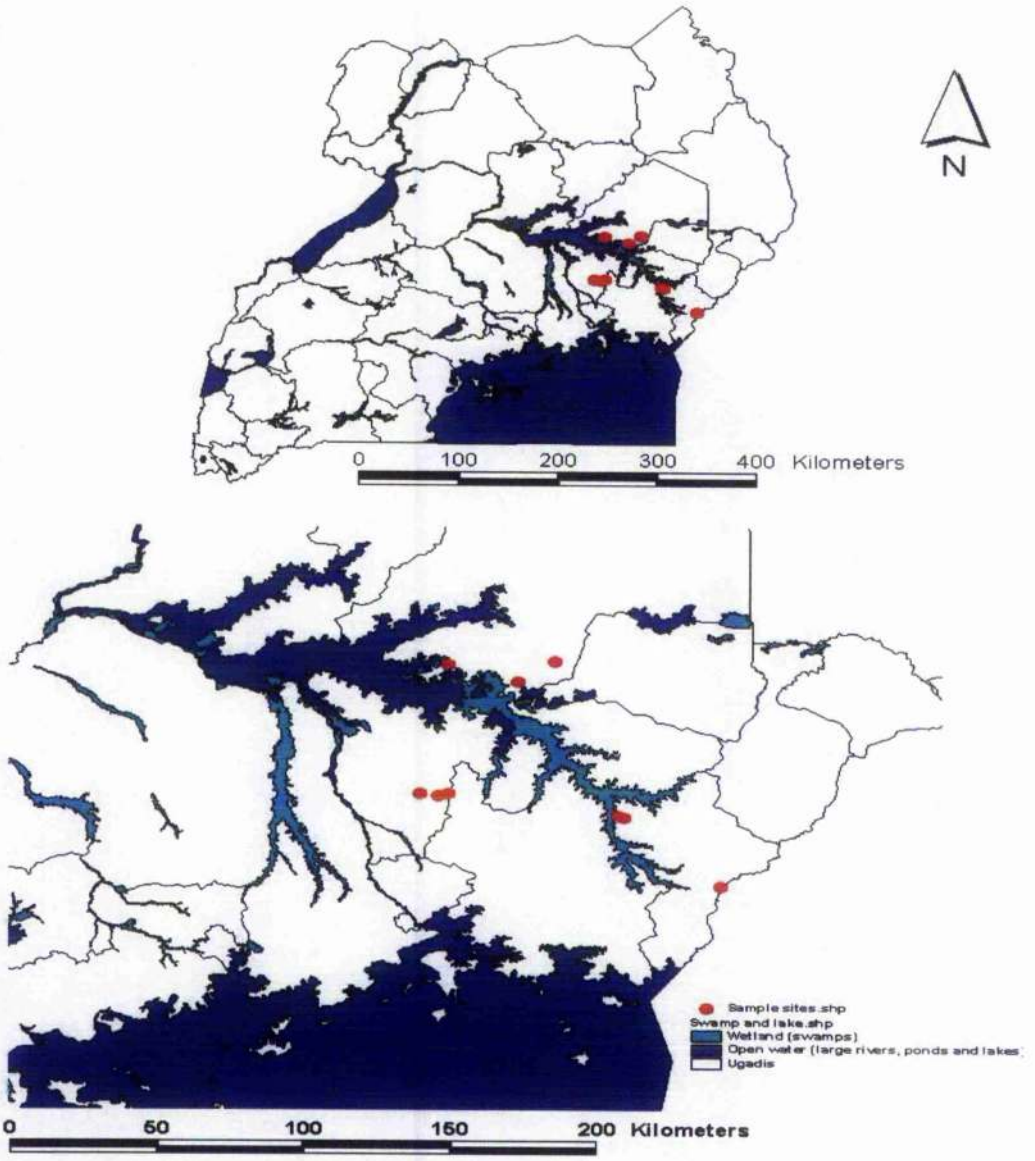


Figure 3.2: Map showing villages (red dots) where the cross-sectional study was conducted

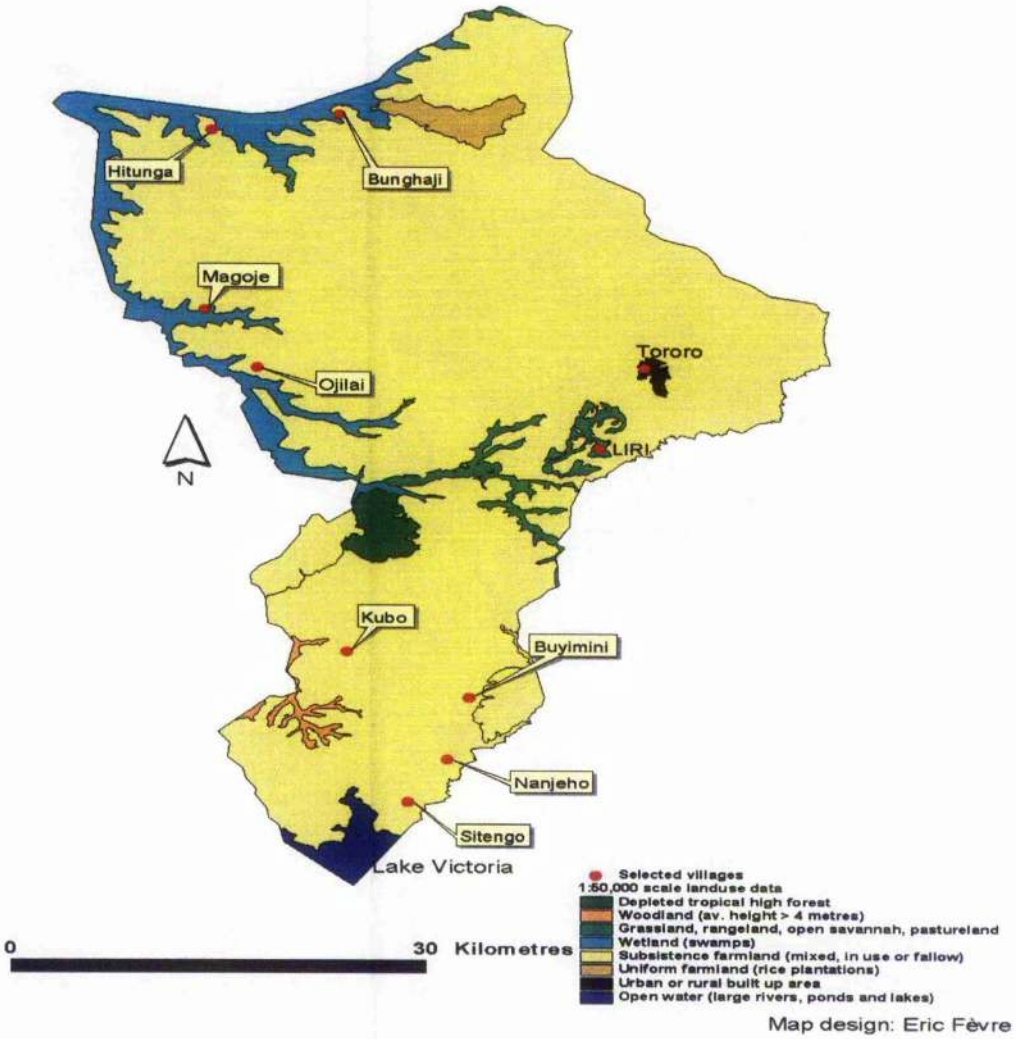


Figure 3.3: Map showing villages (red dots) where the longitudinal study was conducted

Table 3.1: The mean, maximum and minimum number of cattle presented during the village sampling visits

	Villages								
	Tororo district			Busia district					
	Bunghaji	**Hitunga**	**Magoje**	**Ojilai**	**Buyimini**	**Kubo**	**Nanjeho**	**Sitengo**	**Overall**
Mean	65	76	67	68	71	68	69	78	562
Max	79	79	80	78	80	80	80	80	636
Min	48	67	55	52	57	54	59	76	531

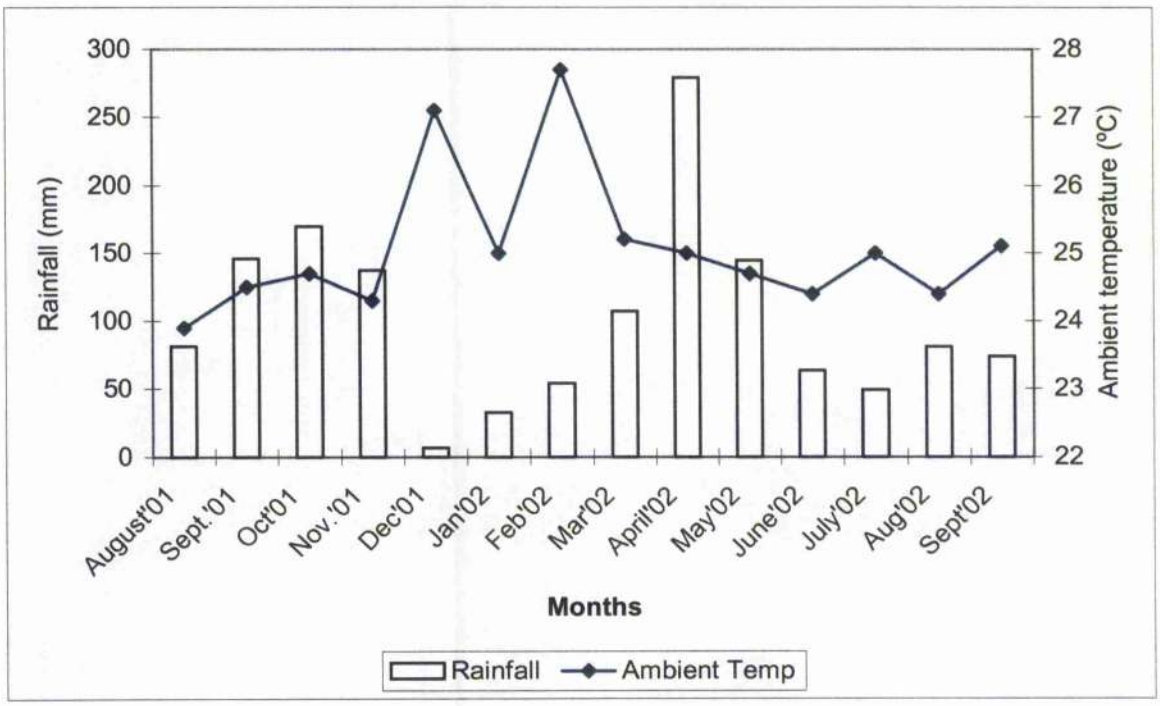


Figure 3.4: Monthly average rainfall and ambient temperature during the longitudinal study (Source: Tororo Meteorological Centre, Uganda, October 2002)

Figure 3.5: Taking rectal temperature, faecal samples, clinical examination and bleeding a cow in the field in Busia district Uganda

3.7 General outline of methodologies

3.7.1 Clinical examination of cattle

General physical examination was conducted on all cattle bled. Parotid, prescapular and prefemoral lymph nodes were palpated to assess whether they were enlarged. The skin coat was examined for any sign of roughness or starring. Oral, vulval and conjunctival mucous membranes were examined for presence of pallor or petechial haemorrhages. Animals were examined for presence of diarrhoea, ocular or nasal discharges. Rectal temperature was taken using a digital thermometer to detect pyrexia. Packed cell volume was measured using the micro-haematocrit reader (Hawksley, England) and haemoglobin concentration was measured using the HemoCue (HemoCue AB, Ängelholm, Sweden) to detect anaemia. Full clinical history of individual cattle and herds was obtained from animal owners. History of any abortions or stillbirths and deaths in the herds was obtained from the cattle owners. In addition, information was collected on owner's and the veterinarian's health assessment for each individual animal.

3.7.2 Assessment of the body condition score

To assess the extent of emaciation of animals, body condition scoring was performed based on a nine score system as described by Nicholson and Butterworth (1986), in which three main conditions (fat-F, medium-M and lean-L) are divided into three categories. The scores include F+, F, F-; M+, M, M-; L+, L and L-, where L- represents 1 and F+ represents 9. Body condition scoring is essentially based on the extent to which either fat is stored or muscle mass has declined. Anatomical parts including the tail-head, brisket and hump, transverse process of the lumbar vertebrae, hips and ribs, and the depth of the *sublumbar fossa* and mass of muscle between the *tuber coxae* and *tuber ischii* were visually inspected for visibility and flesh or fat cover on all animals examined.

3.7.3 Blood

Ear-tagged animals were bled from the jugular vein into two vacutainers (Becton-Dickinson, Vacutainer System, UK), one heparinised and another non-heparinised. About 5 ml of blood was taken from each animal into each tube. Blood samples in heparinised tubes were used for determining the PCV and performing the phase-contrast buffy coat (BCT) and haematocrit centrifugation techniques (HCT). Blood samples in the non-heparinised tubes were used for preparation of serum. An additional blood sample was taken from the marginal ear vein by prick for on-spot determination of haemoglobin and making of thin and thick blood smears.

3.7.4 Lymph node aspirates

Lymph node biopsies were taken in the field from eartagged animals that had pyrexia, enlarged lymph nodes or had other signs suspicious of *T. parva* infections, such as petechial haemorrhages and dyspnoea. Biopsies were taken from enlarged parotid lymph nodes using a 5-ml syringe with a 16-guage needle and placed on a microscope slide. A smear was made, labelled, packed in a slide box and dispatched to the laboratory for examination.

3.7.5 Faecal samples

Faecal samples were taken directly from the rectum. Each sample was placed in a separate plastic bag; clearly labelled with the eartag number of the individual animal, date of collection and name of the village. Samples were dispatched in a cool box to the laboratory, where they were preserved with 1% formalin before being kept at 4°C until examination, to prevent hatching of nematode eggs into larvae. Due to the large number of samples collected at each visit, samples were examined within 5 days after collection.

3.7.6 Examination of samples

All the blood and faecal samples were examined at LIRI, Tororo, Uganda while all the serology was conducted at ILRI, Nairobi, Kenya.

3.7.6.1 Blood examination

Blood samples were examined for trypanosomes using both BCT (Murray *et al.*, 1977) and HCT (Woo, 1969). In addition, Giemsa-stained thin and thick blood films were examined for *Trypanosoma*, *Anaplasma*, *Babesia* and *Theileria* infections. To perform the phase-contrast buffy coat technique, the buffy coat zone was prepared in microhaematocrit tubes filled with about 70 µl of blood, sealed at one end with plasticine and centrifuged for 5 minutes. The capillary tubes were then cut with a diamond-pointed pencil 1 mm below the buffy coat to incorporate the uppermost layer of the red blood cells and 1 cm above to include some plasma. The contents of the tubes were then gently expressed on to a clean slide, mixed and covered with a coverslip. The preparation was then examined under dark ground phase contrast microscopy to detect trypanosomes, identified as *T. brucei*, *T. congolense* and *T. vivax* based on motility. At least 50 microscopic fields were examined per sample.

For the haematocrit centrifugation technique, microhaematocrit capillary tubes were filled with blood (about 70 µl), sealed at one end with plasticine and centrifuged for 5 minutes. The capillary tubes were then wiped clean with tissue paper and placed on a microscope slide. A maximum of twelve capillary tubes were placed on the microscope slide and the spaces between the capillary tubes flooded with oil immersion. The buffy coat-plasma junction of each capillary tube was then examined by microscopy to detect trypanosomes, which were identified as *T. brucei*, *T. congolense* and *T. vivax*. In addition, PCV was measured before the capillary tubes were examined.

Thin and thick blood smears were made from each eartagged animals in the field during sampling. The smears were air-dried, labelled with the eartag number and the date of collection, then packed into slide boxes and dispatched to the laboratory for examination. Before examination, the thick blood films were placed in distilled water for 5 minutes to lyse the erythrocytes while thin films were dipped in methanol for 3 minutes for fixation. The blood smears were stained in 10% Giemsa for 30 minutes. Samples were examined by microscopy under oil immersion for at least 50 fields. The intensity of parasitaemia for theilerial and babesial piroplasms and *Anaplasma* organisms was assessed using a scoring system, whereby + represented 1 organism found in more than 10 fields, ++ represented 1 organism found in more than 1 field but in less than 10 fields, and +++ represented 1 or more organisms found per field. Trypanosomes were identified according to morphology as *T. brucei*, *T. congolense* and *T. vivax*.

3.7.6.2 Examination of lymph node aspirates

Lymph node biopsy smears were fixed for 3 minutes in methanol, then stained in 10% Giemsa for 30 minutes. The biopsies were examined by microscopy under oil immersion for at least 50 fields to detect *T. parva* macroschizonts in the lymphocytes, which is considered confirmatory for East Coast fever (Urquhart *et al.*, 1996).

3.7.6.3 Faecal sample examination

Faecal samples were examined for strongyle eggs using the McMaster method accurate to 50 eggs per gram of faeces as described by Hansen and Perry (1994). Briefly, about 4 grams of faeces was weighed and placed into a beaker. Then 56 ml of flotation fluid (sodium chloride solution) was added. The contents were then thoroughly homogenised and filtered through a tea strainer into another beaker. The filtrate was stirred and a sub-sample was taken with a Pasteur pipette and both sides of the McMaster counting chamber were filled. After allowing the

counting chamber to stand for about 5 minutes, the filtrate was examined by microscopy. All nematode eggs within the engraved area of both chambers were counted, with nematode eggs being identified as 'strongyle-type'. The number of eggs per gram of faeces was calculated by addition of egg counts of the two chambers together and multiplication by 50 (Hansen and Perry, 1994). Egg counts in excess of 1000 were generally considered indicative of heavy infections while those over 500 were indicative of moderate infection (Urquhart *et al.*, 1996).

Faecal samples were examined using the sedimentation technique as described by Hansen and Perry (1994). This involved weighing about 3 grams of faeces in a beaker. Then 40-50 ml of tap water was added. The contents were homogenised and filtered through a tea strainer and the filtrate transferred to a conical flask. The filtrate was allowed to stand for 2 minutes, before the supernatant (approximately 12-15 ml) was drawn off and transferred to a flat-bottomed tube. After sedimentation for a further 2 minutes, the supernatant was again drawn off and a few drops of 5% methylene blue were added. The sediment was then examined for trematode eggs by microscopy. Animals were considered positive for fluke infections when *Fasciola* eggs were detected or for schistosome infection when *Schistosoma* eggs were found irrespective of the count.

3.7.6.4 Serological analysis

Serological analysis of samples was carried out using the antibody detection ELISA for *Theileria parva*, *Anaplasma marginale* and *Babesia bigemina* infections.

3.7.6.4.1 Selection of sera for analysis

Serum samples were collected from cattle examined at each monthly visit. A proportion of sera were selected for analysis using a criterion as follows. All sera collected during the initial sampling visit (day 0) and those collected on experimental days 84, 168, 252 and 336 were

tested. Sera collected during the intervening visits on experimental days 28, 56, 112, 140, 196, 224, 280 and 308 were selected purposively for analysis. For these visits, all sera of young animals (up to the age of 12 months) and a proportion of sera for older animals were selected; sera for older animals previously negative but had become positive or previously positive but had become negative. In addition sera of older animals that originally had PP values within 10 per cent positivity (marginally greater than threshold) above the cut-off point, but on subsequent visits had had a sero-increase of at least 20 PP for *T. parva* and 15 PP for *A. marginale* and *B. bigemina* infections were selected. A sero-increase of at least 20 PP for the *T. parva* test or of at least 15 PP for the *A. marginale* and *B. bigemina* tests was considered significant because it was an equivalent to doubling the antibody titre, which often occurs in older animals that have had a rechallenge (secondary immune response). A secondary immune response is reported to have a dramatic increase in antibody titre that is higher than double the threshold (O'Callaghan, 1998). Ultimately, 5300 sera were analysed using each of the ELISA tests for detection of antibodies against *T. parva*, *A. marginale* and *B. bigemina* infections.

3.7.6.4.2 Assay protocol for the Enzyme-linked immunoassays

Sera were screened for antibodies against *T. parva, A. marginale* and *B. bigemina* infections using ELISA utilising *T. parva* schizont antigen (polymorphic immunodominant molecule), a 19 kD recombinant antigen and a 200 kD recombinant antigen (Katende *et al.*, 1998; Morzaria *et al.*, 1999; Tebele *et al.*, 2000), respectively.

Polysorb micro-ELISA plates (Polysorp, Nunc, Denmark) were utilized for all samples. Plates were coated at concentrations of 150 ng/well, 100 ng/well and 170 ng/well of recombinant antigen solution for ELISAs for detection of *T. parva*, *A. marginale* and *B. bigemina* infections, respectively, diluted to a concentration of 1.0µg/ml in 0.01m Dulbecco's phosphate buffered saline, pH 7.4, and incubated in an incubator/shaker (Labfacility shaker incubator IS89, Insel,

England) at 37°C for 2 hours. Excess antigen solution was discarded, wells dried for 10 minutes in the incubator/shaker and the plates sealed and stored at –20°C until use. Shortly before use, plates were removed from storage and allowed to return to room temperature. Wells were blocked by the addition of 300 µl/well of 1% casein in DPBS followed by incubation for 20 minutes at 37°C. The blocking solution was removed and wells washed with DPBS using a Nunc 12 channel washing bar (Nunc Inter Med, Denmark) fitted to a vacuum pump.

Before testing, strong positive reference control sera (C++) and negative sera (C-) were reconstituted from the lyophilised form by adding 40µl and 100µl of distilled water, respectively. To obtain the weak positive serum (C+), 5µl of C++ was mixed with 80µl, 60µl and 55µl of C- for ELISAs for detection of *T. parva*, *A. marginale* and *B. bigemina* infection, respectively, giving ratios of 1:16, 1:12 and 1:11.

Test and control sera were initially diluted in a 1% skimmed milk solution prepared in DPBS with 0.1% Tween 20 to a working dilution of 1:40 for ELISAs for detection of *T. parva* and *A. marginale* infections and to a working dilution of 1:50 for the *B. bigemina* antibody detection ELISA. Serum dilutions were mixed thoroughly by gentle agitation of each plate on a micro-agitator (Heidolph, France). Then 150µl of a final working dilution of 1:200, for the *T. parva* and *A. marginale* antibody detection ELISAs and of 1:100 for the *B. bigemina* antibody detection ELISA, was transferred to wells of the antigen-coated plates in duplicate. In addition, a pair of conjugate control wells, containing only 1% skimmed milk solution was included on each plate. Plates were incubated for 30 minutes at 37°C in a shaker/incubator for the *T. parva* and *A. marginale* antibody detection ELISAs and for 40 minutes at 37°C for the *B. bigemina* antibody detection ELISA.

Unbound antibodies were discarded by suction using the Nunc 12 channel washing bar and the wells washed by filling and aspiration of wash buffer for 5 times, and draining by inverting and slapping plates onto lint-free paper hand towels. The washing step was followed by a 10-minutes soaking interval. Anti-bovine immunoglobulin horseradish conjugate was diluted to 1:50,000 in DPBS containing 0.1% Tween 20 with 1% skimmed milk and 150µl added to each well. The plates were incubated for 30 minutes at 37 ^{0}C in a shaker/incubator and then washed as previously described.

The colour reaction was developed by addition of 150 µl/well of sodium citrate buffer, pH 4.0, containing 1% hydrogen peroxidase as the substrate and of 40 mM ABTS as the chromogen. After addition of the substrate-chromogen solution, plates were incubated for 30 minutes at 37^{0}C in the shaker/incubator. Thereafter, optical densities (OD) were determined using the Titertek Multiscan Mcc340 spectrophotometer (Titertek Instruments Inc., Huntsville, Alabama, USA) at 405 nm. The OD values were expressed as percentage positivity (PP), which were calculated as follows: (OD of test/OD of strong positive) x 100 (Wright *et al.*, 1993). The cut-off point for the *T. parva* assay was 20PP and for both *A. marginale* and *B. bigemina assays* was 15PP.

Data generated by the microplate reader was transferred automatically to the computer for storage using the computer programme EDI (ELISA Data Interchange, 2.2) (FAO/IAEA, 1997). On each plate were included four wells of internal quality controls; the strong positive control (C++), the weak positive control (C+), negative control (C-) and conjugate control (Cc). These controls were treated in the same way as any test samples with respect to the dilution and tests procedure. The strong positive control, the weak positive control and negative control were included to monitor the consistency among test runs, while the conjugate control was used to monitor the background activity of the system. Each internal control sera had an upper

control limit (UCL) and a lower control limit (LCL). In case of a problem with the plate being tested, the value of the control sera would fall outside the control limits and the sample had to be retested. Each time a plate was read, the EDI programme would determine if the control values fell within the UCL and LCL. The median value of C++ was calculated and all the OD values for test samples were expressed as a percentage of the strong positive control standard automatically by the EDI programme. All the data generated by the EDI programme was eventually downloaded and transferred to a Microsoft Access database, whereby data was related to individual animals, villages, and sampling dates.

3.8 Data collection

Data collection involved recording of data on forms designed to conform to the requirements of individual studies conducted. For cross-sectional and longitudinal studies, field and laboratory data were recorded on two different forms: a field (Table 3.2) and laboratory form (Table 3.3). Each village had one field and laboratory form. Data recorded on field forms included, name of owner, age, breed, sex, tag number, haemoglobin concentration, weight, body condition score, colour of mucous membranes, size of superficial lymph nodes, enlargement of lymph nodes, coat appearance, presence of skin lesions and diarrhoea. Other parameters included health status as assessed by the animal owner and the veterinarian, as well as the level of tick infestation of individual animals by ticks of *Rhipicephalus* spp., *Boophilus* spp and *Amblyomma* spp. For tick counts records, 1 stood for up to 10 ticks, 2 for 11-50 ticks and 3 for over 50 ticks on the whole body. On the laboratory form was recorded the animal tag number, PCV, haemoglobin concentration and the outcome of the parasitological tests for trypanosomes. Other parameters included presence of fluke eggs (*Fasciola* and *Schistosoma*), nematode egg counts, and the outcome of parasitological tests for *Anaplasma* organisms, babesial piroplasms and theilerial piroplasms and schizonts. All data was subsequently extracted from the forms and

entered into spreadsheets of the computer programme Microsoft Excel for storage. Separate files were prepared for each study.

3.9 Data analysis

For purposes of data analysis, animals with a temperature higher than 39.4°C were considered to have fever (Minjauw *et al.*, 1998; Maloo *et al.*, 2001c). Body condition scores lower than 4 (M-) i.e. L+, L and L- were indicative of weight loss. Animals with a PCV lower than 25% or Hb concentration lower than 8 g/dl were considered anaemic (Schalm, 1975). Animals with nematode egg counts higher than 400 eggs per gram of faeces were considered to exhibit the clinical form of gastrointestinal nematode infections (Hansen and Perry, 1994). All animals with a parasitaemia score of at least 1 for *B. bigemina* piroplasms and *A. marginale* organisms were considered positive. Any animal in whose lymph node biopsy macroschizonts were detected and/or had *T. parva* piroplasm parasitaemia score of 1 was considered positive. Animals were considered positive for trypanosome infection based on parasitological detection of trypanosomes, while they were positive for fluke infections based on detection of *Fasciola* eggs and for schistosome infections based on detection of *Schistosoma* eggs. Non-diseased animals constituted a group of healthy animals that did not have detectable parasitaemia for *B. bigemina, A. marginale* and *T. parva or* trypanosomes and fluke eggs and had nematode egg counts of not more than 400 e.p.g.

Table 3.2: A data collection field form

		Hb	Weight	Cond. Score	MM	LN	Coat	Skin Lesions	Diarrhoea	Discharge	Health (Owner)	Health (Vet)	Ticks (Rh)	Ticks (Amb)	Ticks (Boo)	RT
	Tag No	g/dl	Kgs	L, M, F +/-	N, P, PP	Size N/E	N or S	0,1,2	0,1,2	Site Severity	HH, H, S, SS	HH, H, S, SS	0,1,2,3	0,1,2,3	0,1,2,3	°C
1																
2																
3																

Condition score: L-lean, M-medium, F-fat; MM-mucous membranes: N-normal, P-pale, PP-very pale; LN-lymph node: N-normal, E-enlarged; Skin coat: N-normal, S-starring; Skin lesions: 0,1,2-degree of severity; Diarrhoea: 0,1,2-degree of severity; Health status: HH-very health, H-health, S-sick, SS-very sick; Ticks:0-none, 1= <10, 2= 10-50, 3= >50.

Table 3.3: A data collection laboratory form

Tag No	PCV	Hb	Hb Last Month	HCT	BCT	Thick	Thin						LN smear	Fluke eggs	WEC
	%	g/dl	g/dl	Tb Tc Tv TT uF	Tb Tc Tv TT uF	Tb Tc Tv TT uF	Tb Tc Tv	TT	uF	Bab	Tp	Ana	Tp		

Tb-*Trypanosoma brucei*; Tc-*T.congolense*; Tv-*T.vivax*; TT-tsetse-transmitted trypanosomosis; uF-Microfilaria; Bab-*Babesia* spp; Tp- *Theileria parva*; WEC-worm egg counts

Chapter 4 Comparative evaluation of portable haemoglobinometers and conventional haematological measures of anaemia

4.1 Introduction

The presence, absence and degree of anaemia are important diagnostic criteria for bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis (Omuse, 1978; Losos, 1986; van Amstel *et al.*, 1988; Murray and Dexter, 1988; Jain, 1993; de Bont and Vercruysse, 1998). Anaemia is measured by determining the number of red blood cells per µl of blood, haemoglobin concentration in blood (g/dl) and packed cell volume (percent) (Meyer *et al.*, 1992). In veterinary medicine, determination of anaemia relies mainly on the measurement of PCV using the microhaematocrit centrifugation technique, rather than measuring the haemoglobin concentration (Callan *et al.*, 1992). Packed cell volume, the relative volume of erythrocytes determined by centrifugation of blood in a microhaematocrit tube, is the most common clinical test used in assessing and monitoring anaemia and polycythemia in animals (Callan *et al.*, 1992). Changes in the PCV are always accompanied by parallel changes in both red blood cell count and haemoglobin content (Meyer *et al.*, 1992).

Packed cell volume is commonly used to measure the degree of anaemia in trypanosomosis, as usually recorded in association with the buffy-coat technique (Murray *et al.*, 1977). Evidence of anaemia as determined by the reduced PCV is used as a basis for treatment of cases of trypanosomosis. In tsetse-infested areas, treatment is recommended when a PCV of less than 30% is detected in high production dairy cows (Murray and Trail, 1986) or less than 17% at smallholder village location (Maloo *et al.* 1988). Average PCV is closely correlated to trypanosome prevalence in many tsetse-infested areas (Hendrickx *et al.*, 2000), for which

reason it has been used to evaluate the effectiveness of bovine trypanosomosis control programmes in sub-Saharan Africa (Bauer *et al.*, 1999; Magona *et al.*, 2000).

Packed cell volume has been used in evaluation of anaemia in the diagnosis of East Coast fever, anaplasmosis and babesiosis (Omuse, 1978; Egbe-Nwiyi *et al.*, 1997) and also to evaluate the response of different breeds of cattle to experimental *T. parva* infection (Paling *et al.*, 1991). In infections with blood sucking nematodes such as *Haemonchus contortus*, PCV measurement as well as clinical appraisal of mucous membranes are recognised as useful parameters for diagnosis (van Wyk *et al.*, 1997; Eysker and Ploeger, 2000). Monitoring of PCV has also been used to determine the efficacy of drugs against fasciolosis (Suhardono *et al.*, 1991).

The major advantage of PCV determination using the microhaematocrit centrifugation technique is the ability to simultaneously evaluate plasma for total protein concentration, lipaemia, icterus, haemolysis (Callan *et al.*, 1992) and to identify parasites such as trypanosomes (Murray *et al.*, 1977). Packed cell volume also provides a simple and quick means to determine anaemia (Jain, 1993). However, this system offers a number of disadvantages. Plasma may be trapped in the centrifuged microhaematocrit tube, thereby falsely increasing the PCV (Callan *et al.*, 1992). Micro-centrifuges are bulky, fragile when transported and dependent on an AC-power source, limiting their range of use in a variety of veterinary practice situations worldwide (Callan *et al.*, 1992). In the context of delivery of veterinary services in sub-Saharan Africa, PCV determination using the microhaematocrit method requires expensive portable generators and centrifuges as well as vehicles to transport them (Callan *et al.*, 1992), thereby making the PCV method inaccessible to many extension workers and animal health assistants in remote areas of Africa. In addition, PCV is not a reliable measure of anaemia if the animals have an exceptionally high mean cell volume (MCV) (Jain, 1993). In instances of dehydration and splenic contractions, anaemia as determined by PCV may be

masked (Jain, 1993). There is a need for rapid, reliable and cheap tests for anaemia that are able to provide quantitative pen-side results for the immediate diagnosis and management of endemic bovine diseases in sub-Saharan Africa.

Monitoring of haemoglobin as an indicator of anaemia is increasingly being recognised as important in human primary health care in Africa (Wilkinson and Sachs, 1997). Total blood haemoglobin is a mixture of haemoglobin, oxyhaemoglobin, carboxyhaemoglobin and minor amounts of other forms. Accurate measurement of haemoglobin requires preparation of a stable derivative involving all forms of haemoglobin in the blood (Callan *et al.*, 1992). The measurement of blood haemoglobin concentration is normally performed to detect anaemia and polycythaemia in animals, but is rarely used in bovine medicine. The few examples in the literature include diagnosis of East Coast fever, anaplasmosis and babesiosis (Omuse, 1978), schistosomosis (McCauley *et al.*, 1983a), evaluation of drug efficacy against fasciolosis in cattle (Suhardono *et al.*, 1991) and haematological studies on bovine anaplasmosis (Egbe-Nwiyi *et al.*, 1997).

Methods for measurement of blood haemoglobin concentration range from highly accurate spectrophotometric reference methods, as prescribed by the ICSH to inaccurate and imprecise procedures such as the Sahli haemoglobinometer, the Tallqvist blotting-paper and the copper sulphate blood precipitation method (Johns and Lewis, 1989: Wilkinson and Sachs, 1997). Haemoglobin Colour Scales, cyanomethaemoglobin method and portable haemoglobinometers such as the HemoCue and the DIIT Hb-523 are the common methods used for determination of blood haemoglobin concentration.

Measurement of blood haemoglobin concentration is usually performed in laboratories with modern, expensive, non-portable fully automated equipment. Such facilities are not suitable for

parts of the world where the medical or veterinary infrastructure is poor or if available, is inaccessible to a large number of people and their livestock. Hand-held compact, battery powered haemoglobinometers now exist that could facilitate point of care Hb measurements especially under African conditions. In this study, the DHT Hb-535 haemoglobinometer (DHT), the HemoCue (HCU) and the WHO Haemoglobin Colour Scale methods (HCS), which are currently used for measuring haemoglobin in human medicine, were evaluated for their suitability in measuring haemoglobin of bovine blood under field and laboratory conditions. The study was conducted with following objectives:

- To assess the precision, accuracy, linearity and agreement of portable haemoglobinometers using the cyanmethaemoglobin method as a goldstandard.
- To assess sources of imprecision of the different methods under consideration.
- To establish the sensitivity and specificity of the different methods under consideration in the detection of anaemia.
- To correlate haemoglobin measurements and the packed cell volume values as measured under laboratory conditions.
- To establish whether field veterinarians with little or no laboratory experience can easily use the most promising methods.
- To compare the costs associated with the different portable haemoglobinometers.

4.2 Materials and methods

4.2.1 Evaluation of the performance of the haemoglobinometers

The precision of the haemoglobinometers was assessed by making repeated measurements on blood samples. Variability in sample pipetting and diluting thought to be the major sources of imprecision were measured by making two aliquots (dilutions) from each sample and three readings on each dilution. The accuracy and linearity were evaluated by comparing the measurements of the different methods with those of the reference methods. For purposes of

determining the sensitivity and specificity of the methods in the detection of anaemia, anaemia was defined as haemoglobin concentration of less than 8 g/dl as assessed by the cyanmethaemoglobin method.

4.2.2 Sample processing

Cattle samples were obtained from routine sampling of cases at Glasgow University Veterinary School. Each in-coming (original) bovine sample was diluted using normal bovine plasma to obtain four samples of the dilutions: 1 (neat), ¾, ½, and ¼, which were then treated as individual samples. Normal bovine plasma was obtained by centrifuging and decanting off plasma from one of the original bovine samples. In total, 65 samples were used for subsequent testing using the various Hb measurement methods and for determining PCV.

4.2.3 DHT-haemoglobinometer method

The DHT-haemoglobinometer (Developing Health Technology, UK) is a specialised battery-powered photometer for measuring blood haemoglobin concentration by its optical density (Gordon-Keeble Company, 2000). The blood is placed in a standard 10 mm photometric cuvette. The measurement of the optical density is carried out at a wavelength of 523 nm, the crossing point of the absorption curve of various haemoglobin forms. The device is simple to operate, needs no specialized calibration and seems suitable for use in rural settings in developing countries.

This method involved initially making a solution of 0.04% ammonia by adding 0.4 ml of 35% ammonia stock solution to 350 ml of deionised water. Two cuvettes, each containing 2 ml of 0.04% ammonia solution were then prepared for each sample. In each cuvette, 20 µl of blood was added and the solution was mixed carefully, to lyse the erythrocytes. Three readings were taken for each cuvette using the photometer.

The HemoCue method

The HemoCue (HemoCue AB, Ängelholm, Sweden) is a haemoglobinometer system that measures haemoglobin at two wavelengths as azide methaemoglobin, without dilution (von Schrenk *et al.*, 1986). The azide methaemoglobin reaction involves the erythrocyte membranes being disintegrated by sodium deoxycholate, thereby releasing the haemoglobin. Sodium nitrate then converts the haemoglobin iron from the ferrous to the ferric state to form methaemoglobin, which then combines with azide to form azide methaemoglobin (von Schrenk *et al.*, 1986). This method is based on an optical measuring microcuvette of a small volume (10µl) and short light path (0.13 mm distance between the parallel walls of the optical window). Dry reagents are deposited on the inner wall of the microcuvette cavity, and the blood sample, drawn into the cavity by capillary action, is mixed with the reagents spontaneously. The microcuvette is then placed in a HemoCue photometer, in which its absorbance is measured at 565 and 880 nm. The instrument calculates the concentration of the haemoglobin in the sample and displays the result. The advantages of this method are: no technical skills are required, the unit is battery or mains powered, it is quite accurate and robust, compensates for turbidity in samples by measuring at two wavelengths and is easy to use in rural settings. However, this method is associated with operator errors due to inadequate mixing of the blood specimen before sampling, which might be obviated by using a rotating mixer (Neville, 1987). In addition, the HemoCue is too expensive for use in the rural settings in developing countries (van den Broek *et al.*, 1999).

Haemoglobin measurement using this method involved a drop of blood (10 µl) being drawn from the vacutainer into the cavity of a microcuvette by capillary action. The microcuvette was then placed into the HemoCue photometer and three readings were taken for each microcuvette fitted with a red filter. Care was taken to ensure that there were no visible air bubbles inside the microcuvettes, which could lead to discrepant results.

4.2.4 Haemoglobin Colour Scale

The Haemoglobin Colour Scale is a direct matching method which involves placing a drop of whole blood on a piece of filter paper, allowing it to dry and then comparing it with a set of standard colours. New colour scales developed by the WHO (Stott and Lewis 1995; van den Broek *et al.*, 1999) involve a drop of blood being placed on a strip of absorbent paper. After disappearance of the sheen, the colour is compared with a set of six colour standards corresponding to haemoglobin values of 4, 6, 8, 10, 12 and 14 g/dl.

This method has not been evaluated for use in veterinary medicine, but it has been evaluated as a screening method for anaemia in rural antenatal clinics in Malawi, where it was found to have a sensitivity of 50-80%, a specificity of 47-98%, an accuracy of 56-98%, a positive predictive value of 11-66%, a negative predictive value of 58-99% and a good agreement with the Coulter Count measurements (van den Broek *et al.*, 1999). The major advantages of the haemoglobin colour scale are: it is simple to use, well accepted, cheap, gives immediate results and is very suitable for resource limited settings such as in developing countries (van den Broek *et al.*, 1999). New colour scales have a higher sensitivity and positive predictive value compared to the Tallqvist colour scale whose sensitivity and positive predictive value are 60.5% and 46.0% respectively (van den Broek *et al.*, 1999). However, high subjectivity and a large probability for operator errors are the major limiting factors associated with this method.

In this study, this method was performed by taking a drop of blood from each sample aliquot and placing it on the filter paper strip using a capillary tube. After disappearance of the sheen, the colour was compared with a set of six colour standards by holding the test strip behind the scale and the blood spot viewed through the 8-9 mm aperture. Care was taken to hold the colour scale at an angle of about 45° under light, with the light coming behind the investigator.

The colour was compared from the bottom of the scale upwards. The Hb values recorded corresponded to the closest colour standard. Colour standards on the scale correspond to haemoglobin values of 4, 6, 8, 10, 12 and 14 g/dl. Two aliquots were made from each blood sample and the test strip from each aliquot was read once by one observer. To avoid prior bias in making readings, sample analysis by this method was performed before other Hb measurement methods.

4.2.5 Cyanmethaemoglobin method

The cyanmethaemoglobin method (CMH) is one of the most accurate methods available and is considered to be the reference method for blood Hb determination (Callan *et al.*, 1992). In this method, a drop of blood (20µl) was mixed with Drabkin's solution, which contains an agent for lysing red blood cells (e.g. saponin) and another for oxidising the free haemoglobin (e.g. potassium ferricyanimide) to convert it to cyanmethaemoglobin. After a period of about 15 minutes for the conversion reaction to occur, the optical density is read at 540-545 nm (Gong and Backenstose, 1999). The method is useful because it involves cyanmethaemoglobin, a stable derivative that is formed from haemoglobin compounds and is also available commercially as a reference solution for calibration (von Schrenk *et al.*, 1986). However, turbidity caused by incompletely lysed cells, lipid particles, marked leucocytosis, Heinz bodies and hyperparaproteinaemia lead to erroneously high Hb values (Callan *et al.*, 1992). High dilution (1:251) of blood sample with reagent bestows an inherent imprecision on the method, which increases even more when blood is sampled into capillary tubes or is diluted by untrained people (von Schrenk *et al.*, 1986).

In this study, 40 µl of blood was mixed with 20 ml of diluent phosphate buffered saline (pH 7.0 -7.4) measured by the Coulter Dual Dilutor 111 device. The six drops of potassium cyanide (lytic substance) were added to each sample and mixed to allow for haemolysis. The

haemoglobin was then measured using a spectrophotometer (Cecil Instruments Limited-Cambridge, UK) at a wavelength of 540 nm. Two aliquots were taken from each sample and three readings were made for each aliquot. Two aliquots were taken from each sample and three readings were made for each aliquot. The spectrophotometer was standardized by checking with commercially available cyanmethaemoglobin specimen using a calibration curve. The following formula was used for calibration:

$$\text{Cyanmethaemoglobin Haemoglobin} = \frac{\text{Reading of the test X Haemoglobin of the standard}}{\text{Reading of the standard}}$$

4.2.6 Packed cell volume measurement

The packed cell volume was determined to establish the correlation between the Hb concentration and the PCV. Three capillary tubes were filled from each blood sample, with each tube being filled to about 5 mm from the top of the tube. The tubes were then sealed at one end with Cristaseal (Hawksley, England), placed in the micro-haematocrit centrifuge and spun for 5 min at 13,000g, after which, the PCV was read using the micro-haematocrit reader (Hawksley, England).

4.2.7 Use of hand-held haemoglobinometers by field veterinarians with little or no laboratory experience

To determine whether veterinary personnel with little or no laboratory experience can successfully use the haemoglobinometers, three individuals with varying field experience in clinical diagnosis of bovine parasitic diseases in Uganda, were given brief instructions and asked to make two measurements of haemoglobin concentration on each of eight bovine blood samples using the HemoCue and Haemoglobin Colour Scale methods. The DHT method could not be included due to malfunction. Bovine blood samples used were collected from village cattle in Tororo district of Uganda.

4.2.8 Data analysis

For the comparison of haemoglobin measurements using different methods, a form was designed to accommodate three replicate values of haemoglobin concentration for each sample. Data was analysed using the computer packages Microsoft Excel and SAS/Graph software (SAS institute Inc., Cary, NC, USA, 1988). The precision of each method was determined based on the mean difference and the coefficient of variation for replicate samples. The bias was calculated as the mean difference between the readings of the various methods and those of the reference method. The accuracy was calculated as the standard deviation of the differences between the two methods. The linearity of the methods was checked over a haemoglobin concentration range of 0-17 g/dl using regression analysis. Assessment of agreement between the various methods with laboratory reference method was done using Bland-Altman plots (Bland and Altman, 1986; Petrie and Watson, 1999; Paddle, 2002). Analysis of variance was undertaken to assess the overall variance due to imprecision in pipetting and reading.

Sensitivity and specificity were calculated based on the 65 samples using the following formulae:

$$\text{Sensitivity} = \frac{\text{No. of samples detected with Hb} < 8 \text{ g/dl by a method}}{\text{Total samples with Hb} < 8 \text{ g/dl detected by the reference method}} \times 100$$

$$\text{Specificity} = \frac{\text{No. of samples detected with Hb} > 8 \text{ g/dl by a method}}{\text{Total samples with Hb} > 8 \text{ g/dl detected by the reference method}} \times 100$$

The correlation of PCV to Hb was also undertaken using regression analysis. Precision obtained using the Haemoglobin Colour Scale and the HemoCue by field veterinarians was compared.

4.3 Results

Table 4.1 shows the precision of the different methods. The Haemoglobin Colour Scale and HemoCue methods had the best precision, having achieved coefficients of variation (CV) ranging from 2.9 to 8.8% and 3.5 to 10.6%, respectively as compared to the DHT-haemoglobinometer, which had a CV ranging from 7.7 to 23%.

Figures 4.1, 4.2 and 4.3 show the accuracy, bias and linearity of the haemoglobinometers: HCS, HCU and DHT, respectively, in comparison to the reference method. There was a good linearity between the readings of the portable haemoglobinometers: HCS ($R = 0.925$), HCU ($R = 0.920$) and DHT ($R = 0.906$) and those of the reference method (CMH).

Figures 4.4, 4.5 and 4.6 are Bland-Atman plots depicting the agreement between the hand-held haemoglobinometers and the reference method. The mean difference ± 1.96 standard deviation are indicated. The limits of agreements, showing the ranges between which 95% of the results lie, were 2.8 g/dl below and 2.6 g/dl above the reference value for the HCS, 3.9 g/dl below and 2.9 g/dl above for the HCU method, and 7.0 g/dl below and 0.6 g/dl above for the DHT. The DHT had a poorer agreement with the reference method as compared to the other two haemoglobinometers: HCS and HCU.

The proximity of the haemoglobin measurements to the reference results was assessed by determining the proportion of results of the portable haemoglobinometers that lay within 1 g/dl, 1.1–2.0 g/dl and >3.0 g/dl of the reference (Table 4.2). A substantial proportion of the of the results of the HCS (49.2%) and HCU (46.1%) lay within 1 g/dl of the reference value, but a small proportion of the results of the DHT (9.2%) lay within the same range. However, a high proportion of the results of the HCS (87.7%) and HCU (76.9%) lay within 2 g/dl of the

reference value. One-third (32.3%) of the results of the DHT method lay within 2 g/dl of the reference value.

Table 4.2 shows the proportion of variance attributed to various sources of imprecision: diluting and reading of samples. Much of the variance in the measurements of the portable haemoglobinometers: HCS (100%), HCU (97.4%) and DHT (98%), was attributed to diluting.

The sensitivity and specificity of the different hand-held haemoglobinometers in the detection of anaemia are shown in Table 4.4. The sensitivities of the HCS (94%) and HCU (80.5%) were high but that of the DHT was low (52.7%). All the haemoglobinometers had very high specificity: HCS (93%), HCU (96.5%) and DHT (100%).

The correlation between PCV values and Hb measurements obtained using the HCS, HCU, CMH and DHT are shown in Figure 4.7. There was a high correlation between the PCV values and Hb measurements obtained using all methods: HCS (R = 0.974), HCU (R = 0.965), CMH (R = 0.943) and DHT (R = 0.934).

Tables 4.5 and 4.6 show the precision of Hb measurement obtained by field veterinarians in Uganda using the HCS and HCU, respectively. Field veterinarians achieved good precision (8 – 13%) with the HCS and the HCU (1%).

Table 4.7 shows the cost associated with the different hand-held haemoglobinometers evaluated. The HCS was the cheapest method, followed by the DHT and the HCU. The cost of the kit and reagents for analysis of 1000 samples would amount to US$ 22 (€ 19.50) for the HCS, US$ 600 (€ 532) for the DHT and US$ 1100 (€ 975) for the HCU.

Table 4.1: Comparison of the precision of different haemoglobin measurement methods

	CMI	DHT	HCS	HCU
Hb (g/dl)	Coefficient of variation (CV)			
4	10.1 %	23.0 %	8.8 %	10.6 %
6	6.7 %	15.3 %	5.8 %	7.1 %
8	5.0 %	11.5 %	4.4 %	5.3 %
10	4.0 %	9.2 %	3.5 %	4.3 %
12	3.4 %	7.7 %	2.9 %	3.5 %

CMH - Cyanmethaemoglobin method
DHT - Development Health Technology-haemoglobinometer
HCS – WHO Haemoglobin Colour Scale
HCU - HemoCue

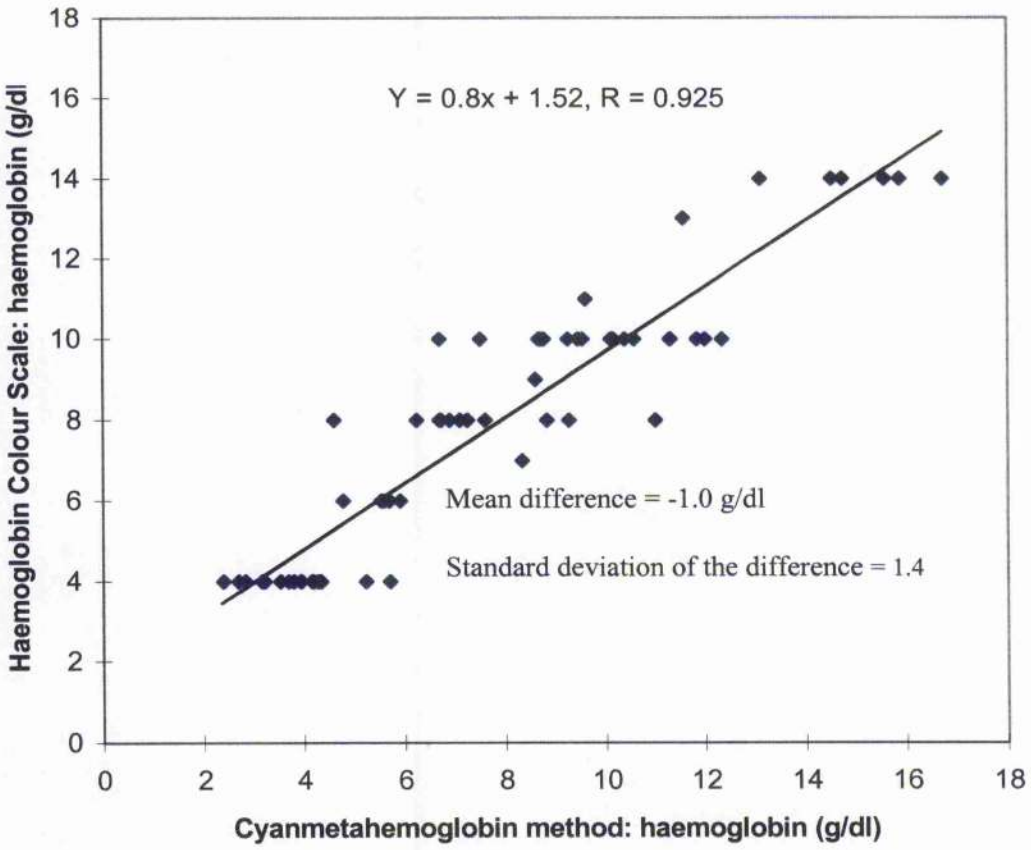


Figure 4.1: Comparison of haemoglobin measurements by the Haemoglobin Colour Scale and the reference method

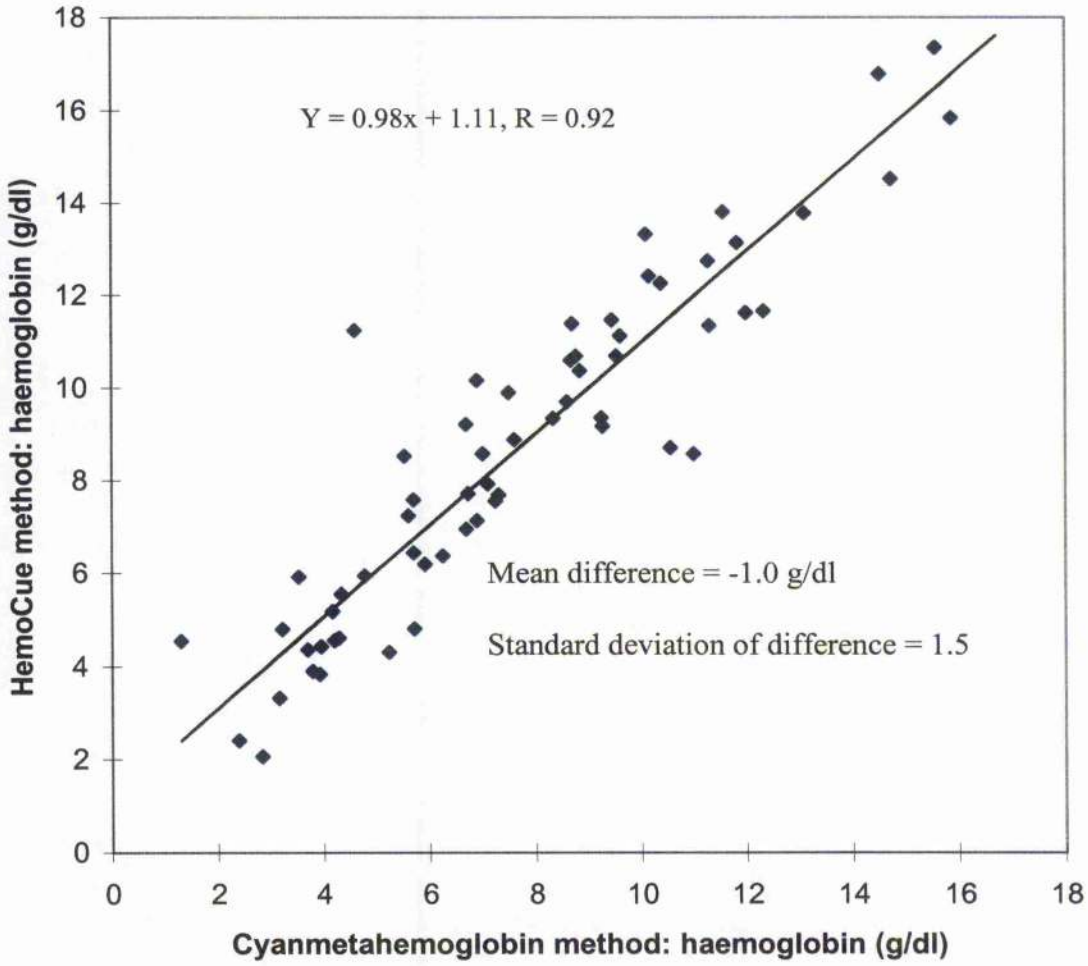


Figure 4.2: Comparison of haemoglobin measurements by the HemoCue and the reference method

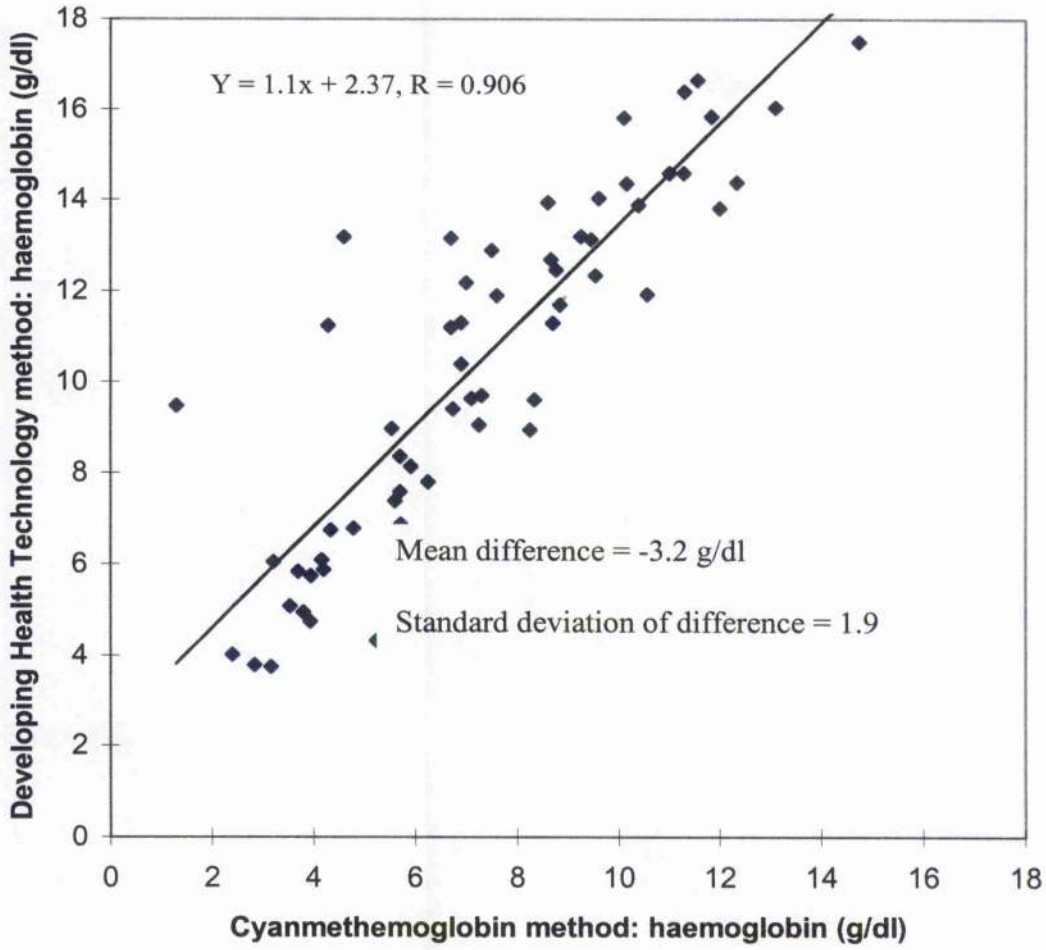


Figure 4.3: Comparison of haemoglobin measurements by the DHT-haemoglobinometer and the reference method

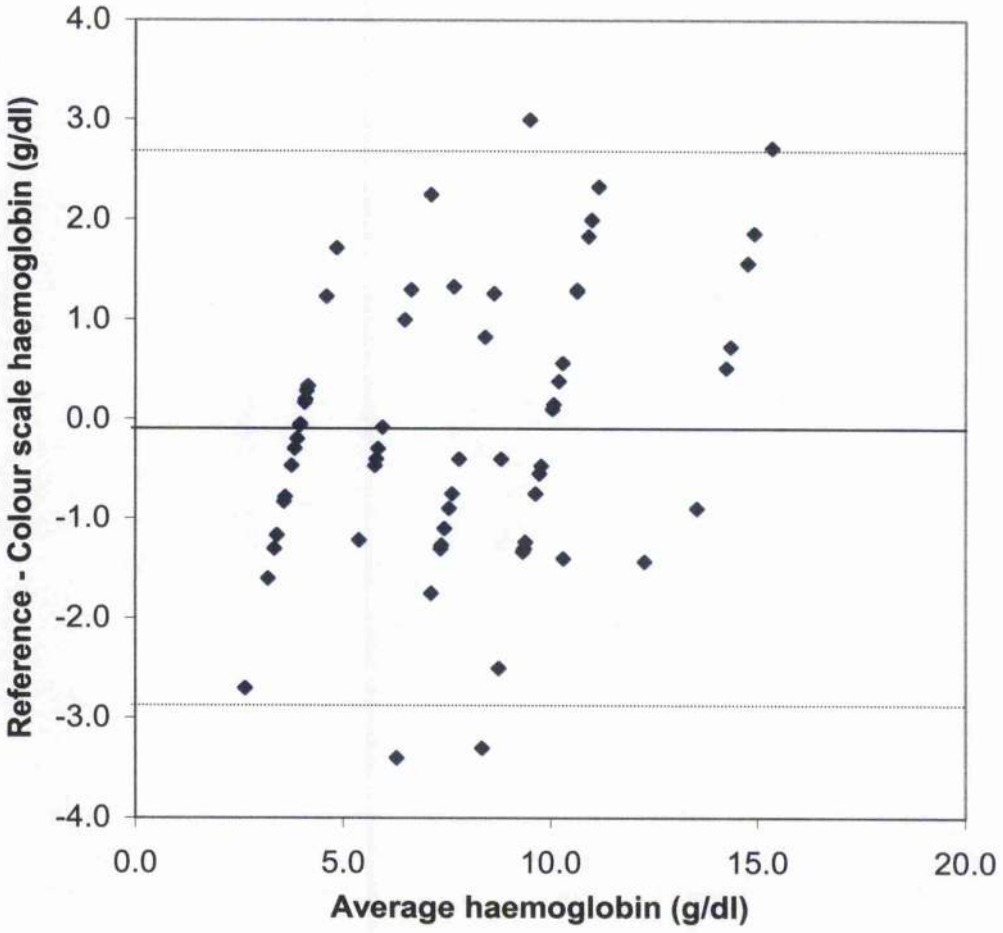


Figure 4.4: Bland-Altman plot depicting the agreement between the Haemoglobin Colour Scale and the reference method. Mean difference = -0.1, Upper limit of agreement = 2.6 and lower limits of agreement = -2.8

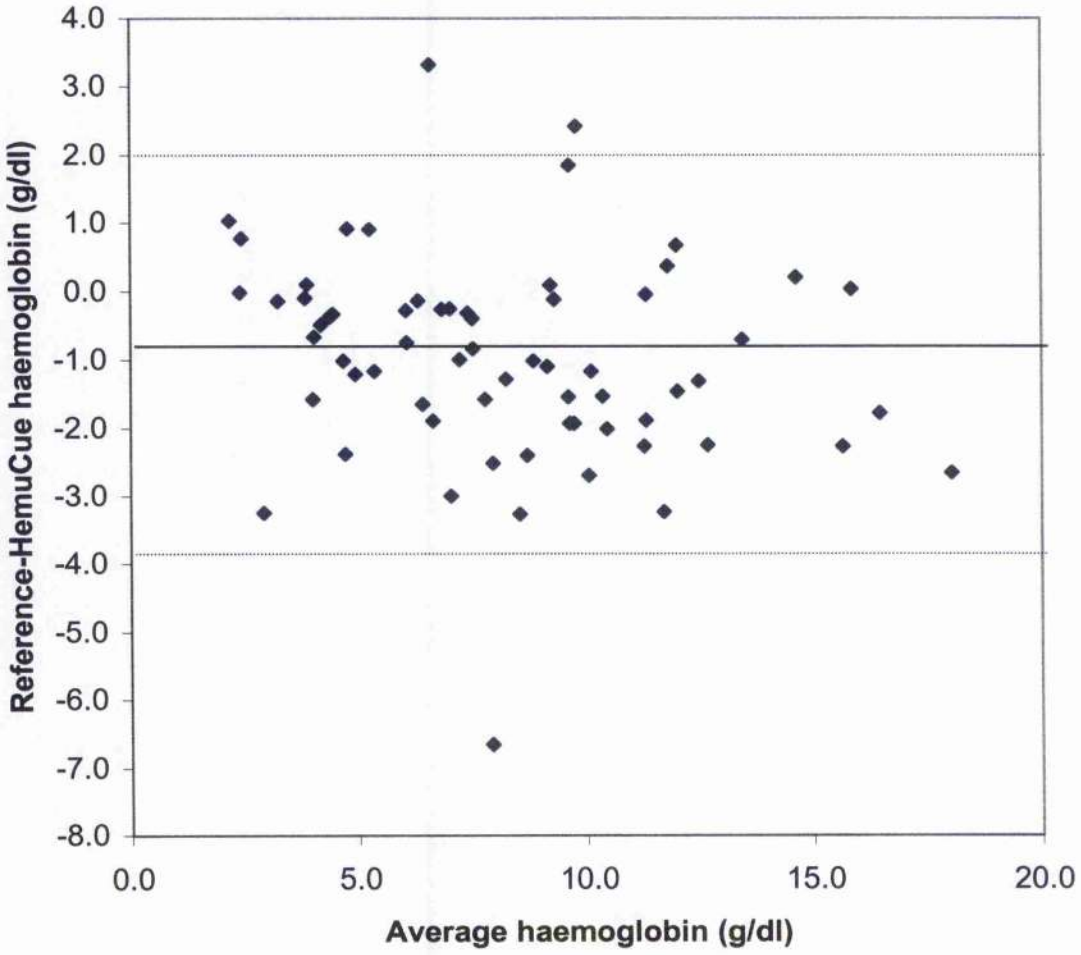


Figure 4.5: Bland-Altman plot depicting the agreement between the HemoCue and the reference method. Mean difference = -0.9, Upper limit of agreement = 2.0 and lower limits of agreement = -3.9

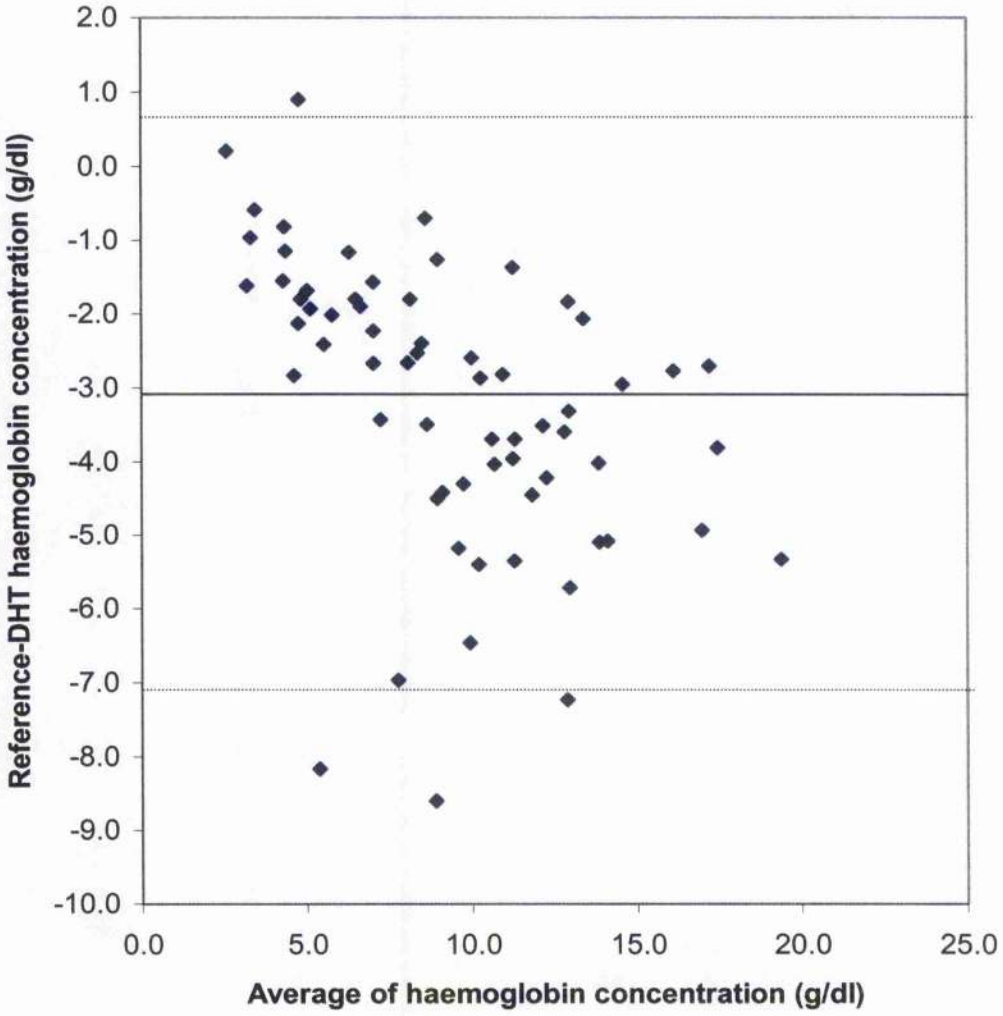


Figure 4.6: Bland-Altman plot depicting the agreement between the DHT-haemoglobinometer and the reference method. Mean difference = -3.2, Upper limit of agreement = 0.6 and lower limits of agreement = -7.0

Table 4.2: Proximity of results of the Haemoglobin Colour Scale, HemoCue and DHT-haemoglobinometer to the reference method

	Proximity of results to the reference method			
	± 1	1.1 – 2.0	2.2 – 3.0	>3.0
HCS	32 (49.2)[a]	25 (38.5)	6 (9.2)	2 (3.1)
HCU	30 (46.1)	20 (30.8)	10 (15.4)	5 (7.7)
DHT	6 (9.2)	15 (23.10	15 (23.1)	29 (44.1)

a – Figures in parentheses are percentages

CMH - Cyanmethaemoglobin method
DHT – Development Health Technology-haemoglobinometer
HCS – WHO Haemoglobin Colour Scale
HCU - HemoCue

Table 4.3: Precision of haemoglobin measurement: proportion of variance attributed to various sources of imprecision

	Variance components		
Hb measurement method	Diluting	Reading	Total
CMH	82.4 %	17.6 %	100 %
DHT	98.0 %	2.0 %	100 %
HCS	100 %	0.0 %	100 %
HCU	97.4 %	2.6 %	100 %

Table 4.4: Sensitivity and specificity of different haemoglobin measurement methods for detection of anaemia

	Sample status		Test performance	
Method	Samples with Hb< 8 (n = 36)	Samples with Hb > 8 (n = 29)	Sensitivity (%)	Specificity (%)
HCS	34	27	94	93
HCU	29	28	80.5	96.5
DHT	19	29	52.7	100

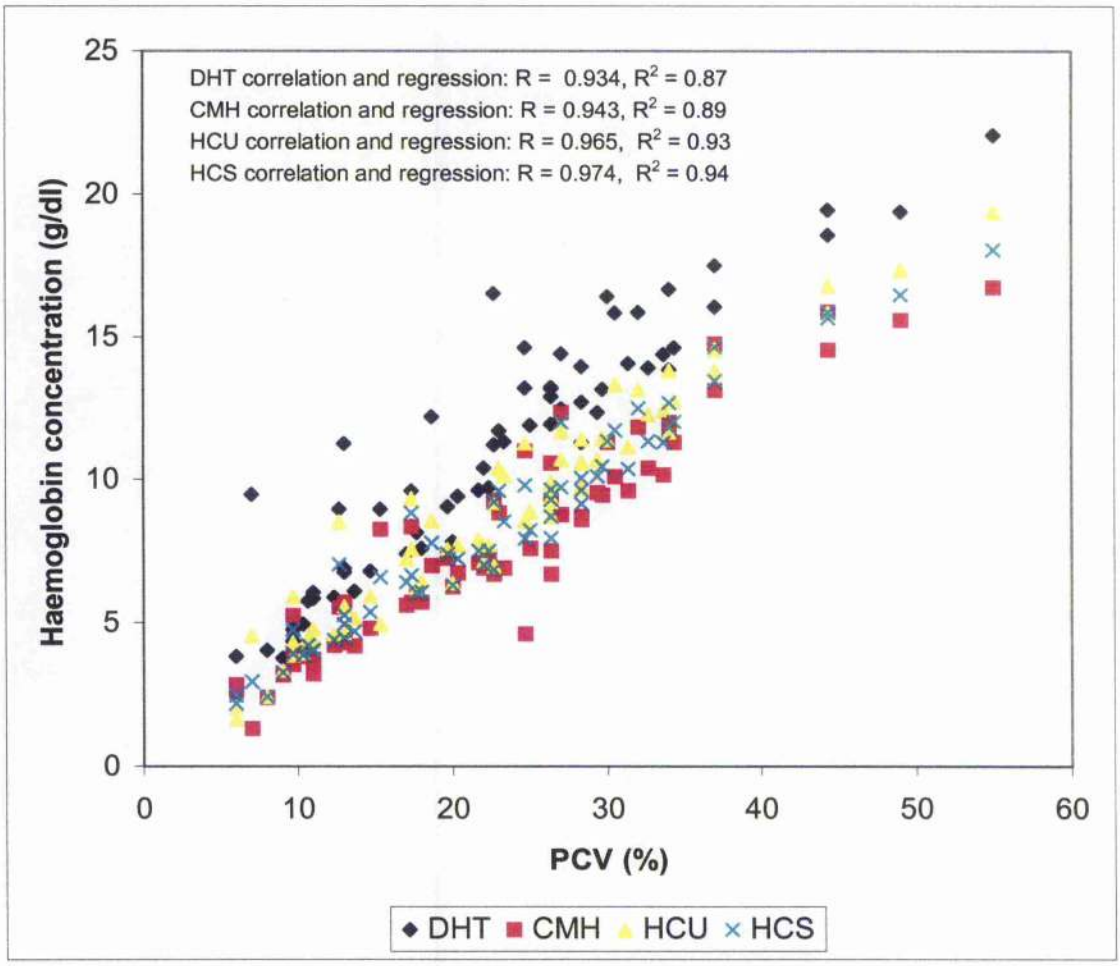


Figure 4.7: Correlation between PCV values and Hb measurements obtained using different methods.

Table 4.5: Precision obtained by field veterinarians in Uganda while measuring haemoglobin of cattle blood using the Haemoglobin Colour Scale

Individual 1			**Individual 2**			**Individual 3**		
Run no. 1	**Run no. 2**	**Diff.**	**Run no. 1**	**Run no. 2**	**Diff.**	**Run no. 1**	**Run no. 2**	**Diff.**
(g/dl)	**(g/dl)**	**(g/dl)**	**(g/dl)**	**(g/dl)**	**(g/dl)**	**(g/dl)**	**(g/dl)**	**(g/dl)**
8	7	-1	6	8	2	8	8	0
8	8	0	8	6	-2	10	10	0
7	6	-1	8	8	0	10	10	0
10	11	1	10	10	0	12	12	0
10	11	1	8	8	0	10	10	0
10	8	-2	10	10	0	12	10	-2
7	7	0	8	8	0	8	8	0
8	7	-1	10	10	0	10	10	0
Mean difference		-0.375			0.05			-0.25
Precision		0.09			0.13			0.08

Table 4.6: Precision obtained by field veterinarians in Uganda while measuring haemoglobin of cattle blood using the HemoCue

Individual 1			Individual 2			Individual 3		
Run no. 1 (g/dl)	Run no. 2 (g/dl)	Diff. (g/dl)	Run no. 1 (g/dl)	Run no. 2 (g/dl)	Diff. (g/dl)	Run no. 1 (g/dl)	Run no. 2 (g/dl)	Diff. (g/dl)
6.8	6.6	-0.2	7.1	7.5	0.4	7.7	7.5	-0.2
9.0	8.9	-0.1	9.6	9.8	0.2	9.9	9.8	0.1
10.6	10.7	0.1	11.5	11.5	0.0	11.6	11.6	0.0
13.0	13.0	0.0	13.7	13.8	0.1	13.6	13.9	0.3
10.3	10.3	0.0	11.2	11.4	0.2	11.5	11.5	0.0
9.7	9.7	0.0	10.5	10.6	0.1	10.7	10.6	-0.1
4.8	4.8	0.0	5.4	5.5	0.1	5.5	5.5	0.0
7.8	8.0	0.2	8.6	8.7	0.1	8.6	8.7	0.1
Mean difference		0.0			0.15			0.0
Precision		0.01			0.01			0.01

Table 4.7: Comparison of costs of portable haemoglobinometers under consideration

	Portable haemoglobinometers		
Costs	**HCS**	**HCU**	**DHT**
Cost of Photometer/Kit	US$ 22 (€ 19.50) [a]	US$ 600 (€ 532)	US$ 600 (€ 532)
Extra running costs per 1000 samples	Nil	US$ 500 (€ 443) [b]	US$ 0.02 (€ 0.017) [c]

a- Price of a kit (Starter Kit ®, COPACK GmbH, Germany) comprised of one Cover-Box with one dispenser containing 200 test-strips, one Colour Scale booklet, an Instruction Manual and extra 4 refill dispensers each containing 200 test strips (giving a total of 1000 test stripes).

b- Each disposable microcuvette costs USD 0.5 (Source: HemoCue, AB, Ängelholm, Sweden).

c- The cost of 0.04 % ammonium solution used for haemolysis of erythrocytes. This haemoglobinometer is produced and sold by Developing Health Technology, Company, UK.

d- Prices quoted are as per December 2002.

4.4 Discussion

In the present study, portable haemoglobinometers used in human medicine, were evaluated for their suitability in measuring haemoglobin of bovine blood under field and laboratory conditions. Although standard laboratory techniques for measuring haemoglobin have similar chemical principles and may apply to all species, instruments that are designed for use on human blood samples and involve a physical principle to measure haematocrit and calculate haemoglobin concentration are likely not to provide accurate results in other species because of the large variation of erythrocyte size among species (Callan *et al.*, 1992). Hence it was necessary to conduct an evaluation of the haemoglobinometers using animal blood samples to assess their usefulness in veterinary medicine.

The Haemoglobin Colour Scale and HemoCue method had better precision than the DHT-haemoglobinometer. The poor precision of the DHT-haemoglobinometer could probably be attributed to high sample dilution, which is known to affect haemoglobinometry (von Schrenk *et al.*, 1986). This step is avoided by the Haemoglobin Colour Scale and HemoCue methods.

There are few reports on evaluation of methods for measurement of haemoglobin concentration in human blood on their accuracy on animal blood samples. However, the correlation coefficient (R = 0.920) obtained between the HemoCue method and the reference (cyanmethaemoglobin) method in this study is comparable to a correlation coefficient of R = 0.946 reported by Callan *et al.* (1992). There was good linear relationship between the readings of the hand-held haemoglobinometers and the reference method. In terms of accuracy and bias, the Haemoglobin Colour Scale had the best results, followed by the HemoCue and the DHT-haemoglobinometer. The accuracy values of 1.5% and 1.9% achieved with cattle blood in this study were similar to 1.5% and 2.0% obtained for human blood by the manufacturers of the HemoCue and DHT-haemoglobinometer, respectively.

Despite the good linear relationship between the readings of the different haemoglobinometers and the reference method revealed by regression analysis, regression analysis is considered to be inappropriate for assessing agreement between different methods of clinical measurement (Bland and Altman, 1986; Paddle, 2002). High correlation does not necessary imply good agreement. Indeed poor agreement between results of the DHT-haemoglobinometer and the reference method was revealed by the Bland-Altman plots, despite high correlation between the results of the two methods. There was a better agreement between the Haemoglobin Colour Scale and the HemoCue method and the reference method than between the DHT-haemoglobinometer and the reference method. With limits of agreement of 2.8 g/dl below and 2.6 g/dl above the reference value for the Haemoglobin Colour Scale, 3.9 g/dl below and 2.9 g/dl above for the HemoCue method, and 7.0 g/dl below and 0.6 g/dl above for the DHT-haemoglobinometer, it was evident that the HemoCue slightly overestimates while the DHT-haemoglobinometer grossly overestimates haemoglobin readings.

Fifty per cent of the results of the Haemoglobin Colour Scale and HemoCue method and less than 10% of those of DHT-haemoglobinometer that lay within 1 g/dl of the reference value could be considered to be quite accurate, but those outside this range would be progressively more inaccurate (Paddle, 2002). Given that 87.7% and 76.9% of the results of the Haemoglobin Colour Scale and the HemoCue, respectively, lay within 2 g/dl of the reference value as compared to 32.5 % for the DHT-haemoglobinometer, the former methods were considered to be more accurate than the latter. The agreement between the Haemoglobin Colour Scale and the reference method obtained in this study was better than 40% within 1 g/dl and 67 % within 2 g/dl reported by van den Broek (1999). However, the agreement between the HemoCue and the reference method achieved in this study was much lower than 95 % within 1 g/dl and 5 % within 2 g/dl reported by Paddle (2002).

Much of the variance in the measurements of portable haemoglobinometers under consideration was attributed to the diluting step, which was the most important source of imprecision. In other studies, sample dilution has also been considered to be one of the steps that reduce the precision of haemoglobinometry and are difficult for untrained personnel to perform (von Schrenk *et al.*, 1986).

The Haemoglobin Colour Scale and HemoCue method had higher sensitivity than the DHT-haemoglobinometer, but all the methods had high specificity for detection of anaemia i.e. haemoglobin concentration below 8 g/dl. The sensitivity and specificity of the Haemoglobin Colour Scale and HemoCue achieved in this study are comparable to existing reports (Neville, 1987; van den Broek, 1999). Literature on previous studies examining the sensitivity and specificity of these methods is scarce, however studies based on human blood have reported sensitivity and specificity of 81% and 76% for the Haemoglobin Colour Scale and 96% and 94% for the HemoCue method, respectively (van den Broek, 1999). Other studies have reported sensitivity and specificity of 88.5% and 77.6% for the HemoCue method (Neville, 1987). The Haemoglobin Colour Scale and HemoCue gave acceptable sensitivity and specificity, but the DHT-haemoglobinometer gave a low sensitivity despite its high specificity (100%). The high specificity of the DHT-haemoglobinometer could be attributed to its property of overestimation of the haemoglobin concentration, hence giving values higher than 8 g/dl in most cases.

There was a highly significant correlation between the PCV values and Hb measurements obtained using all methods. Since the PCV has a direct relationship with the Hb concentration in blood, these results were expected, but it should be noted that the Haemoglobin Colour Scale had the highest correlation coefficient, followed by the HemoCue method, cyanmethaemoglobin method and the DHT-haemoglobinometer. Thus it is clear that PCV

determination often used in veterinary medicine in the detection of anaemia could be substituted with the measurement of Hb concentration.

Field veterinarians achieved good precision with the Haemoglobin Colour Scale and HemoCue method. The tests performed by veterinary field extension staff demonstrated that personnel with little or no laboratory experience could successfully use these haemoglobinometers. This also emphasizes the importance of allowing end-users to evaluate such equipment intended for field use within the local setting, a practice that is in line with a recommendation made by others (Neville, 1987; Gong and Backenstose, 1999; van den Broek, 1999).

The cost of purchasing and running the haemoglobinometers intended for veterinarians practising in developing countries is an important consideration. The Haemoglobin Colour Scale is the cheapest to run. The cost of the HemoCue is the same as that of the DHT-haemoglobinometer, but the extra cost for sample analysis associated with the HemoCue is higher than that of the DHT due to the price of disposable microcuvettes (US$ 0.5 (€ 0.44) per cuvette). The extra cost for the DHT-haemoglobinometer is low; US$ 0.02 (€ 0.017) per 1000 samples for purchasing the ammonium solution. It has been suggested that manufacturers should aim to produce low-cost reusable microcuvettes for the HemoCue or reduce the cost of the disposable microcuvettes to US$ 0.03 (€ 0.026) each, in order to make this method affordable to developing countries (Johns and Lewis, 1989).

Overall, the Haemoglobin Colour Scale is portable, cheap, easy to use, precise and accurate when instructions are followed, but has the element of subjective judgement of results and might be difficult to use by people with colour blindness. On the other hand, the HemoCue is portable, easy to use, has no inter-observer error, precise and accurate, but is expensive. Field experience in Uganda has revealed that the HemoCue photometer is sensitive to temperature

above 30°C (*Anderson and Magona, personal communication*). This could be partly attributed to the hygroscopic nature of the HemoCue cuvettes in that once the sealed container is opened, the cuvettes react to the high humidity in hot and humid climates in the tropics (Sari *et al.*, 2001). The DHT-haemoglobinometer is portable, cheap, simple, but is imprecise and less accurate due to the dilution step involved.

In conclusion, the HemoCue and DHT-haemoglobinometers could be utilized in veterinary medicine in developing countries by both private and government veterinarians in central or district diagnostic laboratories because of the high cost involved, while the Haemoglobin Colour Scale is suitable for use by field veterinarians, animal health workers and community health workers under field conditions due to its low cost and simplicity. Based on the performance and the cost of these methods, the best choice of method would be the Haemoglobin Colour Scale, followed by the HemoCue and the DHT-haemoglobinometer. It is also anticipated that the cost of the HemoCue microcuvettes will decrease with the advent of the application of the HemoCue in both veterinary and human medicine.

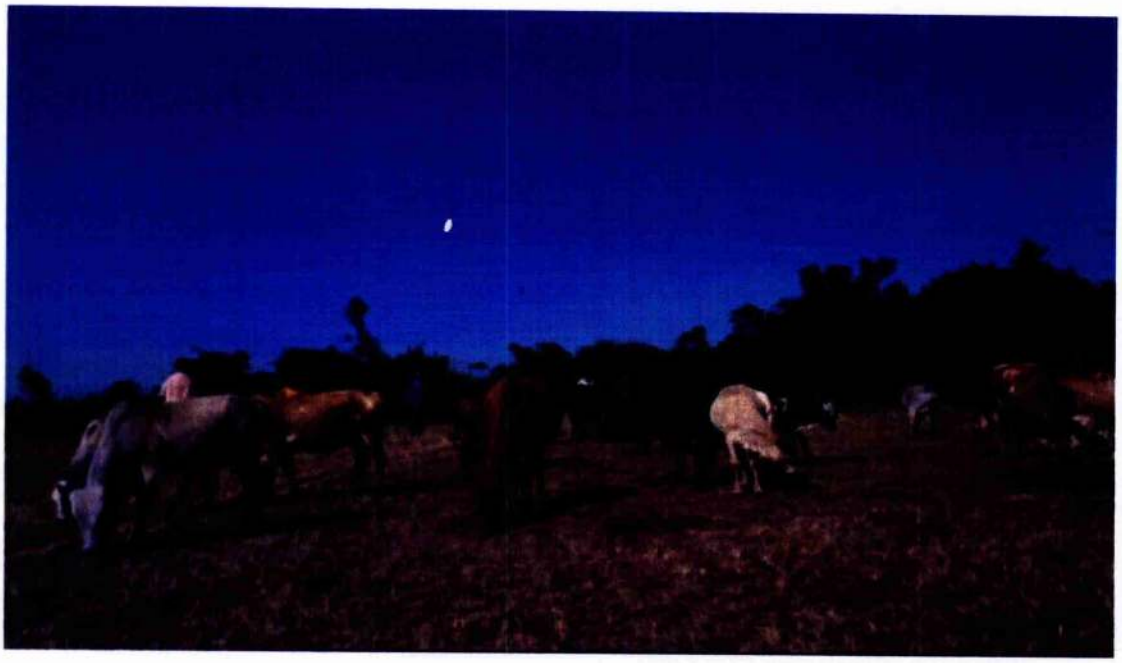


Figure 5.1: A herd of Zebu cattle in Uganda - different individuals have different coat colours. Under tropical conditions, time of the day and coat colour have significant influence on rectal temperature-a surrogate measure for pyrexia. Pyrexia is a characteristic clinical feature of trypanosomosis, East Coast fever, anaplasmosis, babesiosis and cowdriosis.

Chapter 5 Diurnal variations of rectal temperature and sensitivity of parasitological diagnostic tests for trypanosomosis

5.1 Influence of time of the day and coat colour of Zebu cattle on diagnostic value of rectal temperature

5.1.1 Introduction

Pyrexia is a common clinical feature associated with endemic bovine diseases in sub-Saharan Africa such as trypanosomosis, theileriosis, anaplasmosis, babesiosis and cowdriosis. Bovine trypanosomosis is characterized by intermittent fever, which is prominent during the early phases of the disease when the waves of parasitaemia are particularly high (Fiennes, 1970; Holmes *et al.*, 2000). In acute *T. vivax* infections, the body temperature rises up to 40-41°C, while in *T. congolense* infections, the body temperature rises up to 39.4-40°C and pyrexia parallels the parasitaemia, which is usually lower than in *T. vivax* infections (Stephen, 1986). Theileriosis is characterized by pyrexia and in confirmed field cases, the body temperature rises up to 40.1-40.2°C (Omuse, 1978). Pyrexia is also reported to be one of the main clinical signs both in field (Egbe-Nwiyi *et al.*, 1997) and experimental cases (Ajayi *et al.*, 1987) of anaplasmosis. The acute syndrome of babesiosis in cattle is characterized by fever and body temperature usually rises up to 41-45.5°C (Losos, 1986). Cowdriosis in cattle is characterized by pyrexia, with a temperature of 40-42°C lasting one week before dropping to subnormal level before death (Cowdry, 1926).

In veterinary medicine, detection of pyrexia relies on the measurement of rectal temperature. Field veterinarians often measure rectal temperature to detect pyrexia whenever they come across reported cases, regardless of the time of the day. With regards to indigenous cattle breeds in sub-Saharan Africa, there is a wide variation in coat colour between and within

breeds (Felius, 1985). Heat absorption or reflection by the bovine skin differs according to the coat colour (Hansen, 1990).

The usefulness of rectal temperature as a diagnostic feature for endemic bovine diseases may be curtailed by its variations due to factors such as coat colour and diurnal changes in ambient temperature. In view of the above, a study was conducted to assess whether time of the day and coat colour of Zebu cattle influence rectal temperature under tropical conditions.

5.1.2 Materials and methods

Twenty Zebu heifers, aged between 12-18 months, consisting of 10 healthy and 10 unhealthy were selected at LIRI, Tororo, Uganda. The entire group of 20 consisted of four animals with a brown coat, six with a light brown coat, eight with a black coat, one with a grey coat and one with black coat with white spots. Cattle were designated unhealthy based on clinical examination, clinical history and laboratory examination of blood for trypanosomosis and tick-borne diseases. Five serial observations on rectal temperature of 20 animals were made at 05.00 hours, 09.00 hours, 13.00 hours, 17.00 hours and 21.00 hours of the day. The times of days were arbitrarily chosen to cover the entire length of the day. Observations were repeated on 4 occasions a week apart. The influence of the time of the day and coat colour on rectal temperature was assessed using multivariate analysis (ANOVA) with the computer programme Minitab (Minitab Statistical Software Release 13.1, Minitab Inc., Pennsylvania, U.S.A).

5.1.3 Results

Table 5.1 shows the factors that had a significant influence on rectal temperature. The time of the day and coat colour of cattle had a highly significant influence ($P< 0.001$) on rectal temperature.

Figure 5.2 shows the diurnal variation of rectal temperature of a group of healthy cattle and unhealthy ones. For both groups of cattle, the mean rectal temperature rose steadily and reached

a peak at 17.00 hours, and then it declined. It was noted that the mean rectal temperature of unhealthy cattle was significantly higher ($P < 0.05$) than that of the healthy ones from 13.00 to 17.00 hours, but not significant during other times of the day.

Figure 5.3 shows the diurnal variation of rectal temperature of cattle of different coat colours. All cattle independent of the coat colour experienced diurnal variation of the rectal temperature. Grey coat colour was associated with the highest mean temperature, followed by light brown, pure black, pure brown and black spotted white coats.

Results of one-way analysis of variants of rectal temperature of cattle to assess the influence of various coat colours are shown in Table 5.2. The results suggest the influence of different coat colours on rectal temperature was significantly ($P < 0.05$) different. The mean rectal temperature for cattle of different coat colours is further elucidated in Table 5.3. Cattle with a grey coat colour had the highest mean rectal temperature, which was significantly higher than that of cattle with black spotted white coat ($P < 0.05$), but not significantly higher than mean rectal temperature for cattle with light brown, pure black and pure brown coats.

Table 5.1: Multivariate analysis of rectal temperature of cattle (n = 20) to assess the influence of time of the day, coat colour and health status

Source	DF	Seq SS	Adj SS	Adj MS	F	P-value
Time of the day	4	170.769	170.769	42.692	188.66	<0.001
Coat colour	4	8.946	8.626	2.156	9.53	<0.001
Healthy	1	0.027	0.027	0.027	0.12	0.728
Error	390	88.255	88.255	0.226		
Total	399	267.997				

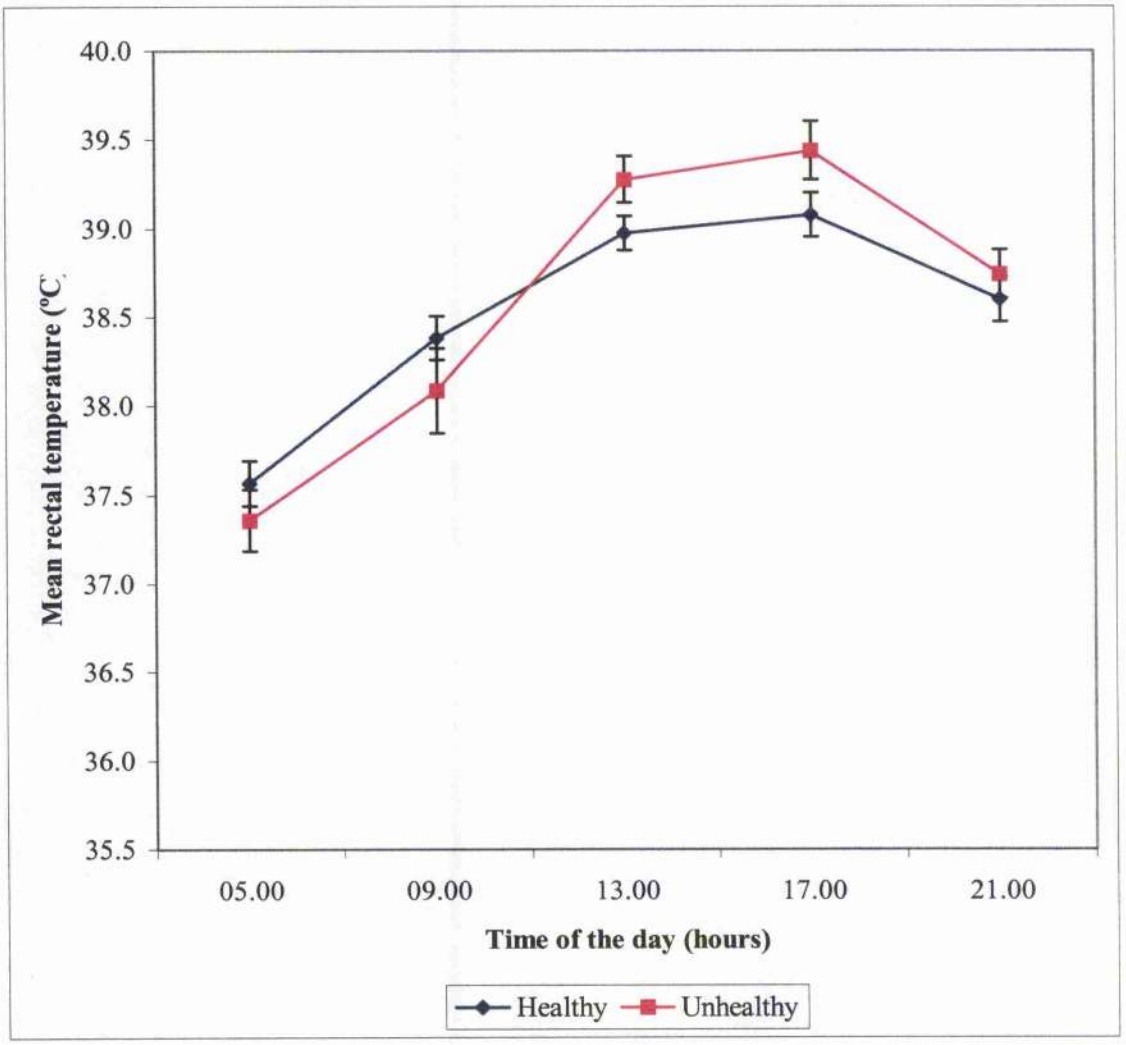


Figure 5.2: Diurnal variation of the mean rectal temperature (± 95% CI) of healthy and unhealthy Zebu cattle

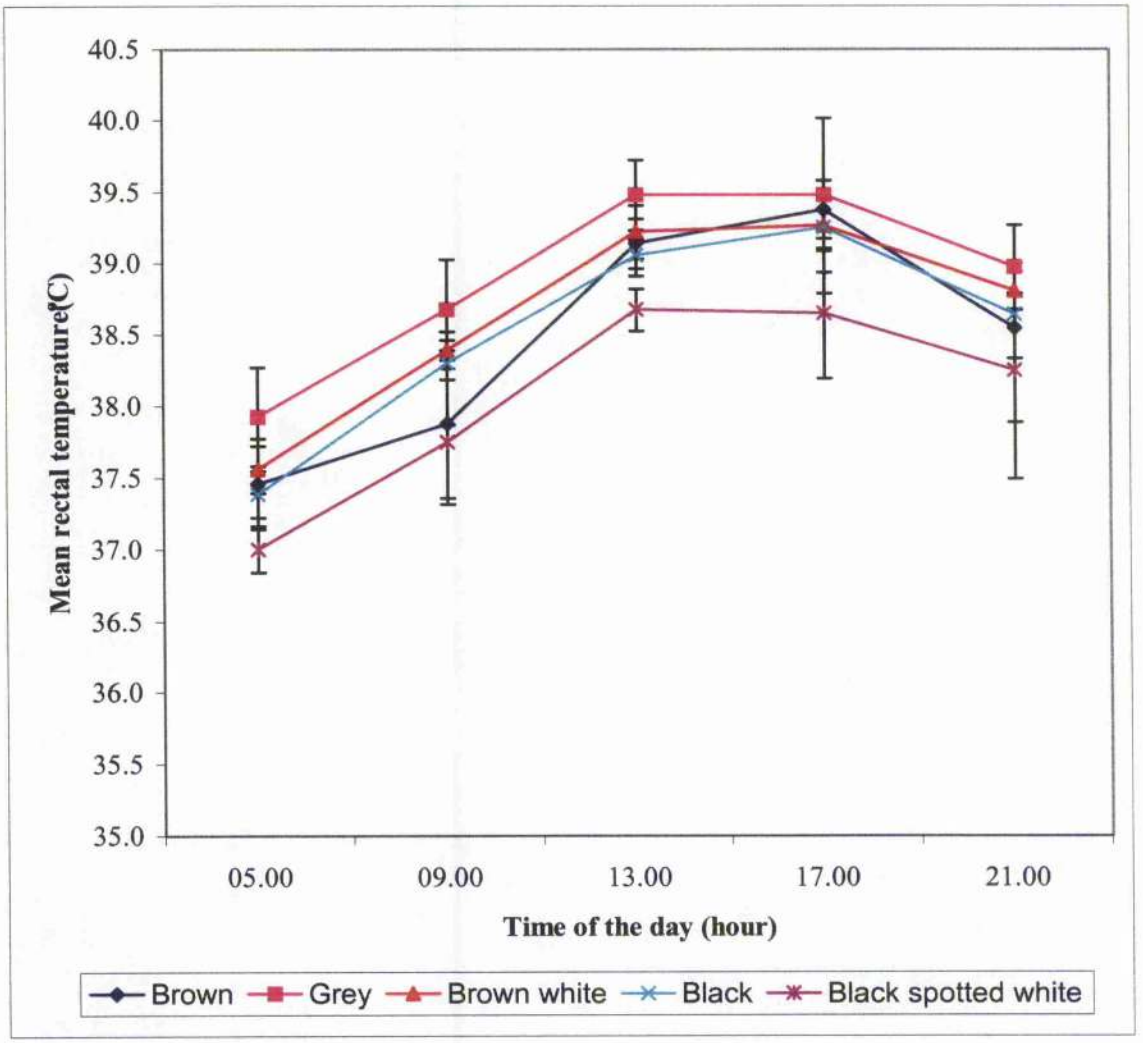


Figure 5.3: Diurnal variation of the mean rectal temperature (± 95% CI) of Zebu cattle of different coat colours

Table 5.2: One-way analysis of variants of rectal temperature of Zebu cattle to assess the influence of various coat colours

Source	DF	SS	MS	F	P
Coat colour	4	8.946	2.237	3.41	<0.05
Error	395	259.051	0.656		
Total	399	267.998			

Table 5.3: Differences in mean rectal temperature of Zebu cattle of different coat colours

				Individual 95% CIs For Mean Based on Pooled StDev
Coat colour	N	Mean	StDev	------+---------+---------+---------+
Brown	80	38.479	0.959	(---*--)
Grey	20	38.905	0.680	(-------*------)
Brown/white	130	38.648	0.752	(--*--)
Black	150	38.527	0.792	(--*-)
Black spotted white	20	38.065	0.765	(------*------)
				------+---------+---------+---------+
Pooled StDev = 0.810				38.00 38.50 39.00 39.50

5.1.4 Discussion

In this study, the effect of time of day and coat colour of Zebu cattle under tropical conditions on rectal temperature and its diagnostic value was investigated. The time of the day and coat colour of cattle had a significant influence on rectal temperature. Diurnal variation of rectal temperature was observed in both healthy and unhealthy cattle as reported by others (Greig and McIntyre, 1979; Hammond and Olson, 1994; Toharmat *et al.*, 1999).

No significant difference between the mean rectal temperature of the unhealthy and healthy groups of cattle was observed during much of the day, apart from during the period 13.00 to 17.00 hours. Similar findings were reported in a study conducted in Nigeria in which no difference in rectal temperature was observed between trypanosome infected and non-infected Muturu cattle, a phenomenon that was attributed to the trypanotolerant nature of this breed (Uza *et al.*, 1998). Differences in rectal temperature might not show up between healthy and unhealthy cattle of tropical breeds such as Zebu also because of their high degree of heat tolerance, which has been associated with their short hair coat (Hammond and Olson, 1994).

Diurnal variation of rectal temperature occurred in Zebu cattle with different coat colours. The grey coat was associated with the highest mean temperature, followed by the light brown (brown-white), pure black, pure brown and black spotted white coat. There was a significant difference between coat colours in terms of their influence on rectal temperature, particularly between the grey coat and the black spotted white. It must be recognised that the grey and black spotted white coat groups had only one animal each. Probably having more animals in each coat colour group could have given a more distinct difference between groups in terms of mean rectal temperature. However, there was an indication of variation in heat tolerance as a result of coat colour. The hair coat colour is reported to be a phenotypic trait that affects the ability of cattle to resist effects of heat stress associated with high incident solar radiation and the ability of cattle hair to absorb light varies with colour (Hansen, 1990). Small diurnal changes in rectal

temperature are said to be an indication of heat tolerance in cattle in a hot, humid environment (Okantah *et al.*, 1993). Increase in rectal temperature is reported to be less in white than in black Holstein cows under Subtropical conditions (Hansen, 1990).

These results indicate that it is important to consider diurnal changes in rectal temperature and differences due to the coat colour of tropical breeds when measuring rectal temperature for assessing pyrexia during clinical diagnosis. Other workers have also reported diurnal variation of rectal temperature of cattle in humid tropics (Amakiri and Funsho, 1979). Rectal temperature was highest between 13.00 and 17.00 hours for both healthy and unhealthy groups. However, this was the period when the unhealthy group had the distinctively higher mean rectal temperature than the healthy group. This period was the most suitable time of the day for veterinarians to detect pyrexia, however there was the likelihood of picking healthy cattle (false positives) that have raised rectal temperature. Given that veterinarians are presented with sick rather than healthy animals by farmers, it is unlikely that picking healthy animals would be a problem. Taking into consideration the influence of time of day and the coat colour of cattle on rectal temperature measurements could improve the assessment of pyrexia and detection of clinical disease during clinical diagnosis of endemic diseases.

5.2 Diurnal variation in sensitivity of parasitological diagnostic tests for trypanosomosis

5.2.1 Introduction

Examination of blood by light microscopy is the most readily applied technique for diagnosis of bovine trypanosomosis in the field. This involves several parasitological techniques often referred to as standard trypanosome detection methods (Wilson, 1969). They include (i) thick, thin and wet blood film examination, (ii) subinoculation of susceptible animals and (iii) concentration methods.

Parasitological tests only detect trypanosomes present in the peripheral blood circulation. During the early phase of infection, the trypanosomes can be found in the peripheral circulation in relatively high numbers. However, the numbers diminish with time, becoming undetectable by parasitological tests during the chronic phase of infection (Masake *et al.*, 1995), when the trypanosomes are sequestrated in various predilection tissue sites such as the spleen, liver and lymph nodes (Losos and Ikede, 1972; Masake and Nantulya, 1991). In cattle, there are always several parasitaemic peaks for approximately the first 10 months post-infection before reaching parasitologically undetectable levels (Masake and Nantulya, 1991). The first peak of parasitaemia, which ranges from 5×10^3 to 5×10^4 trypanosomes per ml of blood, is observed in the second week of infection (Masake and Nantulya, 1991).

The sensitivity of parasitological diagnostic techniques is low (Picozzi *et al*, 2002). The microhaematocrit centrifugation technique is thought to miss 80-90 % of the cases of bovine trypanosomosis (Masake and Nantulya, 1991). A number of factors are said to influence the sensitivity of parasitological tests. The actual numbers of trypanosomes found in a unit of blood will vary with the technique employed to demonstrate them, site of blood whether jugular vein or superficial venule blood, time of day, on the temperature of the animal, skill of the operator,

and the degree of physical activity undergone by the animal prior to bleeding (Stephen, 1986). Presence of mixed infection leads to low detection rate of parasitological methods, since in mixed infection the organisms belonging to the different species give rise to different levels of parasitaemia, making it difficult to identify the species that may be present in low numbers (Nantulya *et al.*, 1992). For example, the levels of *T. congolense* parasitaemia tend to be much lower than that seen in *T. vivax* infection and often more quickly decline, but terminally there may be resurgence of parasitaemia (Stephen, 1986). In *T. brucei* infections, detectable parasitaemia develops 4 to 17 days after the tsetse bite and the peak level of parasitaemia of 10^4 to 5 x 10^5 trypanosomes per ml is attained in 2-3 weeks post-infection. Only 16.3% of the *T. brucei* infections in cattle have detectable parasitaemia within 3 months (Masake *et al.*, 1995). For concentration techniques, they must be read immediately blood samples are collected, at least within 4 to 6 hours, otherwise the number of detectable trypanosomes in the sample declines, especially with *T. congolense* infection (Murray *et al.*, 1977).

Despite the low sensitivity, much of the confirmatory diagnosis of bovine trypanosomosis in the field still relies on parasitological diagnosis. As a common practice when collecting blood samples for parasitological examination, field veterinarians bleed animals at any time of the day, without due regard to the influence of the time of the day animals are bled on the sensitivity of the parasitological tests. In view of this, this study was undertaken to investigate whether time of the day blood samples are taken and examined for trypanosomes under tropical conditions affects the detection rate of parasitological diagnostic tests.

5.2.2 Materials and methods

Ten Zebu heifers, aged between 12-18 months exposed to tsetse challenge were bled from the ear vein at 05.00, 09.00, 13.00, 17.00 and 21.00 hours on four occasions at a weekly interval. Samples were examined using the haematocrit centrifugation technique, the Buffy coat technique and Giemsa-stained smears all conducted in parallel. Making of thick and thin blood smears and bleeding were conducted simultaneously. Fresh blood samples were examined

immediately (within 15 to 20 minutes) they were collected. The influence of time of the day and the different days bleeding was undertaken on the detection of trypanosomes were assessed using multivariate analysis (ANOVA) with the computer programme Minitab (Minitab Statistical Software Release 13.1, Minitab Inc., Pennsylvania, U.S.A).

5.2.3 Results

Parasitological examinations detected that 2 out of 10 cattle had trypanosome infection. Table 5.2 shows the results of multivariate analysis to assess the influence of the day and the time of day animals are bled on the sensitivity of parasitological diagnostic tests (HCT, BCT and Giemsa-stained blood smears) for trypanosomosis. Neither factor had any significant influence ($P > 0.05$) on the sensitivity of the diagnostic tests.

Table 5.3 shows the diurnal sensitivity of parasitological tests for trypanosome infections. Trypanosome infections were detected in samples collected in the mornings (05.00 and 09.00 hours) and in the evenings (17.00 and 21.00 hours), but not in afternoons (13.00 hours). However, there was no significant difference ($P > 0.05$) in diurnal sensitivity of parasitological tests for trypanosome infections. Parasites were detected in 5 out 200 samples, giving a detection rate of 2.5% (Table 5.3).

Table 5.4: Multivariate analysis on the influence of the day and time of the day on the sensitivity of parasitological diagnostic techniques for trypanosomosis

Source	DF	Seq SS	Adj SS	Adj MS	F	P-value
Day	3	1.35	1.35	0.45	1.38	0.295
Time of the day	4	0.5	0.5	0.125	0.38	0.816
Error	12	3.9	3.9	0.325		
Total	19	5.75				

Table 5.5: Diurnal variation of the detection rate of parasitological tests for trypanosomosis

		Numbers		
Time of day	Samples taken	*T. congolense* detected	*T. vivax* detected	Total detected
05.00 hr	40	1	0	1
09.00 hr	40	0	1	1
13.00 hr	40	0	0	0
17.00 hr	40	1	0	1
21.00 hr	40	1	1	2
Overall	200	3	2	5

5.2.4 Discussion

Diurnal variations in the sensitivity of parasitological diagnostic test for bovine trypanosomosis were investigated in this study. The fact that the highest detection rate was observed at 21.00 hours and the lowest at 13.00 hours, implied trypanosome numbers in peripheral blood circulations fluctuated according to a 24-hour rhythm. These results are similar to findings of other studies, where the highest numbers of *T. congolense* parasites circulating in rodent blood was observed at 22.00 and the lowest numbers at 12.00 hours (Hawking, 1976).

The cycle in total trypanosome numbers is reported to depend on the migration of trypanosome between the peripheral and deep blood circulation (Hawking, 1976). In the relationship to the changes in rectal temperature of cattle described earlier, the sensitivity of parasitological tests for trypanosome infections is lowest during the period 13.00-17.00 hours, when rectal temperature is highest (see Section 5.1.3). Probably the high body temperature, especially skin temperature that occurs in cattle during the period 13.00-17.00 hours of the day triggers migration of trypanosomes from the peripheral to the deep blood circulation, hence reducing the number of trypanosomes detectable in peripheral blood by parasitological tests.

Although the general trend highlighted the occurrence of diurnal variations in the parasitaemia of trypanosomes, neither of the factors: time of the day nor the day had significant influence on the detection rate of parasitological tests. This could probably imply that factors such as phase of trypanosome infections: early or chronic phase (Masake and Nantulya, 1991) and presence of mixed trypanosome infections (Stephen, 1986; Nantulya *et al.*, 1992; Masake *et al.*, 1995), among others, have a more significant influence on the level of parasitaemia of the trypanosomes than the time of the day does. Bearing in mind there were few trypanosome-infected animals ($n = 2$) in this experiment, as dictated by unavailability of a sufficient sample size of cattle naturally infected with trypanosomes, this study needs to be conducted with a larger sample (probably more than 10) of infected cattle to fully ascertain the significance of the time of the day on the sensitivity of parasitological tests. Nevertheless, these results indicate

the highest detection rate of parasitological tests for trypanosomosis is achieved when cattle blood samples are taken and examined between 17.00 and 09.00 hours, including 21.00 hours under tropical conditions. Collection and examinations of cattle blood for trypanosome infections at 13.00 hours using parasitological tests should be avoided because it is associated with the lowest detection rate of infection. Overall, these results demonstrated that the sensitivity of parasitological tests for trypanosomosis is low, a finding consistent with those of other studies (Eisler *et al.*, 1998; Picozzi *et al*, 2002). Apart from taking blood samples during a particular time of the day (17.00 and 09.00 hours) to achieve optimal detection rate with microscopy, there is a need to supplement these techniques with more sensitive penside diagnostic tests for trypanosomosis, clinical diagnosis and decision support tools to enable field veterinarians to effectively diagnose and treat cases of trypanosomosis.

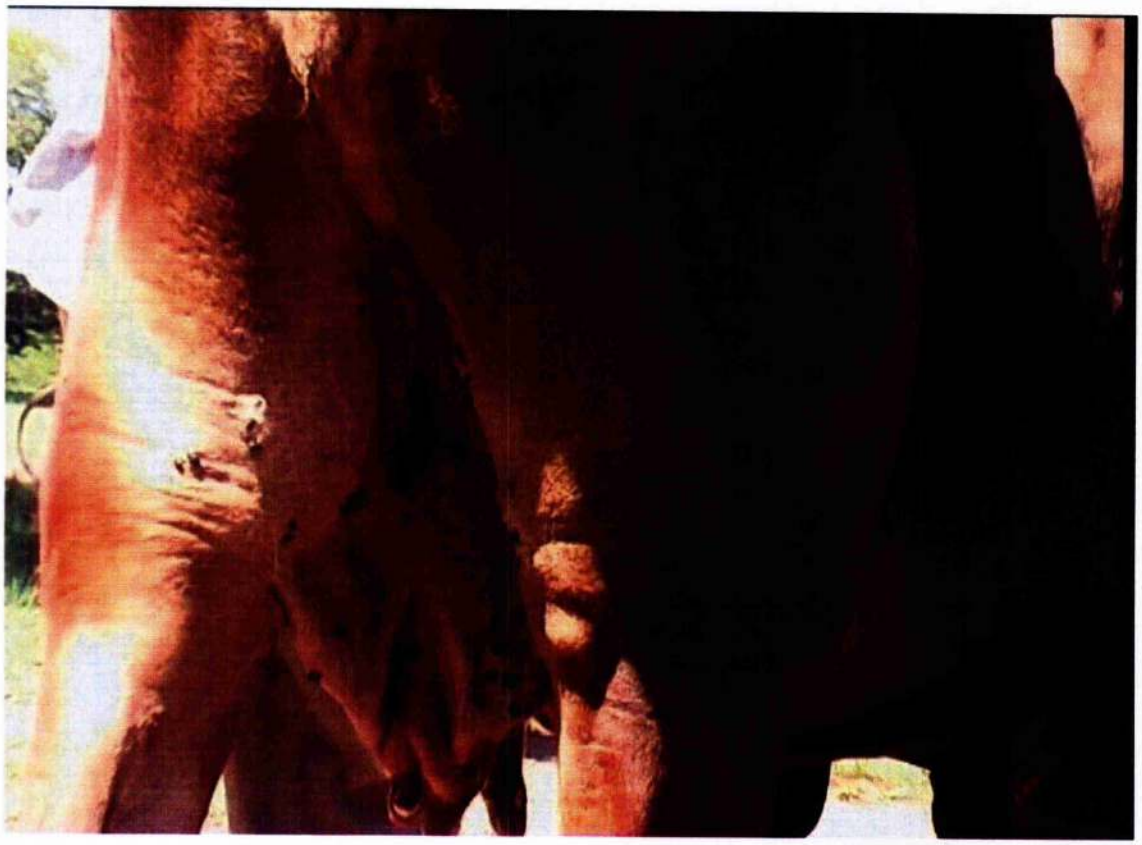


Figure 6.1: High infestation of *Amblyomma variegatum* ticks on a Zebu cow in Uganda during the longitudinal study – despite the infestation of Zebu cattle with these vector ticks for cowdriosis, no cases of cowdriosis were detected. This confirmed the fact that though bovine cowdriosis is often fatal especially in susceptible cattle introduced into *Amblyomma*-infested areas, particularly in imported exotic breeds, cases are rare in indigenous cattle found in endemic areas

Chapter 6 Epidemiology of endemic diseases in cattle kept under a mixed crop-livestock system in Uganda

6.1 Introduction

The epidemiology of various endemic diseases of cattle with particular reference to the prevalence, morbidity and mortality rates and patterns of the major tick-borne diseases (anaplasmosis, babesiosis and theileriosis) are described in this Chapter with reference to the mixed-crop-livestock production system in South East Uganda.

Cattle that are infected with the tick-borne diseases anaplasmosis, babesiosis and theileriosis develop detectable serological responses if animals survive infection. As illustrated in Figure 6.2, calves are borne naïve with respect to tick-borne disease antigen exposure, but they get initial protection against tick-borne diseases by means of passive immunity from maternal antibodies received through colostrum (Burridge and Kimber, 1973; Losos, 1986). Maternal antibodies to babesiosis and anaplasmosis in calves disappear after 9-12 months (De Vos, 1979; Jongejan *et al.*, 1988; Potgieter and Stoltz, 1994), while for theileriosis; they wane after 4-8 months (Norval *et al.*, 1992; Deem *et al.*, 1993; Okello-Onen *et al.*, 1998a; Gitau *et al.*, 2000). After maternal antibodies to tick-borne diseases have waned, animals get a priming immunity response from natural active infection during the initial exposure to tick challenge when they seroconvert.

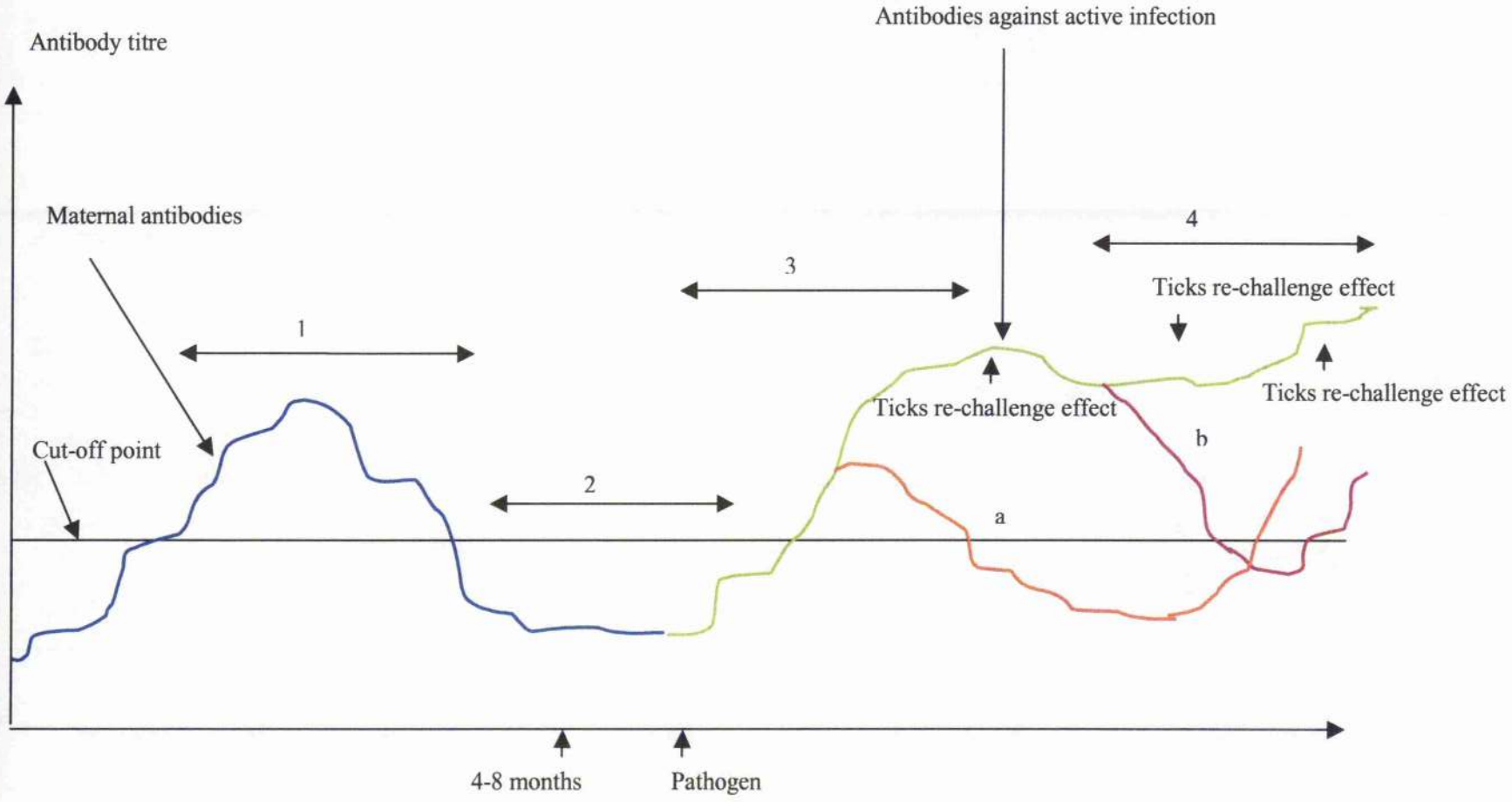
Serological investigation of tick-borne diseases presupposed the following disease scenario for a production system where cattle are extensively exposed to tick challenge (Figure 6.2). The majority of calves up to 4 – 8 months old have maternal antibodies (phase 1), the maternal antibodies later wane (phase 2), then calves get a priming immune response from natural active infection on exposure to tick-borne diseases when the calves seroconvert (phase 3). Phase 4

represents antibody response to tick-borne diseases in adult cattle in which the antibody titre declines or rises depending on the tick challenge. In some individuals e.g. (a) and (b) in Figure 6.2, antibodies may decline especially when the tick challenge is low. However, as tick challenge increases, such animals' immune responses are boosted and thus there is a rise in antibody titre.

Various epidemiologic states of tick-borne diseases occur because of varying innate susceptibility of hosts, varying susceptibility to infection of different tick populations and varying levels of infection in ticks and cattle (Perry and Young, 1995). Indigenous cattle in TBD endemic areas rarely become heavily infested with ticks and thus suffer small or insignificant production losses as a result of tick-borne diseases (Norval *et al.*, 1992; Lawrence *et al.*, 1996; Okello-Onen *et al.*, 1998b). However, indigenous breeds of cattle are at risk of TBDs in situations where they are subjected to intensive tick control or when they are moved from disease-free to endemic areas or during prolonged rains in marginal areas when their immunity declines as a result of low tick survival and challenge (Jongejan *et al.*, 1988; Norval *et al.*, 1992; Deem *et al.*, 1993; Latif *et al.*, 1995; Okello-Onen *et al.*, 2003).

In cases of limited exposure to tick challenge or varying tick challenge, immunity wanes. Field rechallenge depends on geographical location and agroecological factors e.g. periodic unsuitability of the climate for survival and development of *R. appendiculatus* (Perry and Young, 1995). The level of tick challenge is also influenced by low infection rates of *T. parva* in ticks acquired from low parasitaemias in immune carrier cattle (Perry and Young, 1995).

Figure 6.2: A simple model to illustrate the antibody response of cattle to tick-borne infection under field conditions without tick control



Transmission of *B. bigemina* infection might not be affected by seasonal fluctuations of the level of *Boophilus* spp. tick challenge, because during transovarial transmission of *B. bigemina*, transmission occurs during nymphal and adult feeding periods and it can continue through several generations of ticks in the absence of re-infection (Norval *et al.*, 1983). Low parasitaemias of babesiosis often maintained in *Bos indicus* cattle enable them to maintain enzootic stability, but make the critical level of tick infestation required for maintenance of babesial infections in *Bos indicus* cattle higher than in *Bos taurus* cattle (Jongejan *et al.*, 1988). For transmission of *T. parva* infections, common strains of *T. parva* in eastern Africa result in persistent infections of erythrocytes in cattle, creating a carrier status that is a continual source of infection to ticks (Perry and Young, 1995).

For anaplasmosis, animals that recover usually become life-long carriers of the parasites (Norval *et al.*, 1984), however, autosterilization renders animals susceptible to infection again within several months, because clearance of parasite from the blood stream is followed by the disappearance of antibodies to the parasite (Potgieter and Stoltz, 1994), unless such animals get re-challenged by ticks.

An important component of tick-borne serology is the sensitivity and specificity of the diagnostic methods employed. The direct method for diagnosis of TBDs such as light microscopy for identification of the presence of *Babesia*, *Theileria* and *Anaplasma* parasites in Giemsa-stained blood or lymph node biopsy smears does not provide accurate information about prevalence because it has a low sensitivity especially when the infection rate is low, as occurs with carrier animals (Morzaria *et al.*, 1999). Serological methods are better suited for this purpose. Improved ELISAs based on recombinant and synthetic peptide species-specific antigens now exist for TBDs. These assays which are standardised and fully validated, have high sensitivity and specificity (Morzaria *et al.*, 1999).

The ELISA for detection of antibodies against *T. parva* infection utilises the polymorphic immunodominant molecule (PIM) as the diagnostic recombinant antigen (Katende et al, 1998). PIM has a molecular weight of 85 kD (Katende *et al.*, 1998) and is predominantly found on the surface of the schizont stage of *Theileria* parasite but is also present in the sporozoite microspheres (Morzaria *et al.*, 1999). Other candidate diagnostic antigens described for *T. parva* include p67, p104 and p105 (Katende *et al.*, 1998). The p67 molecule is a stage-specific antigen found on the surface of sporozoites and has a molecular weight of 67kD. The p104 molecule is an antigen found in the sporozoite stage and has a molecular weight of 104kD. The p150 molecule is an antigen found both in the sporozoite microspheres and in the schizont stages (Morzaria *et al.*, 1999). However, among all these antigens, PIM is the most sensitive of all the candidate antigens for *T. parva* because 92% of the field sera have antibodies to it and the PIM-ELISA has a sensitivity of over 99% and a specificity of 94-98% (Katende *et al.*, 1998).

The ELISA for detecting antibodies to *A. marginale* utilises a standardized recombinant 19kD antigen of *A. marginale*. The assay has a sensitivity and specificity of over 90% (Morzaria *et al.*, 1999). For detection of antibodies to *B. bigemina*, the assay utilises a standardized recombinant 200 kD antigen of *B. bigemina*. P200 is a merozoite antigen which is reported to be recognized by 98% of the sera collected from cattle in areas where *B. bigemina* infection is endemic. The assay has a sensitivity and specificity of over 90% (Tebele *et al.*, 1995). However, it must be recognized that the immune response to these antigen molecules in cattle is complex.

6.2 Materials and methods

Although from the cross-sectional study, disease prevalence and distribution could be established, it was realised there was a limitation on the range of diseases detected, few cases of

disease were found and incidence of tick-borne diseases often assessed through seroconversion determined by serology, could not be obtained through a cross-sectional study. Thus a longitudinal study design in which the same animals and herds were followed over a long period of time was adopted because it was more appropriate for detection of more diseases and cases, and was more suitable for studying patterns of diseases than the cross-sectional study. During the both the cross-sectional and longitudinal studies, clinical, parasitological and serological data were collected (see Chapter 3).

6.2.1 Data analysis

Parasitological prevalences of the various diseases were calculated and seroprevalences where available, were plotted to assess the disease importance. Prevalence of cases was calculated to assess the morbidity of the various endemic diseases under consideration. Prevalence rather than incidence was chosen since the main focus was to identify disease problems for long-term disease control strategies, for which prevalence is recommended (Thrusfield, 1995). Disease mortality was assessed through calculation of the crude mortality rates. The average population at risk was the average of the population at the beginning of the study (628) and the end of 12 months (549). Clinical signs manifested by cases and their parasitological findings on sampling visits prior to death were used as the basis for determining the probable disease responsible for death. Prevalence and the crude mortality rates were calculated using the following formulae described by Thrusfield (1995):

$$\text{Prevalence} = \frac{\text{Number of individuals having a disease at a particular point in time}}{\text{Number of individuals in the population at risk at that point in time}}$$

$$\text{Crude mortality rate} = \frac{\text{Total number dying}}{\text{Average population at risk}}$$

For purposes of data analysis, morbidity was based on presence of sickness as defined as clinical manifestation of anaemia, weight loss, pallor of mucous membranes, lymph node enlargement, staring coat, diarrhoea, lacrymation and fever in addition to presence of aetiological agents.

Given a test cut-off point of 20 PP for the *T. parva* ELISA and considering animals with 10 PP above the cut-off point as being marginally greater than the threshold (see Chapter 3, Section 3.7.6.4.2), animals initially negative (30 PP and below) were considered to have seroconverted if they had had a sero-increase of at least 20 PP on the subsequent visit. For anaplasmosis and babesiosis, animals initially seronegative (25 PP and below) were considered to have seroconverted if they had had at least a sero-increase of 15 PP on the subsequent visit, taking into account a cut-off point of 15 PP for both diseases and allowing for 10 PP above the cut-off point. Seroconversion rate was calculated using the following formula:

$$\text{Seroconversion rate} = \frac{\text{Number of animals with the first seroconversion}}{\text{Number of animals in the population at risk at that point in time}}$$

The distribution of tick infestation according to villages was examined through plots of the proportion of animals infested with *Rhipicephalus* and *Boophilus* species ticks in the different villages. Seroconversion rates of cattle of different age groups under low tick challenge were compared to those of cattle under high tick challenge using the Chi-square test performed with the computer programme EpiInfo 2000 (Centre for Disease Control, Atlanta, USA).

6.3 Results

6.3.1 Parasitological prevalence of various endemic diseases

Of the 450 cattle examined using parasitological techniques during the cross-sectional study, 44% had fasciolosis, 14.4% had trypanosomosis, 7.6% had theileriosis, 6.4% had parasitic gastroenteritis and 5.3% had anaplasmosis. Cases of concurrent fasciolosis with

trypanosomosis, theileriosis and anaplasmosis were found in 4.4%, 2.9% and 1.8% of the animals, respectively. Concurrent disease involving trypanosomosis, theileriosis, anaplasmosis, babesiosis, parasitic gastroenteritis and fasciolosis was detected in 2.4% of the animals. Cases of cowdriosis were not encountered, despite the abundance of the tick vector *Amblyomma variegatum*.

Of the cumulative 7308 animals sampled over the entire 12 months of the longitudinal study period, 1724, 1577, 995, 490, 318, 78 and 8 were parasitologically positive for anaplasmosis, theileriosis, fasciolosis, parasitic gastroenteritis, trypanosomosis, babesiosis and schistosomosis, respectively. In order of importance regarding the overall parasitological prevalences, the diseases were: anaplasmosis (23.6%), theileriosis (21.6%), fasciolosis (13.6%), parasitic gastroenteritis (6.7%), trypanosomosis (4.4%), babesiosis (1.0%) and schistosomosis (0.1%).

6.3.2 Seroprevalence of major tick-borne diseases

The monthly seroprevalences of *T. parva*, *A. marginale* and *B. bigemina* infections in cattle in the study area are shown in Figure 6.3. The seroprevalence of *T. parva* infection was high, being 77.3% over the entire study period and varied between 68.9% and 85.8%. The seroprevalence of *A. marginale* infection was also high, being 70.6% over the entire study period and varied between 56.2% and 85.6%. For *B. bigemina* infection, the seroprevalence was medium, being 66.5% over the entire study period and varied between 54.9% and 76.9%.

6.3.3 Morbidity of endemic diseases

Of the cumulative 7308 animals examined both clinically and parasitologically over a 12-month study period, 1265, 1161, 517, 247, 233 and 18 cases of anaplasmosis, theileriosis, fasciolosis, parasitic gastroenteritis, trypanosomosis and babesiosis were detected, respectively. In order of importance in terms of their morbidity rates, the diseases were: anaplasmosis

(17.1%), theileriosis (15.6%), fasciolosis (7.0%), parasitic gastroenteritis (3.3%), trypanosomosis (3.2%) and babesiosis (0.2%). Although a few cattle secreted *Schistosoma* eggs, no cases of schistosomosis were found.

6.3.4 Mortality of cattle associated with various endemic diseases

The overall annual crude mortality rate was low (5.2%). East Coast fever was the single most important cause of disease-related mortality, being responsible for 0.8%, followed by anaplasmosis (0.5%). However much of the disease-related mortality of 3.7% was due to multiple diseases involving East Coast fever, anaplasmosis, trypanosomosis, fasciolosis, parasitic gastroenteritis and babesiosis. Much of the mortality involved young animals up to 12 months of age, being 12.4% in animals up to 6 months of age and 11.9% in the 7-12 months age group. In older animals in the age groups of the 13-24 months and over 24 months, mortality rates were low, being 0.7% and 2.4%, respectively.

The major causes of death of animals up to 6 months of age included, East Coast fever and intercurrent disease involving East Coast fever with trypanosomosis, anaplasmosis, parasitic gastroenteritis, fasciolosis and babesiosis. For animals in the age-group 7-12 months, death was mainly due to East Coast fever, anaplasmosis and intercurrent disease involving East Coast fever with anaplasmosis, trypanosomosis, parasitic gastroenteritis and fasciolosis. All the deaths of animals in the age-group 13-24 months were due to unknown causes. For older animals (> 24 months), their major cause of death was anaplasmosis and intercurrent disease involving anaplasmosis with either fasciolosis or trypanosomosis and East Coast fever.

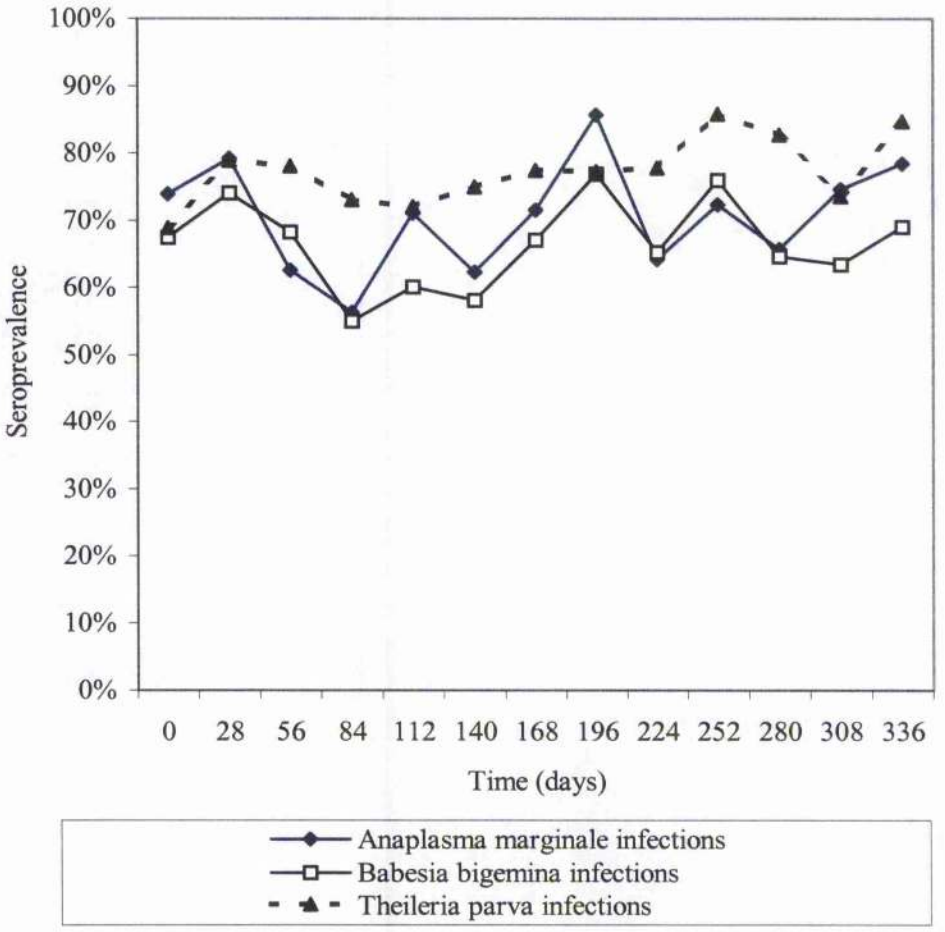
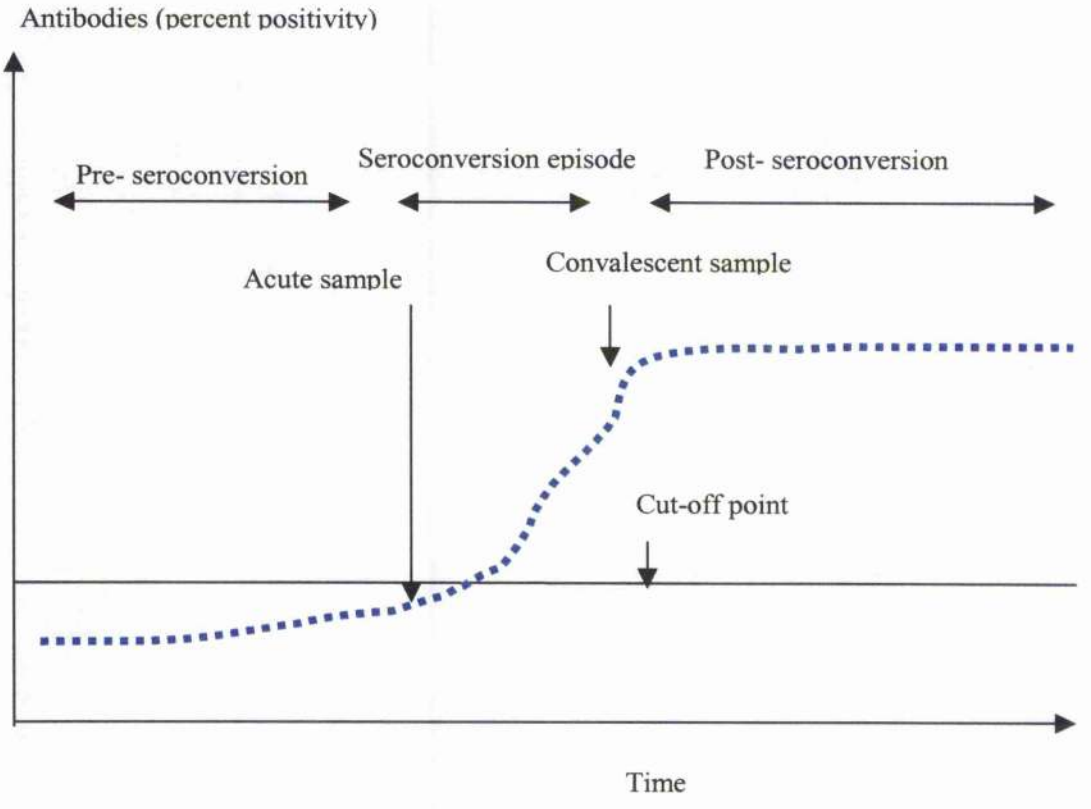


Figure 6.3: Monthly seroprevalence of *T. parva*, *A. marginale* and *B. bigemina* infections during the study period

6.3.5 Serological patterns of tick-borne diseases in cattle under field conditions

Figure 6.4 illustrates classical seroconversion to tick-borne diseases of cattle. Calves initially test negative when born to seronegative dams or when passive maternal antibodies have decayed to a negative level. At this stage calves are said to be in a pre-seroconversion phase. As time goes on such seronegative calves on exposure to tick-borne infection test positive, then they are said to have seroconverted. The seroconversion episode always falls between two points: the acute and convalescent sample. Seroconverted animals are expected to stay positive subsequently during the post-seroconversion period.

Figure 6.4: Schematic illustration of seroconversion to tick-borne diseases



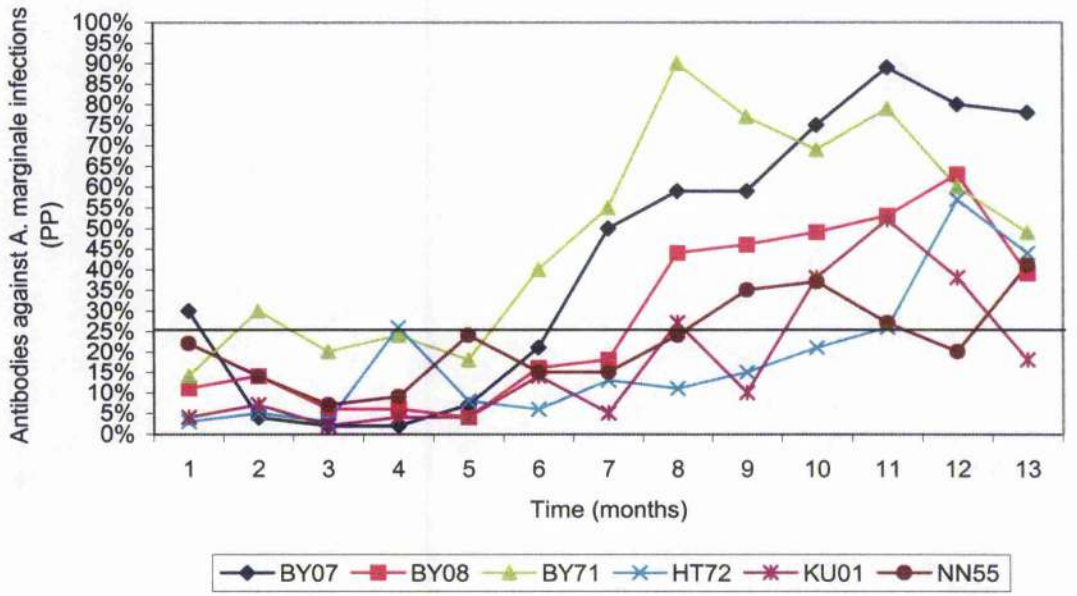
Usually cattle seroconvert once and remain positive, as they grow older especially when exposed to regular tick challenge (see Section 6.1). Seroconversion or primary immune response can only occur in an immunological naïve animal, thus it is only newborn calves that undergo seroconversion. However, as shown in Table 6.1, this study showed some animals may have a secondary immune response (sero-event) under such an extensive production system. Of the cattle examined during the study, 13.0%, 12.2% and 6.0% had secondary immune responses (multiple seroconversions) to *A. marginale*, *B. bigemina* and *T. parva* infections, respectively.

Table 6.1: Distribution of cattle according to the number of seroconversions to *A. marginale*, *B. bigemina* and *T. parva* infections

	Number of animals with different tick-borne infections		
Nature of seroconversion	***A. marginale***	***B. bigemina***	***T. parva***
Multiple seroconversions	84	79	39
Single seroconversion	258	240	207
No seroconversion	306	329	402

Examples of serological profiles of cattle of different age groups that had more than one seroconversion to *A. marginale* infection are shown in Figures 6.5 and 6.6. In Figure 6.5 (a) the general trend was of calves (0-6 months) seroconverting after 6 months, while in Figure 6.5 (b) the trend was of calves (7-12 months) probably already seroconverted having had a decline of antibodies, which then fluctuated around the threshold for several months. In Figure 6.6 (c), antibodies in young cattle (13-24 months) rose above the threshold in some animals but in others the level fluctuated around the threshold. In Figure 6.6 (d), antibody levels in adult cattle (> 24 months) also fluctuated around the threshold.

a



b

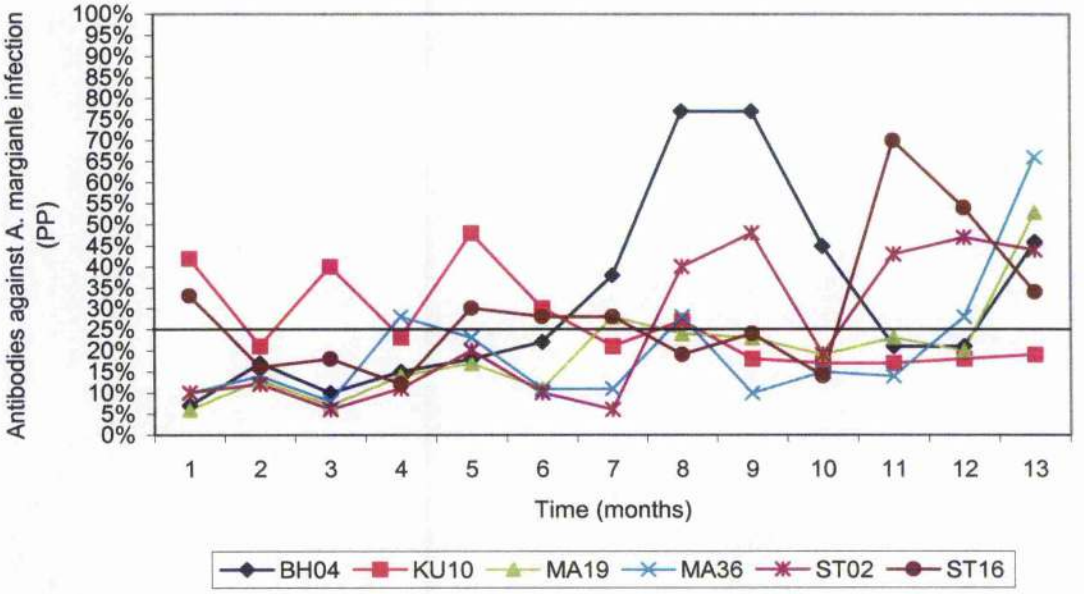
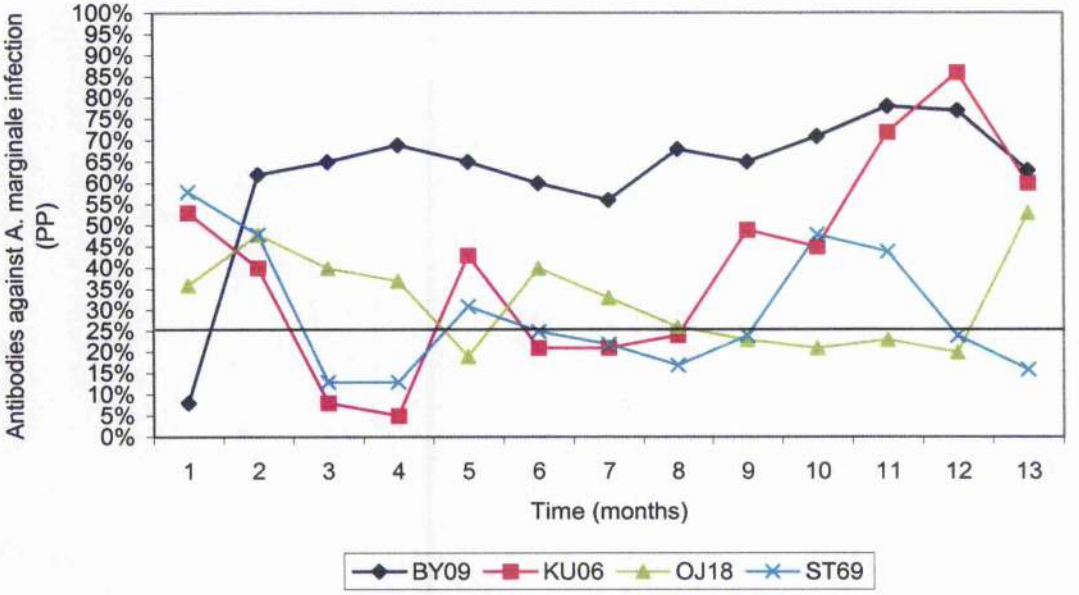


Figure 6.5: Selected profiles of antibodies against *A. marginale* infections in cattle aged 0-6 months (a) and 7-12 months (b) in Uganda. The bold line represents the threshold for seroconversion (25PP)

c



d

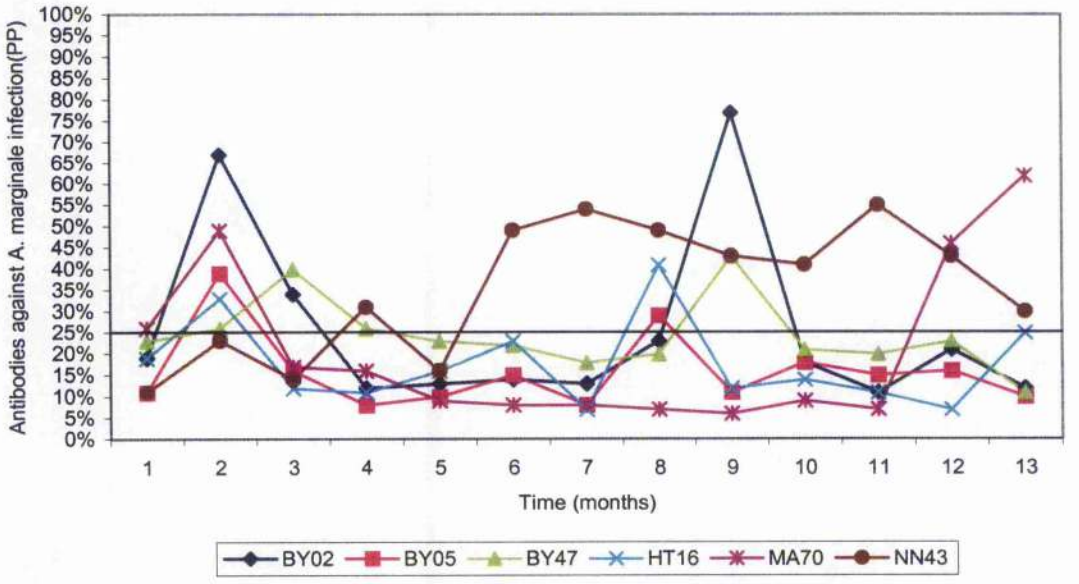
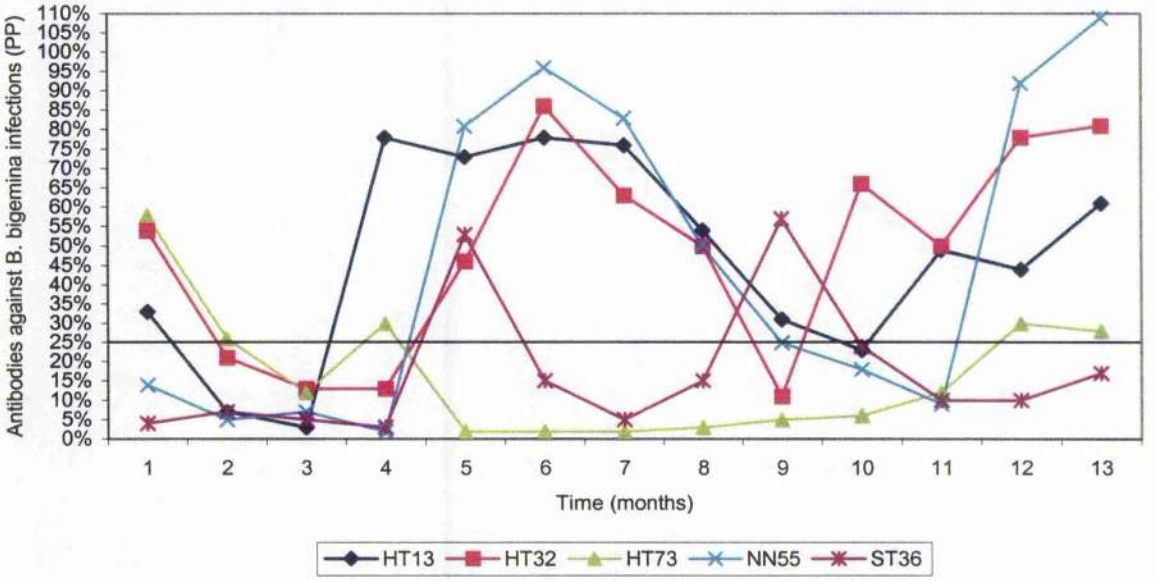


Figure 6.6: Selected profiles of antibodies against *A. marginale* infections in cattle aged 13-24 months (c) and > 24 months (d) in Uganda. The bold line represents the threshold for seroconversion (25PP)

Examples of serological profiles of cattle of different age groups that had more than one seroconversion to *B. bigemina* infection are shown in Figures 6.7 and 6.8. In calves up to 6 months old, the antibody (maternal) level declined and was followed by seroconversion to *B. bigemina* infection and subsequent fluctuation of antibody level around the threshold (Figure 6.7a). In Figure 6.7 (b) some calves of 7-12 months old experienced a rise in antibody level probably as a result of seroconversion, but other calves had antibody levels that remained below the threshold throughout the study period. In Figure 6.8 (c) young cattle of 13-24 months old, experienced a decline followed by a rise in antibody level. In Figure 6.8 (d) some adult cattle (> 24 months) maintained the antibody levels below the threshold throughout the study period, while others in the same age group experienced a decline followed by a rise in the antibody level.

Examples of serological profiles of cattle of different age groups that had more than one seroconversion to *T. parva* infection are shown in Figures 6.9 and 6.10. In Figure 6.9 (a) some calves of up to 6 months old experienced a decline of maternal antibodies followed by seroconversion, but for others that were initially negative seroconverted. In Figure 6.9 (b) calves of 7-12 months old, had fluctuating antibody levels around the threshold. In Figure 6.10 (c) young cattle of 13-24 months old experienced a fluctuation of antibody levels around the threshold throughout the study. In Figure 6.10 (d) some adult cattle of > 24 months old experienced a decline followed by a rise of antibody level, while others experienced a persistent decline of the antibody level, which eventually remained low.

a



b

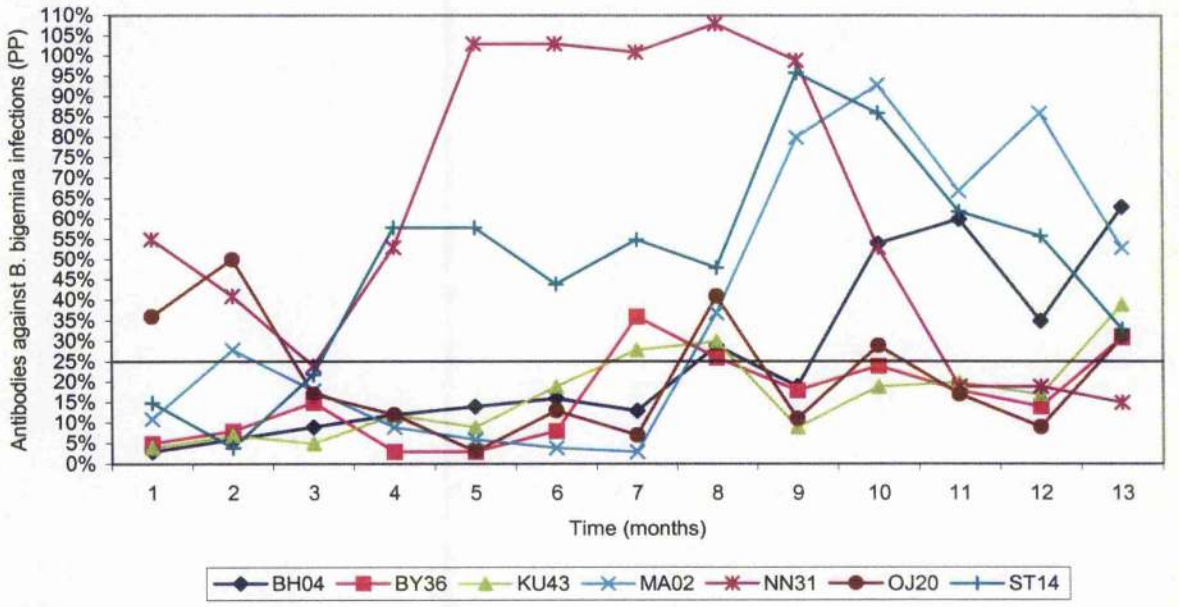
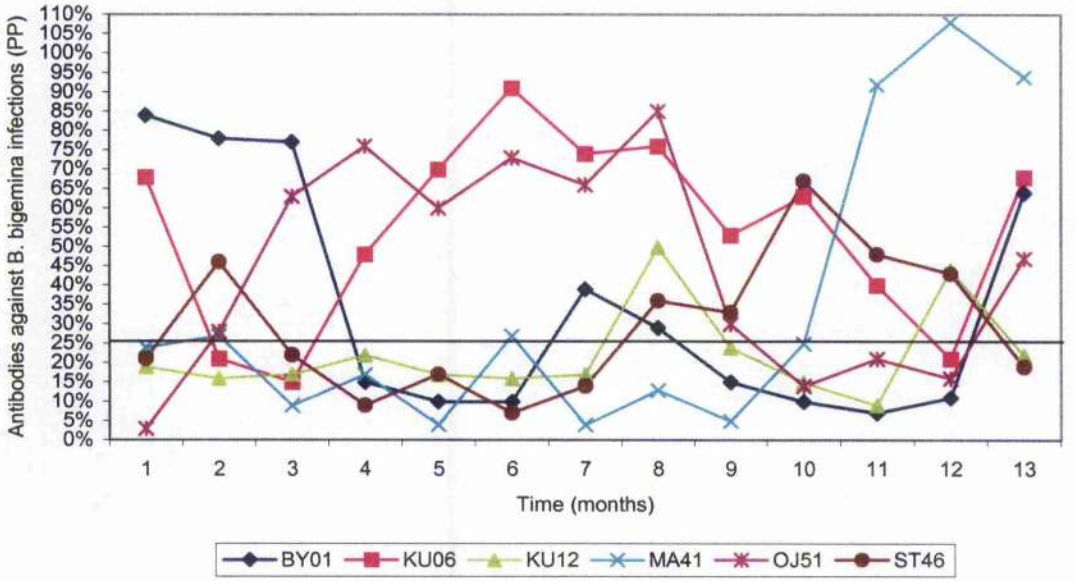


Figure 6.7: Selected profiles of antibodies against *B. bigemina* infections in cattle aged 0-6 months (a) and 7-12 months (b) in Uganda. The bold line represents the threshold for seroconversion (25PP)

c



d

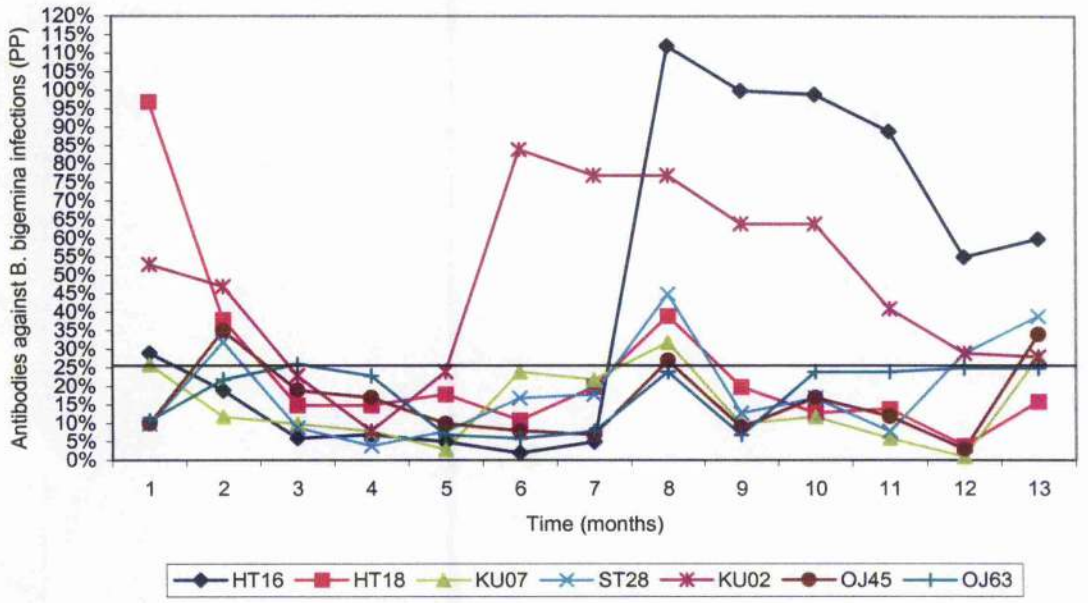
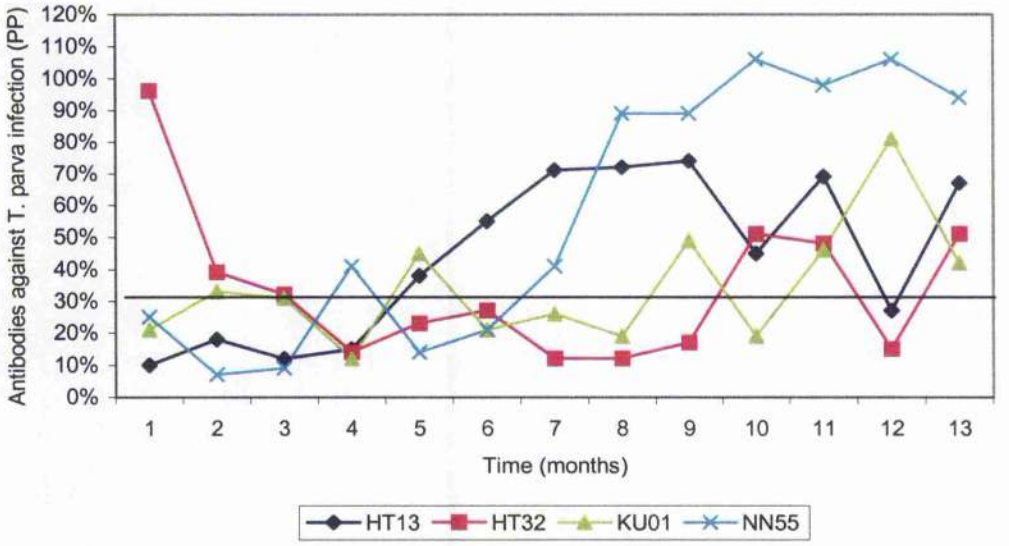


Figure 6.8: Selected profiles of antibodies against *B. bigemina* infections in cattle aged 13-24 months (c) and > 24 months (d) in Uganda. The bold line represents the threshold for seroconversion (25PP)

a



b

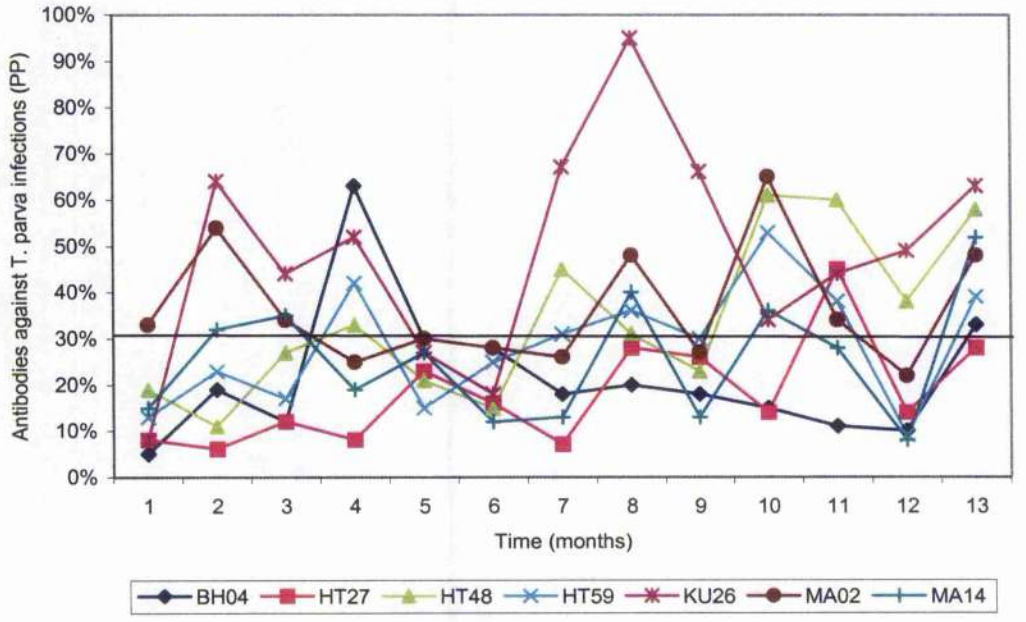
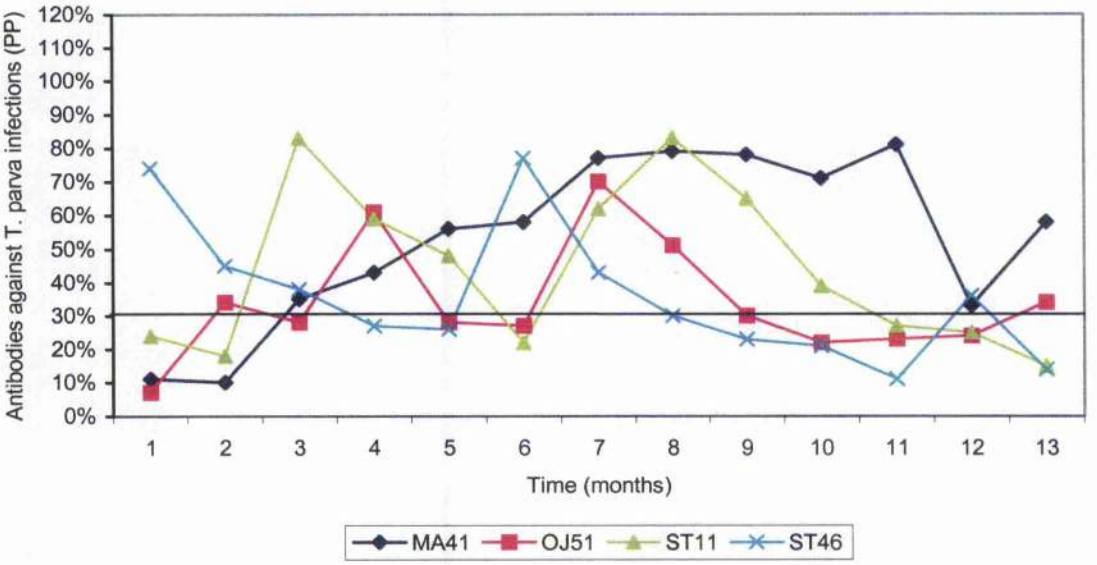


Figure 6.9: Selected profiles of antibodies against *T. parva* infections in cattle aged 0-6 months (a) and 7-12 months (b) in Uganda. The bold line represents the threshold for seroconversion (30PP)

c



d

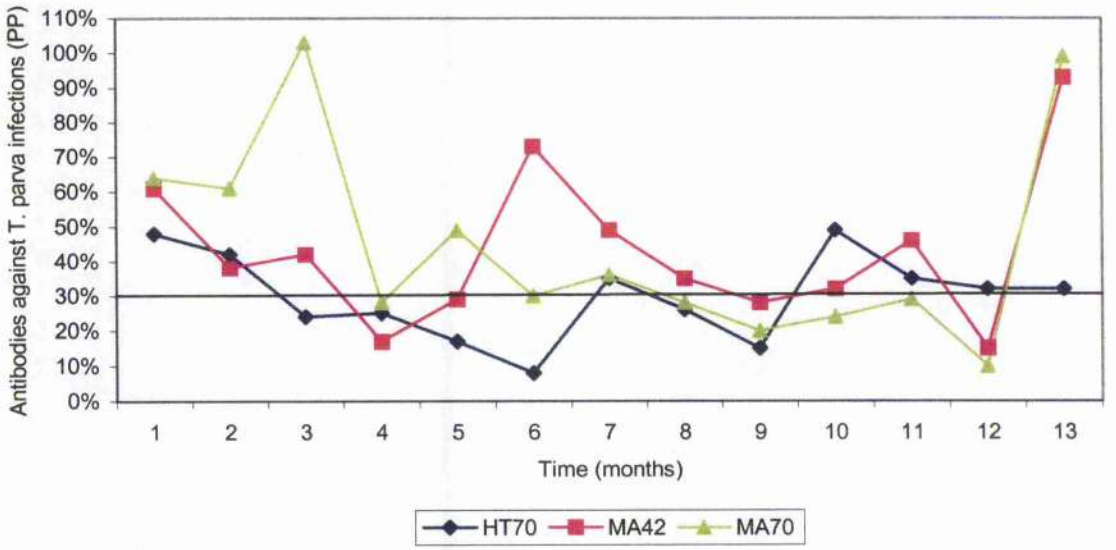


Figure 6.10: Selected profiles of antibodies against *T. parva* infections in cattle aged 13-24 months (c) and > 24 months (d) in Uganda. The bold line represents the threshold for seroconversion (30PP)

The proportion of cattle in different study villages infested with *Rhipicephalus appendiculatus* is shown in Figure 6.11. Whereas over 90% of the cattle in some villages: Buyimini, Kubo, Nanjeho, Ojilai and Sitengo were infested with *R. appendiculatus* ticks throughout the study period, in others: Bunghaji, Hitunga and Magoje, fewer animals (10-80%) were infested. The former set of villages had a high tick challenge and the latter set of villages had low a tick challenge as regards *R. appendiculatus*.

The proportion of cattle in different study villages infested with *Boophilus* spp. is shown in Figure 6.12. Unlike *R. appendiculatus*, the distinction between villages in terms of the distribution of *Boophilus* spp was not very clear. However, 40-80% of the cattle in some villages: Buyimini, Ojilai, Nanjeho, Hitunga and Kubo were infested with *Boophilus* spp. ticks throughout the study period, while in other villages: Sitengo, Magoje and Bunghaji fewer animals (10-40%) were infested. For *Boophilus* spp. ticks, the former villages had a high tick challenge while the latter villages had a low tick challenge.

The proportion of cattle infested with *R. appendiculatus* in the low and high tick challenge zones are compared in Figure 6.13. The proportion of cattle infested with *R. appendiculatus* in the high tick challenge zone was significantly higher than of the low tick challenge zone ($P < 0.05$).

The proportion of cattle infested with *Boophilus* spp. ticks in the low and high tick challenge zones are compared in Figure 6.14. The proportion of cattle infested with *Boophilus* sp. ticks in the high tick challenge zone was significantly higher than that of the low tick challenge zone ($P < 0.05$).

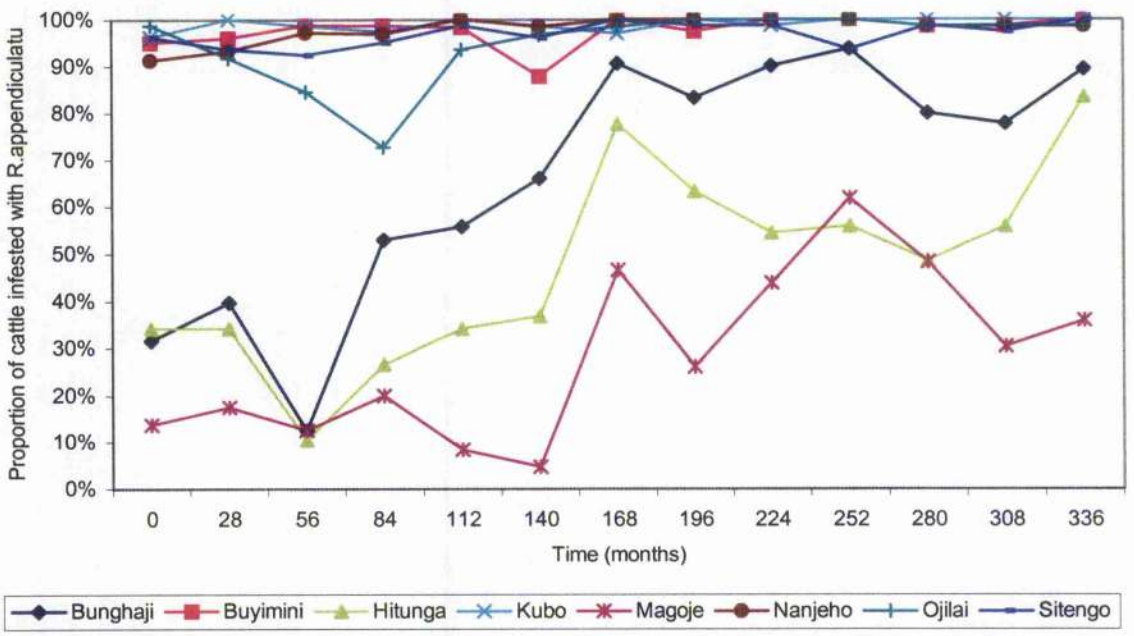


Figure 6.11: Village-level distribution of *R. appendiculatus* infestation of cattle

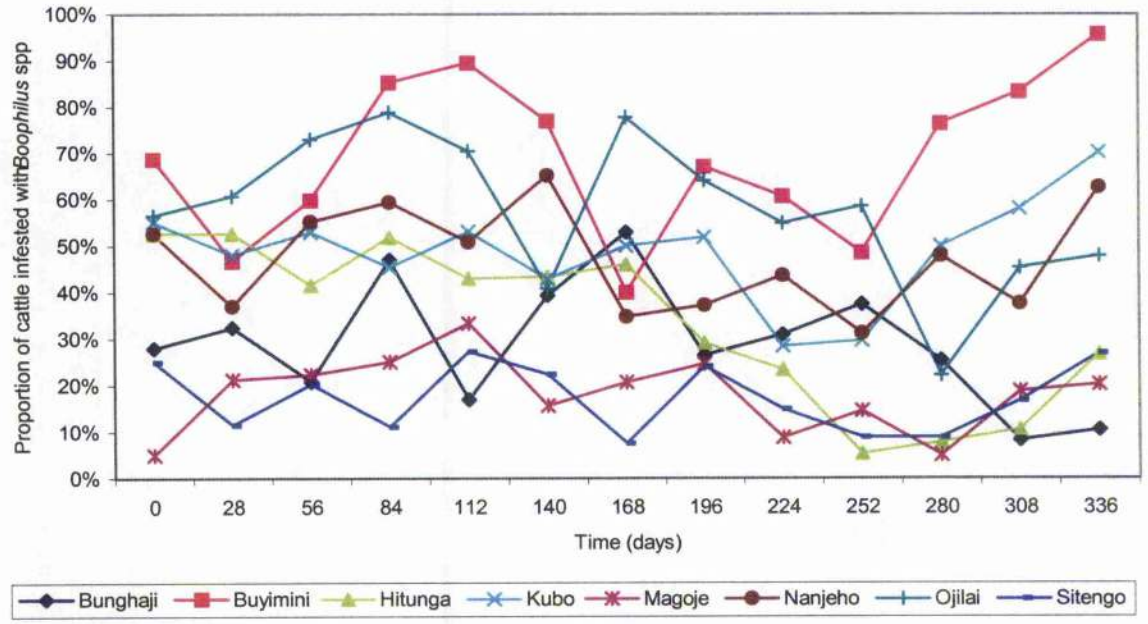


Figure 6.12: Village-level distribution of *Boophilus* sp. infestation of cattle

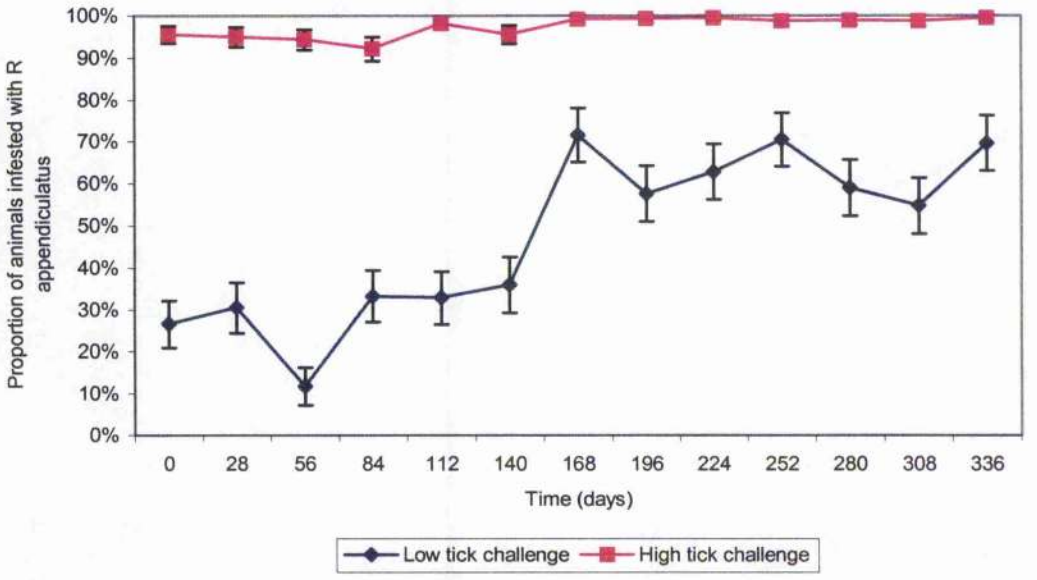


Fig. 6.13: Monthly proportion of cattle infested with *R appendiculatus* (± 95% CI) in the high and low tick challenge zones

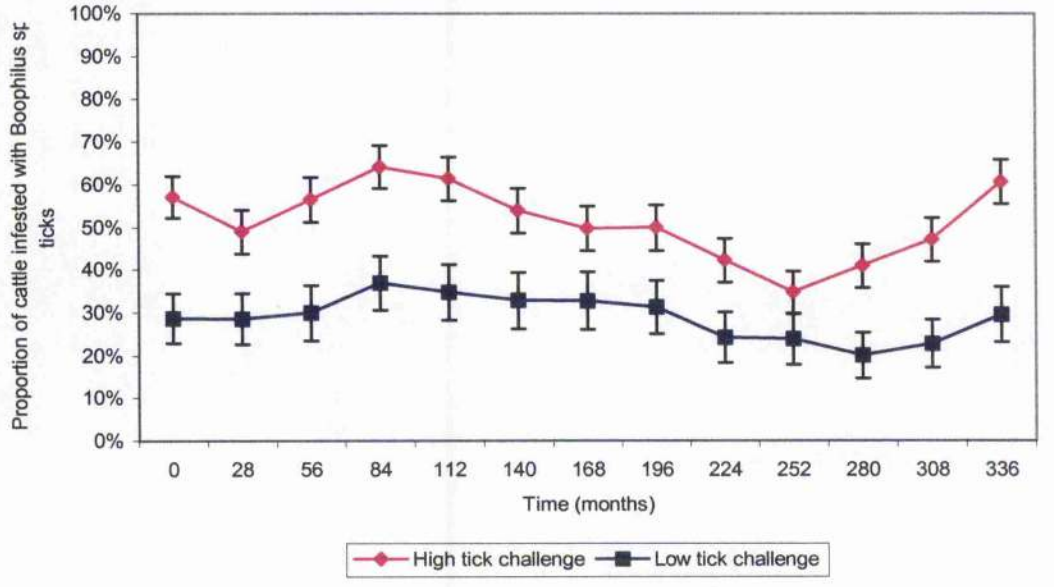


Fig. 6.14: Monthly proportion of cattle infested with *Boophilus* sp. ticks (± 95% CI) in the high and low tick challenge zones

Seroconversion rates of cattle of different age groups to *A. marginale*, *B. bigemina* and *T. parva* infections in the high and low tick challenge zones are shown in Figures 6.15, 6.16 and 6.17, respectively. In Figure 6.15, seroconversion rates to *A. marginale* infection of cattle of all age groups under low tick challenge were higher than those of their counterparts under high tick challenge (0-6m, 14% vs 11.4%; 7-12 m, 11.9% vs 8.5%; 13-24m, 9.5% vs 6.6%; >24m, 11.4% vs 7.8%). However, there was no statistically significant difference between the two zones in terms of seroconversion rates of cattle of all age groups (6m, $\chi^2 = 0.58$, DF = 1, P= 0.44; 7-12m, $\chi^2 = 3.0$, DF = 1, P=0.08; 13-24m, $\chi^2 = 2.2$, DF = 1, P= 0.13; >24m, $\chi^2 = 3.7$, DF = 1, P= 0.05). Independent of the tick challenge, calves up to 6 months old had a high seroconversion rate. In subsequent age groups, there was a decline up to the age of 13-24 months, followed by a rise in older animals (> 24 months).

As regards *B. bigemina* infection (Figure 6.16), apart from calves up to 6 months old, seroconversion rates of cattle of different age groups under high tick challenge were higher than those of their counterparts under low tick challenge (7-12m, 11.2% vs 9.6%, 13-24m, 10.3% vs 10.1%, >24m, 8.1% vs 4.9%). A statistically significant difference was observed between seroconversion rates of older cattle (> 24 m) ($\chi^2 = 4.5$, DF = 1, $P < 0.05$). Otherwise, there was no significant difference between seroconversion rates of cattle of other age groups of the two zones (0-6m, $\chi^2 = 2.6$, DF = 1, P = 0.10; 7-12m, $\chi^2 = 0.67$, DF = 1, P= 0.41; 13-24m, $\chi^2 = 0.006$, DF = 1, P= 0.93). On the contrary, calves of up to 6 months old under low tick challenge had a higher seroconversion rate (16%) than those in the high tick challenge zone (9.1%). In terms of the general trend, under low tick challenge seroconversion rates decreased with age, while under high tick challenge seroconversion rates increased with age up to 24 months then declined.

For *T. parva* infections (Figure 6.17), seroconversion rates were high and similar in calves of 6 months old both under low and high tick challenge. Under low tick challenge, seroconversion

rates remained at the same level in all age groups, but under high tick challenge, they decreased with age. Seroconversion rates of cattle older than 6 months under low tick challenge were higher than those of their counterparts under high tick challenge (7-12m, 9% vs 4.9%; 13-24m, 10.2% vs 4.7%; > 24m, 9.9% vs 3.8%). This difference between the seroconversion rates of cattle older than 6 months of the two zones was significant (7-12m, $\chi^2 = 11.7$, DF = 1, $P < 0.05$; 13-24m, $\chi^2 = 11.2$, DF = 1, $P < 0.05$; > 24m, $\chi^2 = 27.2$, DF = 1, $P < 0.001$).

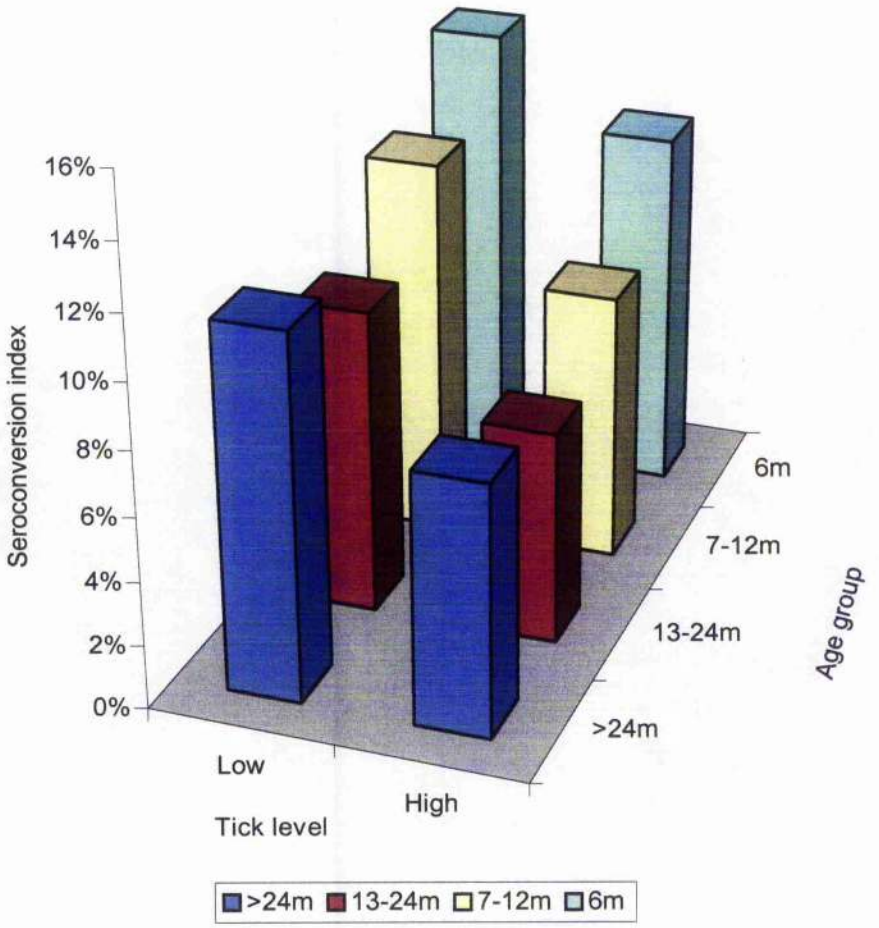


Fig. 6.15: Seroconversion rates of cattle of different age groups to *A. marginale* infection in the high and low tick challenge zones

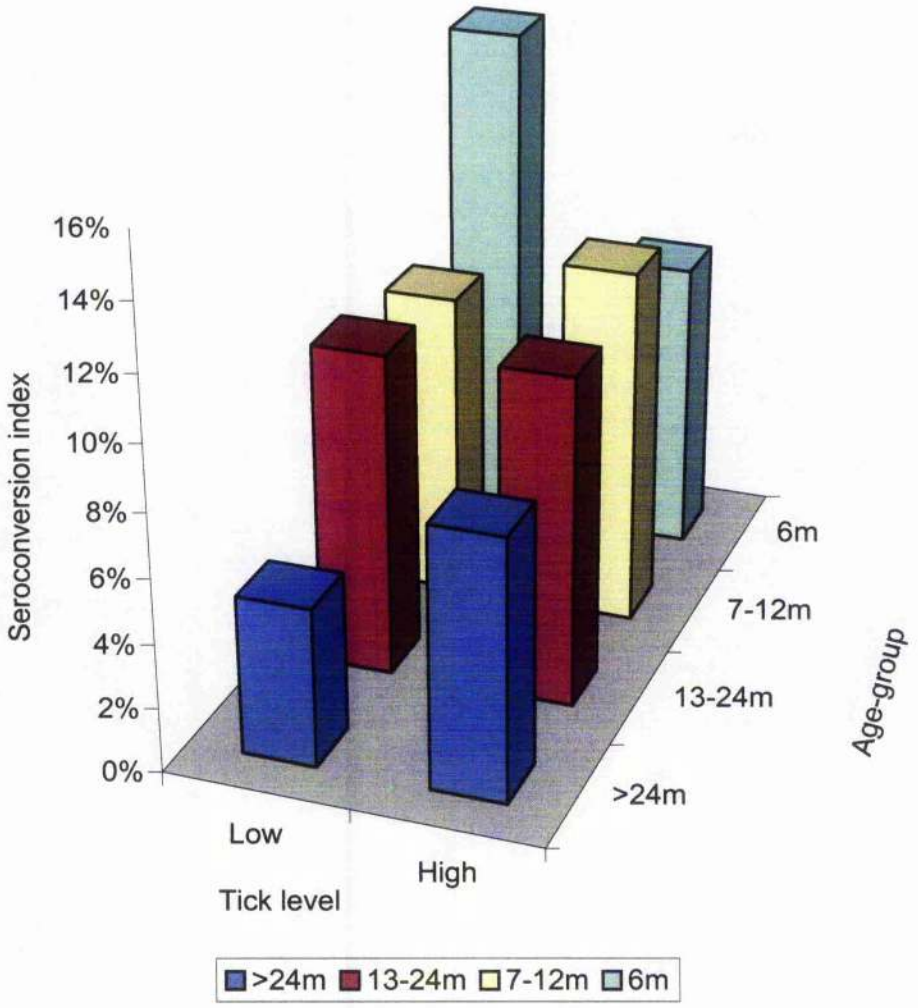


Fig. 6.16: Seroconversion rates of cattle of different age groups to *B. bigemina* infection in the high and low tick challenge zones

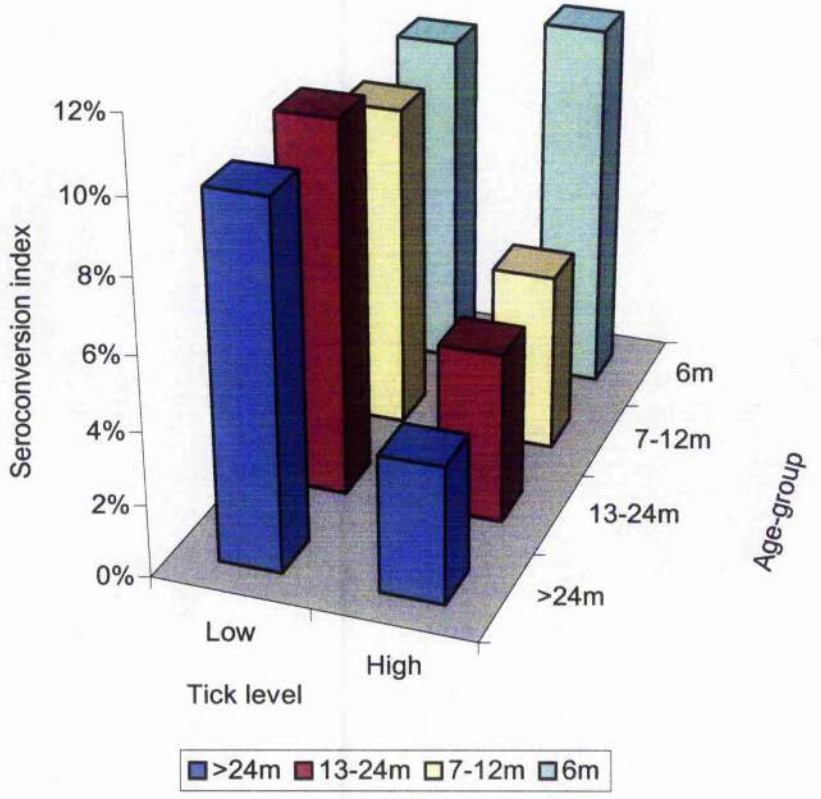


Fig. 6.17: Seroconversion rates of cattle of different age groups to *T. parva* infection in the high and low tick challenge zones

6.3 Discussion

The importance of various endemic diseases in cattle in South East Uganda regarding their prevalence, morbidity and mortality rates was investigated in a prospective study. The endemic diseases detected in order of importance regarding parasitogical prevalence were: anaplasmosis (23.6%), theileriosis (21.6%), fasciolosis (13.6%), parasitic gastroenteritis (6.7%), trypanosomosis (4.4%), babesiosis (1.6%) and schistosomosis (0.1%). The parasitological prevalences found were comparable to 29% obtained previously by Ssenyonga *et al.*, (1991) for anaplasmosis, 48% reported for theileriosis (Magona and Mayende, 2002) and 0.6% reported for schistosomosis (Magona *et al.*, 1999), but were lower than 16% reported for babesiosis (Magona and Mayende, 2002), 29-36% for fasciolosis (Magona *et al.*, 1999), 25% for trypanosomosis (Okuna *et al.*, 1996) and 22-61% for parasitic gastroenteritis (Magona and Mayende, 2002). A small proportion of cattle (1.8-4.4%) examined during the cross-sectional study were suffering from concurrent diseases.

Moderate to high seroprevalences of 68.9-85.8%, 56.2-85.6% and 54.9-76.9% for *T. parva*, *A. marginale* and *B. bigemina* infections, respectively, were found. This probably implied that cattle populations studied were extensively exposed to *T. parva*, *A. marginale* and *B. bigemina* infections. High seroprevalences for *T. parva*, *A. marginale* and *B. bigemina* infections have been attributed to the widespread exposure of large proportions of cattle to East Coast fever, babesiosis and anaplasmosis with the resultant development of clinical or mild diseases and attainment of a state of endemic stability to tick-borne diseases (Gitau *et al.*, 2000; Maloo *et al.*, 2001b).

A low overall annual crude mortality rate (5.2%) was found in the study area. East Coast fever was found to be the single most important cause of disease-related mortality (0.8%), followed by anaplasmosis (0.5%). This was consistent with previous estimates that East Coast fever is responsible for 79% while anaplasmosis is responsible for only 11% of cattle deaths

attributable to tick-borne diseases in Uganda (Anon., 1992). However much of the disease-related mortality of 3.7% was due to multiple diseases involving East Coast fever with anaplasmosis, trypanosomosis, fasciolosis, parasitic gastroenteritis and babesiosis. Underpinning the factor that suppression of the immune response by one pathogen prevents the host from mounting a normal response against other invading pathogens as was also demonstrated in other studies assessing effects of intercurrent trypanosomosis and gastrointestinal nematode infections (Kaufmann *et al.*, 1992; Dwinger *et al.*, 1994).

Much of the mortality involved young animals up to 12 months of age, with mortality rates of 12.4% and 11.9% for animals up to 6 months and 7-12 months of age, respectively. Older animals of 13-24 months and over 24 months had low mortality rates (0.7% and 2.4%, respectively). These rates in young animals were similar to a mortality rate of 19% observed in animals before the age of 1 year reported in other studies elsewhere (Ganaba *et al.*, 2002).

The major causes of death of young animals up to 12 months of age included, East Coast fever and intercurrent disease involving East Coast fever with trypanosomosis, anaplasmosis, parasitic gastroenteritis, fasciolosis and babesiosis. East Coast fever has been reported in other studies elsewhere to cause a mortality rate of 14% in 10-months old crossbred calves (Jacobsen, 1983) and 13.5% in Zebu calves up to 1 year old (Okello-Onen, 1996). Clinical helminth infections have also been reported in other studies to cause additional stress that aggravates mortality of indigenous Zebu cattle (Omara-Opyene, 1985; Waruiru *et al.*, 1998; Rubaire-Akiiki *et al.*, 1999). It is also noteworthy that the immunosuppressive effect of trypanosomosis (Dwinger *et al.*, 1994) could have exacerbated the effects of East Coast fever in intercurrent disease involving trypanosomosis and East Coast fever.

Anaplasmosis and intercurrent disease involving anaplasmosis with either fasciolosis or trypanosomosis were the major causes of death in cattle of over 24 months old. In other studies,

stress resulting from chronic subclinical trypanosomosis due to *T. vivax* or *T. congolense* has been reported to lead to recrudescence of parasitaemia and clinical anaplasmosis in premune carrier animals (Fox *et al.*, 1993) with resultant mortality in adult cattle. Manifestation of fasciolosis in cattle is often subclinical leading to retarded growth and reduced productivity, however it is reported to make affected animals more susceptible to other infections (Waruiru *et al.*, 2000) such as anaplasmosis. A combination of anaplasmosis, trypanosomosis and fasciolosis could cause extremely severe anaemia culminating into death.

It was evident that moderate parasitological prevalences of the various endemic diseases found in cattle: anaplasmosis (23.6%), theileriosis (21.6%), fasciolosis (13.6%), parasitic gastroenteritis (6.7%), trypanosomosis (4.4%), babesiosis (1.6%) and schistosomosis (0.1%) were associated with low morbidity rates: anaplasmosis (17.1%), theileriosis (15.6%), fasciolosis (7.0%), parasitic gastroenteritis (3.3%), trypanosomosis (3.2%) and babesiosis (0.2%). The low disease morbidity observed could be attributed in part to a degree of genetic resistance to both ticks and tick-borne diseases possessed by indigenous cattle (Norval *et al.*, 1992; Lawrence *et al.*, 1996). It could also be attributed to the fact that most helminth infections are subclinical under adequate nutritional levels and clinical disease only occurs when the nutritional level subsequently declines (Waruiru *et al.*, 1993).

Only a few cattle secreted *Schistosoma* eggs and no cases of schistosomosis were found either. The low detection rate of infection was probably, in part, attributed to in-depth diagnostic procedures using the more sensitive syringe filtration or the Teesdale smear technique not being undertaken because of the low prevalence of the disease that did not warrant so. However, the low prevalence of bovine schistosomosis could also be attributed to the highly focalised nature of this disease in endemic areas due to the underlying aggregated distribution of intermediate snail hosts (de Bont and Vercruysse, 1998) that could have led to missing of infections in the villages studied.

Cases of cowdriosis were not encountered, despite abundance of the tick vector *A. variegatum*. Usually most cases of cowdriosis are diagnosed after death (Cowdry, 1926). In addition, there were no appropriate tools available to undertake cowdriosis serology. However, bovine cowdriosis is often fatal especially in susceptible cattle introduced into *Amblyomma*-infested areas, but cases are rare in indigenous cattle found in endemic areas (Losos, 1986).

Regarding seroconversion, a few cattle in the study area experienced secondary immune response to *A. marginale* (13%), *B. bigemina* (12.2%) and *T. parva* infections (6%). Probably this was due to seasonal variation in the tick challenge at village level or tick control measures and management practices such as keeping animals under stall-feeding that are reported to limit exposure to infection and create susceptible populations of cattle (Perry and Young, 1995; Maloo *et al.*, 2001c). In absence of rechallenge, antibodies to *T. parva* generated in a primary immune response (seroconversion) decline to negative levels within 6 months post-infection (O'Callaghan, 1998). However, none of the cattle in the study area were kept under stall-feeding system and not much in terms of tick control was practised in the production system studied. It is possible there could have been some irregular tick control such as tick hand picking carried out on the few animals that had multiple seroconversions. Definitely, seasonal variation of the abundance of *Rhipicephalus* and *Boophilus* species ticks on cattle was observed in some villages (see Figure 6.11 and 6.12).

Serological profiles of cattle of different age groups that had more than one seroconversion were examined. For *A. marginale* infections, most calves up to 6 months of age and a proportion of calves of 7-12 months old experienced seroconversion, but for calves of 7-12 months old that had already seroconverted, experienced a decline of antibodies. Older animals in the age groups 13-24 and >24 months experienced fluctuation of antibodies around the threshold. Most of these animals belonged to villages with a low tick challenge as regards

Boophilus decoloratus and *Rhipicephalus evertsi evertsi* that are major vectors of *A. marginale* infections in Uganda (Okello-Onen *et al.*, 1999). Under conditions of low or fluctuating vector populations and limited parasite reservoirs, clearance of *A. marginale* parasites from the blood stream is known to occur, followed by disappearance of antibodies to the parasites (Potgieter and Stoltz, 1994).

For serological profiles of *B. bigemina* infections, most calves up to 6 months of age initially experienced a decline of antibodies (maternal) to negative levels, followed by seroconversion. Antibody levels remained high for about 6 months before dropping to negative levels, which probably implied these calves had high levels of maternal antibodies. It was also observed that most of the cattle of age groups: 7-12, 13-24 and >24 months whose antibody levels fluctuated around the threshold belonged to villages with low tick challenge of *Boophilus* spp. ticks. Fluctuation in antibody level was probably due to low vector populations that were probably too low to constitute a critical level of tick infestation required for maintenance and transmission of babesial infections in *Bos indicus* cattle (Jongejan *et al.*, 1988).

For serological profiles of *T. parva* infections, generally calves up to 6 months old experienced seroconversion, while cattle of older age groups (7-12m, 13-24m and >24m) experienced fluctuation of antibody levels around the threshold. Most of these animals belonged to villages with a low tick challenge of *R. appendiculatus*. Fluctuation of antibody levels against *T. parva* infection in cattle has been attributed to a combination of low vector population with low sporozoite infection rates (Perry and Young, 1995; Maloo *et al.*, 2001c). This finding suggests that vector populations and probably the sporozoite infection rates in these villages were not sufficient to maintain rechallenge of cattle older than 6 months.

The seroconversion rates to *A. marginale* infection of cattle of all age groups under low tick challenge were higher than those of their counterparts in the high tick challenge zone. These

differences in seroconversion rates to *A. marginale* infections could probably be attributed to existence of endemic stability under high tick challenge and endemic instability in the low tick challenge zone. It has been postulated that with endemic stability the force of infections increases with increasing age (Coleman *et al.*, 2001) and thus the proportion of susceptible population decreases, which leads to decrease in seroconversion rate. This implied that the force of infection was lower, while the proportion of susceptible population and the seroconversion rate were higher under low tick challenge as compared to high tick challenge.

The general trend of seroconversion was similar between cattle under the low and high tick challenge probably because *A. marginale* infections are transmitted by several vectors including *B. decoloratus* and *R. evertsi evertsi* (Okello-Onen *et al.*, 1999), and the biting flies (Ristic, 1968; Potgieter and Stoltz, 1994) whose distribution could have cut across the villages studied. This similarity in trend of seroconversion under low and high tick challenge could also be explained by the existence of domestic reservoirs, existence of life-long carriers of *A. marginale* infections after clinical disease and the occurrence of endemic stability even where there is very low tick challenge, thus maintaining high infection rates in vectors (Norval *et al.*, 1984).

As regards seroconversion to *B. bigemina* infection, generally older cattle under high tick challenge had higher seroconversion rates than their counterparts under low tick challenge. This finding is probably explained by the fact that low abundance of *Boophilus* spp. ticks has been reported on Zebu cattle in Uganda (Okello-Onen *et al.*, 2003), which has been attributed to Zebu cattle having a higher degree of host resistance to *Boophilus* spp. than other tick species (Kaiser *et al.*, 1982; Mattioli *et al.*, 1995). With low tick intensity, any increment in *Boophilus* intensity would increase chances of transmission of *B. bigemina* infection. Moreover, low parasitaemias in *Bos indicus* cattle increase the critical level of tick infestation required for the maintenance of babesial infections in *Bos indicus* as compared to *Bos taurus* (Jongejan *et al.*,

1988). Hence transmission is favoured by a higher intensity of *Boophilus* infestation under high tick challenge than low tick challenge.

Contrary to older cattle, calves of up to 6 months old under the low tick challenge had a higher seroconversion rate to *B. bigemina* infection than those under high tick challenge. This probably implied that there were a higher proportion of susceptible calves under low tick challenge than high tick challenge, which probably implied that more calves were borne to seronegative dams under low tick challenge as compared to high tick challenge. Thus fewer calves under the low tick challenge had sufficient passive transfer of maternal antibodies through colostrum than those under high tick challenge (O'Callaghan, 1998). With a large proportion of naïve and susceptible calves, the rate of seroconversion would be high even with the slightest increase in the tick intensity.

Under the high tick challenge, it was observed that the seroconversion rate in calves of up to 6 months old was low and seroconversion increased with age. Given that seroconversion rates to *B. bigemina* increased with increasing tick intensity, the observation that seroconversion rates increased with age, probably implied older cattle (7-12m, 13-24m and >24m) had higher tick intensity than calves below 6 months old. Differences in age-related infestation of Zebu cattle by *Boophilus* spp. ticks have been reported by others in Uganda (Okello-Onen *et al.*, 2003) and have been attributed to differences in age-related resistance to *Boophilus* spp. ticks by *Bos indicus* cattle (Jongejan *et al.*, 1988; Okello-Onen *et al.*, 2003).

Regarding *T. parva* infection, generally, seroconversion rates remained at the same level in all age groups under low tick challenge, but decreased with age under high tick challenge. This trend seemed to suggest that probably there existed endemic stability under high tick challenge and endemic instability under low tick challenge. Under endemic stability, the force of infection increases with age (Coleman *et al.*, 2001) leading to reduction in the proportion of

susceptible individuals and decrease in seroconversion rate. Increase in the force of *T. parva* infections under high tick challenge was probably associated with the continuous challenge due to development of several generations of *R. appendiculatus* per year, accompanied with the likely high level of *T. parva* sporozoite infection rates in adult ticks (Perry and Young, 1995; Maloo *et al.*, 2001c). On the contrary, under low tick challenge, most likely there was a less sustained tick challenge and therefore a certain proportion of cattle of all ages remained susceptible and seroconverted whenever there was a rise in tick challenge and *T. parva* sporozoite infection rate.

In conclusion, anaplasmosis, theileriosis, fasciolosis, parasitic gastroenteritis, trypanosomosis and babesiosis are the most important endemic bovine diseases under the mixed crop-livestock production system in South East Uganda. In reference to patterns of tick-borne diseases, there exists seasonal variation in *Rhipicephalus* spp. and *Boophilus* spp. tick challenge in some locations in South East Uganda. As a consequence of this variation in tick challenge, indigenous cattle in these locations experience high seroconversion rates for *A. marginale* and *T. parva* infections, but low seroconversion rates for *B. bigemina* infections, implying a large proportion of them are susceptible to tick-borne diseases during seasons when there is insufficient exposure to tick challenge. As an implication to disease control, the highest incidence of clinical disease due to tick-borne diseases is expected in these locations with low tick challenge, especially during seasons when tick abundance increases.

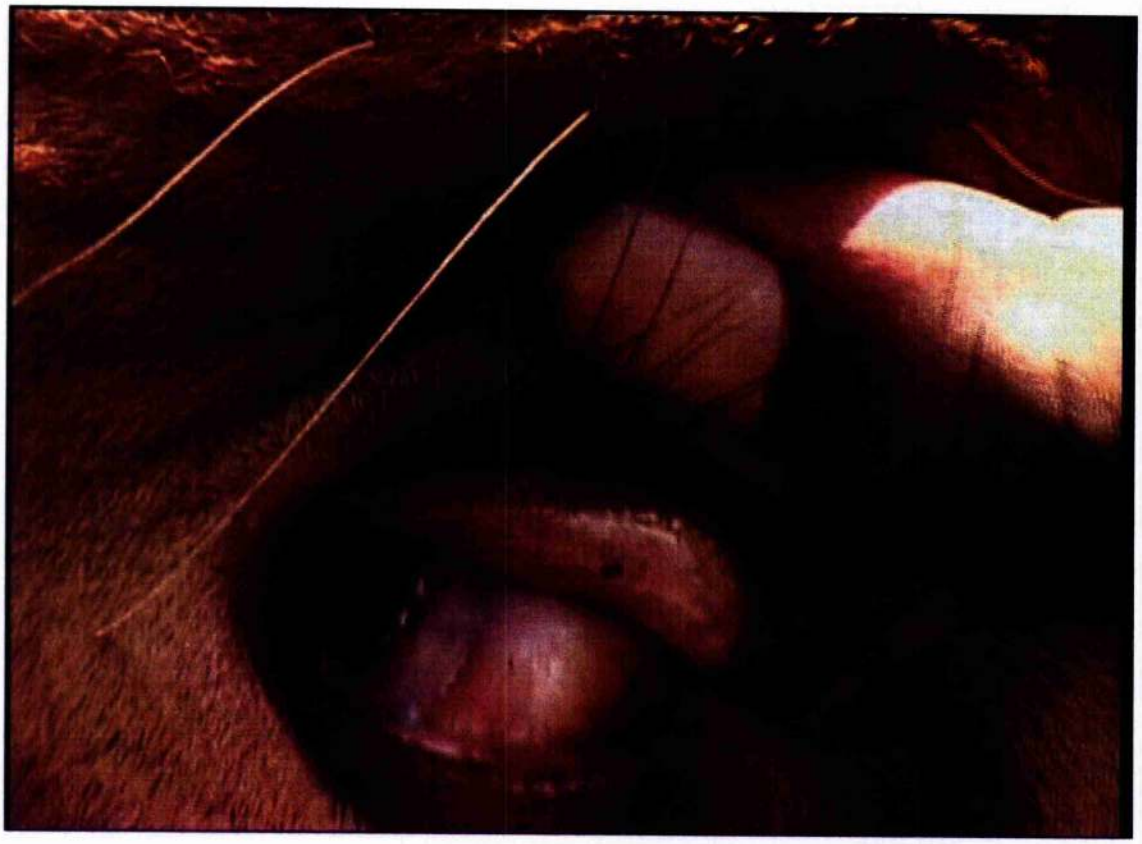


Figure 7.1: Checking pallor of ocular mucous membrane of a cow in Uganda - pallor of mucous membranes is an indication of anaemia, a clinical feature associated with trypanosomosis, anaplasmosis, babesiosis, haemonchosis and fasciolosis

Chapter 7 Field studies on clinical signs of bovine endemic diseases in Uganda

7.1 Introduction

Designing practical decision support tools to aid in differential diagnosis of livestock diseases requires scientifically confirmed data on probability of a disease given the clinical signs (Morley, 1991). Examples of diagnostic decision support tools in veterinary medicine designed out of such data include BOVID (Blood *et al.*, 1990) and a model for bovine clinical biochemistry measurements (Knox *et al.*, 1998). However, there is paucity of datasets containing quantitative information on clinical signs of livestock diseases. The pathogenesis and characteristic clinical signs of livestock diseases have been described in several textbooks of veterinary medicine (Fiennes, 1970; Ristic, 1981; Losos, 1986; Radostits *et al.*, 1994), but often the probability of all signs occurring in all disease states is unknown (Morley, 1991). For instance, it has been recognized that similar clinical signs can arise in animals with different diseases and clinical signs can vary in different animals with the same disease within a population (McKendrick *et al.*, 2000). To design decision support tools that can tackle this complex situation of differential diagnosis requires data on clinical signs from field cases. However, such datasets indicating clinical signs with significant association to presence of aetiological agents and of high diagnostic value for the respective diseases are scarce. Therefore the aim of this study was to collect such data for developing urgently required decision support tools for diagnosis of endemic bovine diseases in sub-Saharan Africa.

This Chapter describes findings of field studies conducted on cattle in South East Uganda to identify the most important clinical signs associated with bovine pathogens in field cases with particular interest on trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis. In addition, analysis was performed to

identify clinical signs and risk factors that could best be used to predict the probability of seroconversion to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in cattle.

7.2 Materials and Methods

Clinical, parasitological and serological data collected during the longitudinal study described in Chapter 3 were utilised in this chapter.

7.2.1 Data analysis

For purposes of data analysis, disease cases were individual animals that had at least one clinical signs in addition to presence of aetiological agents. Non-diseased individuals constituted a group of healthy animals as defined in Chapter 3 Section 3.9. The chi-square test was performed to establish the strength of association between clinical signs observed and presence of aetiological agents for the various diseases, using the computer programme EpiInfo 2000 (Centre for Disease Control, Atlanta, USA).

Comparisons of important clinical features between seroconverted and non-seroconverted cattle to *A. marginale*, *B. bigemina* and *T. parva* infections during the acute phase, convalescent phase and entire seroconversion episode (see Chapter 6, Section 6.3.5) were undertaken using binary logistic regression, performed on both binary and continuous variables. Chi-square analysis was also performed for all the binary form of clinical features to establish important differences between seroconverted and non-seroconverted cattle to all the three diseases.

The binary independent variables for logistic regression included, tick challenge at village level, sex, weight loss, fever, anaemia, pallor of mucous membranes, lymph node enlargement, staring coat, diarrhoea and lacrymation. For purposes of analysis, tick challenge took the values, 1 for high and 0 for low (see Chapter 6, Figures 6.13 and 6.14). Sex took the values 1 for male and 0 for female. For independent variables, weight loss, fever, anaemia, pallor of

mucous membranes, lymph node enlargement, staring coat, diarrhoea and lacrymation, present took the value 1 and absence took the value 0. Continuous independent variables included, *Rhipicephalus* spp. and *Boophilus* spp. intensity on individual animals, rectal temperature, packed cell volume, haemoglobin concentration, lymph node size, age and condition score. Tick intensity had the values 0, 1, 2 and 3, which represented no ticks, up to 10 ticks, 11-50 ticks and over 50 ticks, respectively. Rectal temperature had continuous values in degrees Celsius and packed cell volume had continuous values in percentage. Haemoglobin concentration had continuous values in g/dl and lymph node size was in centimetres. Age, a categorical variable, had values 0 for up to 6 months old, 1 for 7-12 months, 2 for 13-24 months and 3 for > 24 months. Condition score had values of 1 to 9 (see Chapter 3, Section 3.7.2).

Rhipicephalus spp. and *Boophilus* spp. intensity, rectal temperature, packed cell volume, haemoglobin concentration, lymph node size and condition score of cattle that seroconverted to *A. marginale*, *B. bigemina* and *T. parva* infections during the acute phase, convalescent and entire seroconversion episode were compared to those of non-seroconverted cattle using the student t-test.

Logistic regression was performed using the computer programme Minitab (Minitab Statistical Software, Minitab Inc., Pennsylvania, U.S.A). The binary logistic model equation for predicting the probability of presence of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infection in cattle was as follows:

Logit (seroconversion) = $b_0 + b_1$ (Tick challenge) + b_2 (Age) + b_3 (Sex) + b_4 (Anaemia) + b_5 (Weight loss) + b_6 (Lymph node enlargement) + b_7 (Staring coat) + b_8 (Diarrhoea) + b_9 (Lacrymation) + b_{10}(Fever) + b_{11}(Pallor of mucous membranes) (1)

In the model, tick challenge was also substituted with *Rhipicephalus* spp. intensity for *T. parva* analyses or with both *Rhipicephalus* spp. and *Boophilus* spp. intensity for *A. marginale* analyses or with *Boophilus* spp. intensity for *B. bigemina* analyses. Anaemia, weight loss,

lymph node enlargement and fever were also substituted with PCV, condition score, lymph node size and rectal temperature, respectively.

7.3 Results

7.3.1 Association of clinical signs to presence of aetiological agents of endemic diseases under consideration

The strength of association between clinical signs observed and presence of aetiological agents for trypanosomosis, anaplasmosis, theileriosis, babesiosis, fasciolosis and parasitic gastroenteritis in cattle examined in South East Uganda is shown in Tables 7.1 to 7.6. Anaemia, pallor of mucous membranes, fever, enlarged lymph nodes, lacrymation, staring coat and weight loss had a significant association ($P < 0.05$) to presence of trypanosomes (Table 7.1). Anaemia, pallor of mucous membranes, fever, weight loss and staring coat had a highly significant association ($P < 0.001$) to presence of *A. marginale* parasites (Table 7.2). Fever, enlarged lymph nodes, lacrymation, anaemia, pallor of mucous membranes, staring coat and diarrhoea had a significant association ($P < 0.05$) to the presence of *Theileria spp.* piroplasms and /or macroschizonts (Table 7.3).

Anaemia, pallor of mucous membranes and diarrhoea had a significant association ($P < 0.05$) to the presence of *Babesia* spp. piroplasms (Table 7.4). Anaemia, pallor of mucous membranes, weight loss, staring coat and diarrhoea had highly significant association ($P < 0.001$) to presence of *Fasciola* eggs (Table 7.5). Weight loss, staring coat, anaemia and pallor of mucous membranes had highly significant association ($P < 0.001$) to the presence of worm eggs of over 400 e.p.g, but diarrhoea did not have a significant association ($P = 0.92$) (Table 7.6). A few cattle secreted *Schistosoma* eggs on few occasions, but no clinical signs had any significant association to presence of *Schistosoma* eggs.

Table 7.1: Strength of association between clinical signs observed and presence of trypanosomes in cattle in South East Uganda

	No. of cattle					
Clinical sign	**Infected**	**Non-infected**	χ^2	**OR**	**95% CI**	**P**
Fever						
Present	30	46	164.59	12.66	7.61-21.01	<0.001
Absent	204	3961				
Lacrymation						
Present	41	339	21.16	2.30	1.59-3.32	<0.001
Absent	193	3668				
Diarrhoea						
Present	3	50	0.07	1.03	0.25-3.45	0.79
Absent	231	3957				
Staring coat						
Present	91	994	22.30	1.93	1.46-2.56	<0.001
Absent	143	3013				
Enlarged lymph nodes						
Present	119	1112	56.17	2.69	2.05-3.54	<0.001
Absent	115	2895				
Pallor						
Present	34	110	90.05	6.02	3.91-9.24	<0.001
Absent	200	3897				
Weight loss						
Present	9	72	3.92	2.19	1.01-4.59	<0.05
Absent	225	3935				
Anaemia						
Present	74	188	273.05	9.40	6.79-12.98	<0.001
Absent	160	3819				

OR = Odds ratio, CI = confidence interval, P= level of significance

Table 7.2: Strength of association between clinical signs observed and presence of presence of *Anaplasma marginale* parasites in cattle in South East Uganda

		No. of cattle					
Clinical sign		**Infected**	**Non-infected**	χ^2	**OR**	**95% CI**	**P**
Fever							
	Present	61	46	63.44	4.36	2.91-6.55	<0.001
	Absent	1204	3961				
Staring coat							
	Present	585	994	209.61	2.61	2.28-2.98	<0.001
	Absent	680	3013				
Pallor							
	Present	184	110	251.12	6.01	4.67-7.75	<0.001
	Absent	1081	3897				
Weight loss							
	Present	86	72	81.02	3.99	2.86-5.56	<0.001
	Absent	1179	3935				
Anaemia							
	Present	401	188	703.96	9.43	7.78-11.43	<0.001
	Absent	864	3819				

OR = Odds ratio, CI = Confidence interval, P= level of significance

Table 7.3: Strength of association between clinical signs and presence of *Theileria* spp. piroplasms and /or macroschizonts in cattle in South East Uganda

	No. of cattle					
Clinical sign	Infected	Non-infected	χ^2	OR	95% CI	P
Fever						
Present	55	46	58.66	4.28	2.83-6.49	<0.001
Absent	1106	3961				
Lacrymation						
Present	167	339	35.1	1.82	1.48-2.23	<0.001
Absent	994	3668				
Diarrhoea						
Present	28	50	7.44	1.96	1.19-3.19	<0.05
Absent	1133	3957				
Staring coat						
Present	581	994	269.39	3.04	2.65-3.49	<0.001
Absent	580	3013				
Enlarged lymph nodes						
Present	469	1112	67.19	1.76	1.54-2.03	<0.001
Absent	692	2895				
Pallor						
Present	176	110	263	6.33	4.90-8.18	<0.001
Absent	985	3897				
Weight loss						
Present	83	72	86.8	4.21	3.01-5.88	<0.001
Absent	1078	3935				
Anaemia						
Present	352	188	62.9	8.84	7.26-10.77	<0.001
Absent	809	3819				

OR = Odds ratio, CI = confidence interval, P= level of significance

Table 7.4: Strength of association between clinical signs and presence of *Babesia* spp. piroplasms in cattle in South East Uganda

	No. of cattle					
Clinical sign	**Infected**	**Non-infected**	χ^2	**OR**	**95% CI**	**P**
Fever						
Present	1	46	0.02	2.53	-	0.88
Absent	34	3961				
Lacrymation						
Present	3	339	0.08	1.01	0.25-3.48	0.77
Absent	32	3668				
Diarrhoea						
Present	4	50	20.11	10.21	2.94-31.86	<0.001
Absent	31	3957				
Staring coat						
Present	13	994	2.2	1.79	0.85-3.73	0.13
Absent	22	3013				
Pallor						
Present	4	110	6.64	4.57	1.34-13.88	<0.05
Absent	31	3897				
Weight loss						
Present	0	72	0.03	0	0.00-7.81	0.87
Absent	35	3935				
Anaemia						
Present	15	188	98.1	15.24	7.28-31.70	<0.001
Absent	20	3819				

OR = Odds ratio, CI = Confidence interval, P= level of significance

Table 7.5: Strength of association between clinical signs and presence of *Fasciola* eggs in cattle in South East Uganda

	No. of cattle					
Clinical sign	Infected	Non-infected	χ^2	OR	95% CI	P
Diarrhoea						
Present	23	50	17.86	2.93	1.72-4.95	<0.001
Absent	622	3957				
Staring coat						
Present	293	994	117.01	2.52	2.12-3.00	<0.001
Absent	352	3013				
Pallor						
Present	71	110	99.23	4.38	3.17-6.05	<0.001
Absent	574	3897				
Weight loss						
Present	30	72	19.8	2.67	1.69-4.20	<0.001
Absent	615	3935				
Anaemia						
Present	104	188	121.49	3.91	3.00-5.08	<0.001
Absent	541	3819				

OR = Odds ratio, CI = Confidence interval, P= level of significance

Table 7.6: Strength of association between clinical signs and presence of gastrointestinal nematode egg counts of > 400 e.p.g in cattle in South East Uganda

	No. of cattle					
Clinical sign	Infected	Non-diseased	χ^2	OR	95% CI	P
Diarrhoea						
Present	4	50	0.01	0.92	0.28-2.67	0.92
Absent	344	3957				
Staring coat						
Present	180	994	116.46	3.25	2.59-4.08	<0.001
Absent	168	3013				
Pallor						
Present	49	110	113.75	5.81	4.00-8.41	<0.001
Absent	299	3897				
Weight loss						
Present	23	72	32.53	3.87	2.32-6.41	<0.001
Absent	325	3935				
Anaemia						
Present	79	188	177.34	5.97	4.41-8.06	<0.001
Absent	269	3819				

OR = Odds ratio, CI = Confidence interval, P= level of significance

7.3.2 *Clinical signs and risk factors for prediction of seroconversion to A. marginale, B. bigemina and T. parva infections in cattle*

7.3.2.1 Seroconversion to A. marginale infection in cattle

The results of binary logistic analysis shown in this section were based on the model equation 1(see Section 7.2.1). Clinical features for predicting the probability of presence of seroconversion to *A. marginale* in cattle during the acute phase, convalescent phase and entire seroconversion episode, respectively, are shown in Tables 7.7 to 7.9.

Rhipicephalus spp. and *Boophilus* spp. intensity on individual animals, rectal temperature and PCV were the significant clinical features ($P < 0.05$) for prediction of seroconversion to *A. marginale* infection during the acute phase (Table 7.7). The overall model was highly significant ($P < 0.001$) according to the model chi-square statistic. Rectal temperature had a significant positive coefficient while PCV, *Rhipicephalus* spp. and *Boophilus* spp. intensity had significant negative coefficients. These results suggest that increase in *Rhipicephalus* spp. and *Boophilus* spp. intensity was associated with a decrease in the risk of seroconversion to *A. marginale*. Every unit increase in PCV was associated with a 0.97 fold decrease in the risk of seroconversion to *A. marginale* and every unit increase in rectal temperature was associated with 1.23 fold increase in the risk of seroconversion.

The level of tick challenge at village level was highly significant ($P < 0.001$) for prediction of seroconversion to *A. marginale* infections during the convalescent phase (Table 7.8). The overall model was significant ($P < 0.05$) and level of tick challenge had a significant negative coefficient. From these results, the risk of seroconversion to *A. marginale* infection by cattle under high tick challenge was 0.64 fold less than those under low tick challenge.

For the entire seroconversion episode, *Rhipicephalus* spp. intensity, rectal temperature and PCV were significant ($P < 0.05$) for prediction of seroconversion to *A. marginale* infection

(Table 7.9). The results suggest that every unit increase in *Rhipicephalus* spp. intensity was associated with a 0.87 fold decrease in the risk of seroconversion and every unit increase in PCV was associated with a 0.98 fold decrease in risk of seroconversion. However, every unit increase in rectal temperature was associated with 1.15 fold increase in risk of seroconversion to *A. marginale* infection.

The level of tick challenge was confirmed through further analysis to be a significant factor for prediction of seroconversion to *A. marginale* infection during the entire seroconversion episode. Cattle under low tick challenge had a highly significant risk of seroconversion than those under high tick challenge (χ^2= 19.7, DF = 1, P < 0.001).

Further analysis using the student t-test showed that seroconverted cattle had significantly lower mean PCV during the acute phase (T-value = 2.29, D.F. = 327, P < 0.05), but not during the convalescent phase (T-value = 1.33, D.F. = 335, P = 0.18). Seroconverted cattle had significantly higher mean rectal temperature than non-seroconverted ones during the acute phase (T-value = -2.10, D.F. = 311, P < 0.05), but not during the convalescent phase (T-value = -0.86, D.F. = 324, P = 0.38). The mean infestation intensities of seroconverted cattle with *Rhipicephalus* spp. was significantly lower than that of non-seroconverted cattle during the acute phase (T-value = 2.84, D.F. = 325, P < 0.05), but not during the convalescent phase (T-value = 0.47, D.F. = 313, P = 0.64). Likewise, the mean infestation intensities of seroconverted cattle with *Boophilus* spp. was significantly lower than that of non-seroconverted ones during the acute phase (T-value = 312, D.F. = 375, P < 0.05), but not during the convalescent phase (T-value = -0.03, D.F. = 343, P = 0.97).

Overall, predictors for seroconversion to *A. marginale* infection in cattle during entire seroconversion episode included *Rhipicephalus* spp. intensity, rectal temperature and PCV. For the acute phase, they included *Rhipicephalus* spp. and *Boophilus* spp. intensity on individual

animals, rectal temperature and PCV. For the convalescent phase, the level of tick challenge at village level was the only predictor.

Table 7.7: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *A. marginale* infection in cattle during the acute phase

	Dependent variable				
Independent variables	**Seroconversion n = 2034**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P**	**OR**	**95% CI**
Constant	-8.4680	3.8560	<0.05		
Rhipicephalus spp. intensity	-0.2078	0.0853	<0.05	0.81	0.69 - 0.96
Boophilus spp. intensity	-0.2692	0.1274	<0.05	0.76	0.60- 0.98
Rectal temperatures	0.2063	0.0983	<0.05	1.23	1.01 - 1.49
PCV	-0.0334	0.0138	<0.05	0.97	0.94 - 0.99
Chi-square statistic for overall model = 23.222, DF = 4, P < 0.001					

P = level of significance, OR = odds ratio, CI = confidence interval

Table 7.8: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *A. marginale* infection in cattle during the convalescent phase

	Dependent variable				
Independent variables	**Seroconversion n = 2095**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P**	**OR**	**95% CI**
Constant	-1.6776	0.1079	<0.001		
Tick level	-0.4501	0.1375	<0.001	0.64	0.49 - 0.83
Chi-square statistic for overall model = 14.698, DF = 3, P-Value = <0.05					

P = level of significance, OR = odds ratio, CI = confidence interval

Table 7.9: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *A. marginale* infection in cattle during the entire seroconversion episode

	Dependent variable				
Independent variables	Seroconversion n = 2283				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P**	**OR**	**95% CI**
Constant	-5.7270	2.9330	<0.05		
Rhipicephalus spp. intensity	-0.1411	0.0610	<0.05	0.87	0.77 - 0.98
Rectal temperature	0.1403	0.0749	<0.05	1.15	0.99 - 1.33
PCV	-0.0240	0.0105	<0.05	0.98	0.96 - 1.00
Chi-square statistic for overall model = 14.698, DF = 3, P-Value = <0.05					

P = level of significance, OR = odds ratio, CI = confidence interval

7.3.2.2 Seroconversion to B. bigemina infection in cattle

Clinical features for prediction of seroconversion to *B. bigemina* infection in cattle during the acute phase, convalescent phase and the entire seroconversion episode are shown in Tables 7.10 to 7.12. The level of tick challenge at village level, weight loss, staring coat, lacrymation and age were significant ($P < 0.05$) for prediction of seroconversion to *B. bigemina* infection during the acute phase (Table 7.10). The overall model was highly significant ($P < 0.001$) and the level of tick challenge, weight loss and staring coat had significant positive coefficients, while lacrymation and age had significant negative coefficients. These results suggest that the risk of seroconversion of cattle to *B. bigemina* infections under high tick challenge was 1.43 fold more than that of cattle under low tick challenge. Cattle manifesting weight loss were 2.28 fold more likely to have seroconverted to *B. bigemina* infection that those that did not. In addition, cattle with a staring coat were 1.44 fold more likely to have seroconverted to *B. bigemina* infection that those with a normal coat. Furthermore, cattle manifesting lacrymation were 0.47 fold less likely to have seroconverted to *B. bigemina* infection than those that did not. The risk of seroconversion decreased with increasing age. Progression of animals from one age group (up to 6 months) to another (7-12 months) was associated with a 0.87 fold decrease in risk of seroconversion to *B. bigemina* infection.

The level of tick challenge, anaemia, lacrymation and age were significant ($P < 0.05$) for prediction of seroconversion to *B. bigemina* during the convalescent phase (Table 7.11). The overall model was highly significant ($P < 0.001$). The level of tick challenge and anaemia had significant positive coefficients, while lacrymation and age had significant negative coefficients. The results suggest that the risk of seroconversion to *B. bigemina* infection of cattle under high tick challenge was 1.56 fold more than under low tick challenge. Cattle with anaemia were 2.82 fold more likely to have seroconverted to *B. bigemina* infection than those without. In addition, cattle with lacrymation were 0.54 fold less likely to have seroconverted to *B. bigemina* infection than those without. Progression of animals from one age group (up to 6

months) to another (7-12 months) was associated with a 0.85 fold decrease in risk of seroconversion to *B. bigemina* infection.

The level of tick challenge, anaemia, staring coat, lacrymation and age were significant ($P < 0.05$) for prediction of seroconversion to *B. bigemina* infection during the entire seroconversion episode (Table 7.12). The overall model was highly significant ($P < 0.001$). The level of tick challenge, anaemia and staring coat had significant positive coefficients, while lacrymation and age had significant negative coefficients. From these results, the risk of seroconversion to *B. bigemina* of cattle under high tick challenge was 1.5 fold more than under low tick challenge. Cattle with anaemia were 1.78 times more likely to have seroconverted to *B. bigemina* infection than those without. In addition, cattle with a staring coat were 1.37 times more likely to have seroconverted to *B. bigemina* infection that those with a normal coat. On the contrary, cattle with lacrymation were 0.52 fold less likely to have seroconverted to *B. bigemina* infection than those without. Overall, progression of animals from one age group (up to 6 months) to another (7-12 months) was associated with a 0.86 fold decrease in risk of seroconversion to *B. bigemina* infection.

Further analysis confirmed that cattle under high tick challenge were at a higher risk of seroconversion to *B. bigemina* infection than their counterparts under a low tick challenge ($\chi^2 = 16.4$, DF = 1, $P < 0.001$). Staring coat had a highly significant association to seroconversion to *B. bigemina* infection ($\chi^2 = 12.5$, DF = 1, $P < 0.001$). Weight loss also had a significant association ($\chi^2 = 7.3$, DF = 1, $P < 0.05$) and anaemia too had a significant association to seroconversion to *B. bigemina* infection ($\chi^2 = 7.3$, DF = 1, $P < 0.05$). In addition, lacrymation had a significant association with seroconversion to *B. bigemina* infection ($\chi^2 = 11.2$, DF = 1, $P < 0.05$).

Comparison between seroconverted and non-seroconverted cattle indicated that cattle that had seroconverted to *B. bigemina* infection had significantly lower mean PCV than non-seroconverted ones during the convalescent phase (T-value = 3.51, D.F. = 288, P < 0.05), but not during the acute phase (T-value = -0.11, D.F. = 299, P = 0.91). Seroconverted cattle had significantly higher mean rectal temperature than non-seroconverted ones during the acute phase (T-value = -2.11, D.F. = 299, P < 0.05), but not during the convalescent phase (T-value = -1.47, D.F. = 289, P = 0.14). In addition, seroconverted cattle had significantly lower mean body condition score than non-seroconverted ones during the acute phase (T-value = 2.89, D.F. = 281, P < 0.05), but not during the convalescent phase (T-value = -1.11, D.F. = 295, P = 0.26). Furthermore, seroconverted cattle had significantly higher mean infestation intensity of *Boophilus* spp. than non-seroconverted cattle during the acute phase (T-value = -2.09, D.F. = 299, P < 0.05), but not during the convalescent phase (T-value = -0.41, D.F. = 282, P < 0.67).

Overall, the important predictors of seroconversion to *B. bigemina* during the entire episode included the level of tick challenge at village level, staring coat, weight loss, lacrymation and anaemia. For the acute phase, they included the level of tick challenge, weight loss, staring coat and lacrymation, while for the convalescent phase, they included the level of tick challenge, lacrymation and anaemia.

Table 7.10: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *B. bigemina* infection in cattle during the acute phase

	Dependent variable				
Independent variables	**Seroconversion n = 2149**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-2.1209	0.1828	< 0.001		
Tick level	0.3579	0.1476	<0.05	1.43	1.07-1.91
Weight loss	0.8228	0.3185	<0.05	2.28	1.22 - 4.25
Staring coat	0.3661	0.1534	<0.05	1.44	1.07 - 1.95
Lacrymation	-0.7544	0.3075	<0.05	0.47	0.26 - 0.86
Age	-0.1414	0.0654	<0.05	0.87	0.76 - 0.99

Chi-square statistic for overall model = 38.130, DF = 5, P < 0.001

Table 7.11: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *B. bigemina* infection in cattle during the convalescent phase

	Dependent variable				
Independent variables	**Seroconversion n = 2148**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-2.0683	0.1764	< 0.001		
Tick Level	0.4450	0.1491	<0.05	1.56	1.17 - 2.09
Anaemia	1.0352	0.231	<0.05	2.82	1.79 - 4.43
Lacrymation	-0.6093	0.298	<0.05	0.54	0.30 - 0.98
Age	-0.1581	0.0656	<0.05	0.85	0.75 - 0.97

Chi-square statistic for overall model = 41.296, DF = 5, P < 0.001

Table 7.12: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *B. bigemina* infection in cattle during the entire seroconversion episode

	Dependent variable				
Independent variables	**Seroconversion n = 2384**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-1.4036	0.1368	< 0.001		
Tick level	0.4036	0.1103	< 0.001	1.50	1.21- 1.86
Anaemia	0.5747	0.1992	<0.05	1.78	1.20 - 2.62
Staring coat	0.3147	0.1146	<0.05	1.37	1.09 - 1.71
Lacrymation	-0.6571	0.2212	<0.05	0.52	0.34 - 0.80
Age	-0.1528	0.0499	<0.05	0.86	0.78 -0.95
Chi-square statistic for overall model = 59.597, DF = 6, P < 0.001					

7.3.2.3 Seroconversion to T. parva infection in cattle

Clinical features for prediction of seroconversion to *T. parva* infection in cattle during the acute phase, convalescent phase and the entire seroconversion episode are shown in Tables 7.13 to 7.15. The level of tick challenge, lymph node enlargement and rectal temperature were significant ($P < 0.05$) for prediction of seroconversion to *T. parva* infection during the acute phase (Table 7.13). The overall model was highly significant ($P < 0.001$). The level of tick challenge had a significant negative coefficient, while lymph node enlargement and rectal temperature had significant positive coefficients. These results suggest that the risk of seroconversion to *T. parva* infection for cattle under high tick challenge was 0.39 fold less than for those under low tick challenge. Cattle with enlarged lymph nodes were 1.39 times more likely to have seroconverted to *T. parva* infection than those with normal lymph nodes. In addition, every unit increase in rectal temperature was associated with 1.24 fold increase in the risk of seroconversion to *T. parva* infection.

The level of tick challenge and lymph node enlargement were significant ($P<0.05$) for prediction of seroconversion to *T. parva* infection during the convalescent phase (Table 7.14). The overall model was highly significant ($P <0.001$). The level of tick challenge had a significant negative coefficient while lymph node enlargement had a significant positive coefficient. From these results, the risk of seroconversion to *T. parva* infection for cattle under high tick challenge was 0.40 times more than for those under low tick challenge. Cattle with enlarged lymph nodes were 1.39 times more likely to have seroconverted to *T. parva* infection than those with normal lymph nodes.

Rhipicephalus spp. intensity, lymph node enlargement and PCV were significant ($P < 0.05$) for prediction of seroconversion to *T. parva* infection in cattle during the entire seroconversion episode (Table 7.15). The overall model was highly significant ($P < 0.001$). *Rhipicephalus* spp. intensity and PCV had significant negative coefficients, while lymph node enlargement had a

positive coefficient. The results indicate that every unit increase in *Rhipicephalus* spp. intensity was associated with a 0.68 fold decrease in risk of seroconversion to *T. parva* infection. Furthermore, every unit increase in PCV was associated with a 0.98 fold decrease in risk of seroconversion to *T. parva* infection.

Further analysis confirmed that cattle under low tick challenge were at a higher risk of seroconversion to *T. parva* infection than their counterparts at high tick challenge (χ^2 = 70.3, DF = 1, P < 0.001). There was a significant association between lymph node enlargement and seroconversion to *T. parva* infection (χ^2 = 6.4, DF = 1, P < 0.05) and between fever and seroconversion to *T. parva* infection (χ^2 = 4.2, DF = 1, P < 0.05). These factors were important during the acute phase and entire seroconversion episode.

Comparisons between seroconverted and non-seroconverted cattle revealed that seroconverted cattle had significantly higher mean rectal temperature during the convalescent phase (T-value = 2.05, D.F. = 236, P < 0.05), but not during the acute phase (T-value = -1.74, D.F. = 231, P = 0.08). In addition, seroconverted cattle had a significantly lower mean infestation intensity of *Rhipicephalus* spp ticks than non-seroconverted ones during both the acute phase (T-value = 5.26, D.F. = 251, P < 0.001) and convalescent phase (T-value = 3.71, D.F. = 236, P < 0.001).

Overall, *Rhipicephalus* spp. intensity, lymph node enlargement and PCV were important predictors for seroconversion to *T. parva* infection in cattle. The level of tick challenge at village level, lymph node enlargement and rectal temperature were important predictors during the acute phase. While the level of tick challenge and lymph node enlargement were important during the convalescent phase.

Table 7.13: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *T. parva* infection in cattle during the acute phase

	Dependent variable				
Independent variables	**Seroconversion n = 2653**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-10.2890	4.0470	<0.05		
Tick Level	-0.9379	0.1489	<0.001	0.39	0.29 - 0.52
Lymph node enlargement	0.3299	0.1648	<0.05	1.39	1.01 - 1.92
Rectal temperature	0.2166	0.1056	<0.05	1.24	1.01 - 1.53
Chi-square statistic for overall model = 44.932, DF = 3, P < 0.001					

Table 7.14: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *T. parva* infection in cattle during the convalescent phase

	Dependent variable				
Independent variables	**Seroconversion n = 2655**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-1.9983	0.1181	< 0.001		
Tick level	-0.9081	0.1487	< 0.001	0.40	0.30 - 0.54
Lymph node enlargement	0.3267	0.1651	<0.05	1.39	1.00 - 1.92
Chi-square statistic for overall model = 38.838, DF = 2, P < 0.001					

Table 7.15: Maximum likelihood estimates of binary logistic model of clinical features for prediction of seroconversion to *T. parva* infection in cattle during the entire seroconversion episode

	Dependent variable				
Independent variables	**Seroconversion n = 2778**				
Predictor (x_i)	**Coefficient (b_i)**	**SE (b_i)**	**P-value**	**Odds Ratio**	**95% CI for odds ratio**
Constant	-0.6334	0.3947	0.109		
Rhipicephalus spp. intensity	-0.3889	0.0685	< 0.001	0.68	0.59 -0.78
Lymph node enlargement	0.2615	0.1241	<0.05	1.30	1.02 - 1.66
PCV	-0.0243	0.0117	<0.05	0.98	0.95 -1.00
Chi-square statistic for overall model = 41.481, DF = 3, P < 0.001					

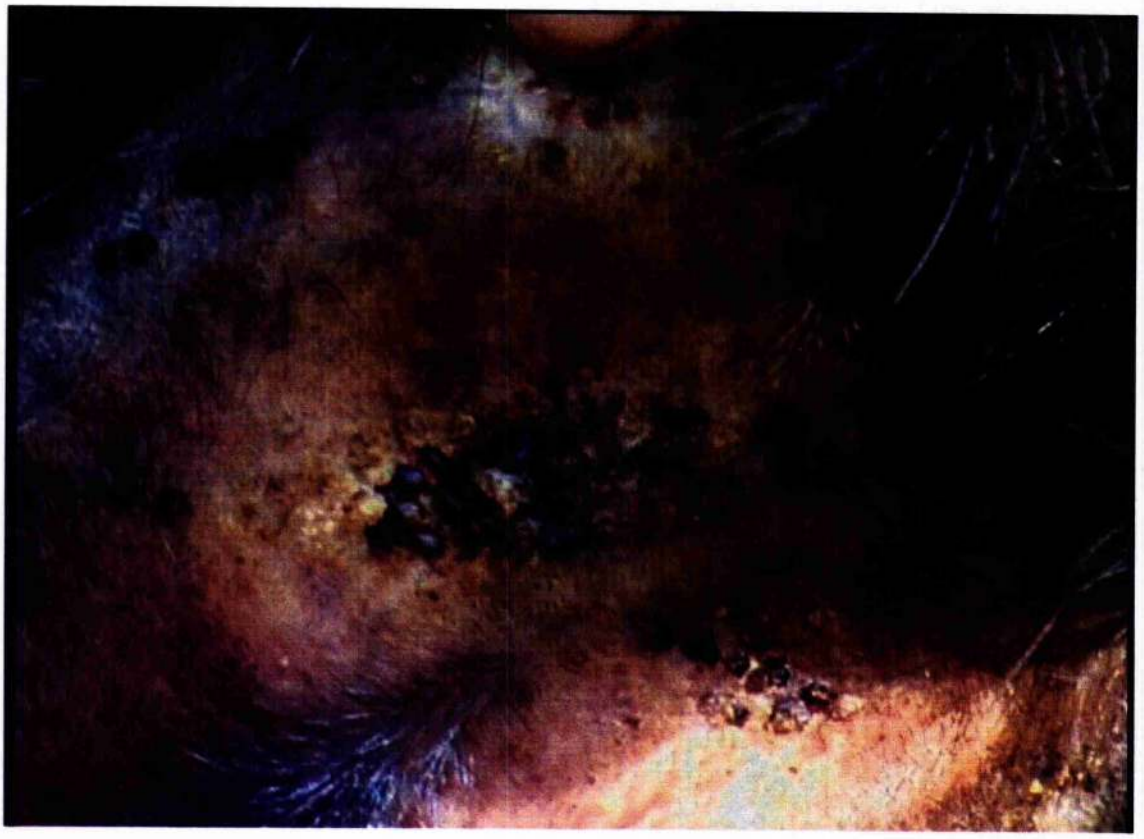


Figure 7.2: High infestation of *Rhipicephalus appendiculatus* in the ear of a cow in Uganda during the longitudinal study – the intensity of *R. appendiculatus* and fever were found to be the significant indicators for presence of seroconversion to *Theileria parva* infection in Zebu cattle

7.3 Discussion

The association of clinical signs to presence of actiological agents for trypanosomosis, anaplasmosis, theileriosis, babesiosis, fasciolosis and parasitic gastroenteritis in cattle under field conditions in South East Uganda was assessed in this study. In addition, the association of clinical signs and risk factors with seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infections in cattle was also analysed. For trypanosomosis, anaemia, pallor of mucous membranes, fever, enlarged lymph nodes, weight loss, staring coat and lacrymation were the clinical signs that had significant association. These clinical signs were thus of high diagnostic value for trypanosomosis. Anaemia is regarded as the principal clinical signs of trypanosomosis in cattle, often coupled with fluctuating fever, which corresponds to the waves of parasitaemia (Fiennes, 1970; Holmes *et al.*, 2000). Other clinical signs include weight loss, enlarged lymph nodes, pallor of mucous membrane and petechial haemorrhages (Fiennes, 1970; Stephen, 1986; Murray and Dexter, 1988; Holmes *et al.*, 2000).

For anaplasmosis, anaemia, pallor of mucous membranes, fever, weight loss and staring coat had highly significant association. These clinical signs are considered to be highly diagnostic for anaplasmosis, usually associated with both the acute and chronic forms of anaplasmosis (Ajayi *et al.*, 1987; Potgieter and Stoltz, 1994; Egbe-Nwiyi *et al.*, 1997). Staring coat was an unusual sign, but it is often closely associated with weight loss.

For theileriosis (East Coast fever), fever, enlarged lymph nodes, diarrhoea, lacrymation, anaemia, pallor of mucous membranes, weight loss and staring coat had significant associations. These clinical signs are therefore of high diagnostic value for East Coast fever. Fever, enlarged lymph nodes, diarrhoea and lacrymation are signs associated with cases of East Coast fever (Shannon, 1977; Omuse, 1978; Kiptoon *et al.*, 1983; Mbassa *et al.*, 1998;), but anaemia, pallor of mucous membranes, weight loss and staring coat were unusual clinical signs. Intense anaemia in East Coast fever cases was first observed at postmortem by Bruce *et al.* (1910) and it has also been reported in chronic cases of East Coast fever (Omuse, 1978; Mbassa

et al., 1994) observed in more resistant breeds of cattle such as Zebu and in animals with haemotropic parasite infections (Kiptoon *et al.*, 1983). Weight loss (emaciation) and staring coat have been reported in cases of East Coast fever in Zebu calves (Bruce *et al.*, 1910).

For babesiosis, anaemia, diarrhoea and pallor of mucous membranes had a significant association and were the only signs of high diagnostic value. Anaemia and pallor of mucous membranes in addition to high fever (41-45.5°C), haemoglobinuria and jaundice are important signs associated with acute babesiosis in cattle (Ristic, 1981; Losos, 1986). However, given the low pathogenicity of some *Babesia* species such as *B. bigemina* as compared to *B. bovis*, there is a variation in clinical signs and the disease syndromes (Losos, 1986). Anaemia, pallor of mucous membranes and diarrhoea in addition to weight loss are major signs associated with the chronic syndrome of babesiosis in cattle (Urquhart *et al.*, 1996).

For fasciolosis, anaemia, pallor of mucous membranes, weight loss, staring coat, diarrhoea and lacrymation had highly significant association and are of high diagnostic value. Anaemia, pallor of mucous membranes, weight loss and staring coat are important signs associated with fasciolosis (Urquhart *et al.*, 1996; Egbe-Nwiyi and Chaudrai, 1996; Bowman and Lynn, 1999). Weight loss and anaemia are usually associated with mild or chronic fasciolosis in cattle (Urquhart *et al.*, 1996; Magona and Mayende, 2002). Diarrhoea and lacrymation have been reported in field cases of intercurrent disease in Zebu cattle in Uganda involving fasciolosis with either theileriosis or trypanosomosis and parasitic gastroenteritis (Anon. 1997a).

For parasitic gastroenteritis, anaemia, weight loss, pallor of mucous membranes and staring coat had highly significant association. Anaemia, weight loss or emaciation, pallor of mucous membranes and staring coat are considered to be of high diagnostic value for parasitic gastroenteritis (Kaufmann *et al.*, 1989; Waruiru *et al.*, 1993; Eysker and Ploeger, 2000). High worm burdens predominantly of *Haemonchus contortus*, *Bunostomum phlebotum* and *Oesophagostomum radiatum* in gastrointestinal tracts of weaner calves were found to be

associated with manifestation of weakness, staring coat and extreme pallor of mucous membrane (Kaufmann *et al.*, 1989). Weight loss is an important clinical sign of chronic gastrointestinal nematode infections in older cattle, while anaemia, oedema, diarrhoea and anorexia are associated with severe cases of parasitic gastroenteritis observed in younger cattle (Eysker and Ploeger, 2000).

Diarrhoea, a sign considered to be of high diagnostic value for parasitic gastroenteritis (Kaufmann *et al.*, 1989; Waruiru *et al.*, 1993; Eysker and Ploeger, 2000), did not have a significant association to worm eggs per gram of faeces of field cases. Ganaba *et al.* (2002) also found no correlation between worm eggs per gram of faeces and presence of diarrhoea in calves in a study conducted in Burkina Faso. Overt parasitic gastroenteritis that leads to diarrhoea has been reported to be rare in traditionally managed indigenous cattle in Uganda (Sauvage *et al.*, 1974). Instead, the chronic form of parasitic gastroenteritis is more common and is usually associated with gradual weight loss and increased susceptibility to other diseases (Omara-opyene, 1985; Eysker and Ploeger, 2000).

Although some cattle secreted *Schistosoma* eggs on few occasions, no clinical signs had any significant association to presence of *Schistosoma* eggs. Bovine schistosomosis generally occurs as mild disease even when high prevalences of detected parasites in slaughter cattle are encountered and most schistosome infections in endemic areas occur at a subclinical level (de Bont and Vercruysse, 1998).

Important clinical signs and risk factors that could best be used to predict the probability of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infection in cattle were assessed. In the original experiment it was envisaged an informer would be contacted midway the sampling to report animals which had been sick since the previous sampling. This would considerably increase the chances of detecting animals with clinical disease because the clinical course of the disease fell entirely between two samplings. However, this system of consulting a village

informer proved difficult for logistical reasons. As a result the statistical power of the analysis was probably less than would have been possible if these intermediate consultations had been conducted. The impact of this effect would depend on the duration of a particular sign. The likelihood of detecting a significant association between any clinical sign and seroconversion would depend on the average duration of that sign in affected animals. Nevertheless, important differences between seroconverting and non-seroconverting animals to *A. marginale*, *B. bigemina* and *T. parva* infections in terms of clinical signs were found.

The level of tick challenge at village level, *Rhipicephalus* spp. and *Boophilus* spp. infestation intensity on individual animals, rectal temperature and packed cell volume were the significant predictors for presence of seroconversion to *A. marginale* infection in cattle. Indeed the level of challenge of *Rhipicephalus evertsi evertsi* in addition to that of *Boophilus* spp. is reported to determine the distribution of bovine anaplasmosis and its seroprevalence (Omuse, 1978; Jongejan *et al.*, 1988; Deem *et al.*, 1993; Latif *et al.*, 1995).

Following primary infection, incubation of anaplasmosis in adult cattle takes on average 26 days. Initially there is fever followed by severe anaemia, which takes over 4-15 days (Potgieter and Stoltz, 1994). Packed cell volume, a measure of anaemia and rectal temperature, a measure of fever, are important clinical manifestations of anaplasmosis (Ajayi *et al.*, 1987; Potgieter and Stoltz, 1994; Egbe-Nwiyi *et al.*, 1997). *Anaplasma* antigen-antibody complexes often occur in erythrocytes of infected cattle and are associated with erythrophagocytosis, which leads to anaemia. Both antiparasitic and anterythrocytic antibodies are reported to appear during *A. marginale* infection (Egbe-Nwiyi *et al.*, 1997). This explains the intimate associations between packed cell volume, a measure of anaemia and seroconversion to *A. marginale* infection. It should also be noted that anaemia and fever are the most long-lasting clinical signs associated with acute anaplasmosis, the more reason they are suitable for predicting presence of seroconversion to *A. marginale* infections.

Increasing intensity of *Rhipicephalus* spp and *Boophilus* spp. infestation of cattle was associated with a decrease in risk of seroconversion to *A. marginale* infection. This trend of seroconversion risk could probably have been attributed to increasing force of infection (Coleman *et al.*, 2001) associated with increasing tick intensity, which consequently was associated with decreasing proportion of cattle susceptible to *A. marginale* infection with the population.

It was noted that each phase of seroconversion to *A. marginale* infections had different predictors. Whereas *Rhipicephalus* spp. intensity on individual animals, rectal temperature and PCV were important predictors during the entire episode of seroconversion, *Rhipicephalus* spp. and *Boophilus* spp. intensity on individual animals, rectal temperature and PCV were important at the beginning of seroconversion (acute phase) and the level of tick challenge at village level was important at the end of seroconversion (convalescent phase).

For *B. bigemina* infection in cattle, the level of tick challenge at village level, *Boophilus* spp. intensity on individual animals, anaemia, weight loss, staring coat, lacrymation and age were the significant predictors of seroconversion. Anaemia, weight loss and staring coat are important signs associated with the chronic syndrome of babesiosis in cattle (Losos, 1986; Urquhart *et al.*, 1996), which appears to be the commonest form among indigenous Zebu cattle studied.

Lacrymation had a significant association to seroconversion to *B. bigemina* infection and decreasing intensity or absence of lacrymation had a positive effect on presence of seroconversion. The association between lacrymation and seroconversion to *B. bigemina* infection is not clear. However, lacrymation has been found by others (Khan and Dandiya, 1983) to be a response to pharmacological stimulus. During pathogenesis of babesiosis, pharmacologically active inflammatory substances such as plasma kinin and its precursor enzyme kallikrein are released (Losos, 1986). Probably lacrymation is a response to kallikrein

or other pharmacologically active agents released during pathogenesis of babesiosis; an issue that requires further investigation.

Boophilus spp. intensity had a significant positive coefficient, implying the risk of seroconversion to *B. bigemina* infections increased with increasing tick intensity. *Babesia bigemina* is readily transmitted transovarially by both *Boophilus decoloratus* and *Boophilus microplus* (De Vos, 1979) and in Uganda *B. decoloratus* is the most important vector (Okello-Onen *et al.*, 1998a).

Progression of animals from one age group (up to 6 months) to another (7-12 months) was associated with a decreasing risk of seroconversion to *B. bigemina* infection. Although this is contrary to fact that there is inverse age susceptibility in *B. bigemina* infection with young animals possessing innate resistance while older animals are fully susceptible (Losos, 1986; Jongejan *et al.*, 1988), there is increasing chance of exposure to tick challenge under this production system as animals become older. Hence the majority of adult cattle become protected with antibodies against *B. bigemina* infection, which subsequently reduced the proportion of susceptible individuals and the risk of seroconversion among older cattle.

It must be recognised, however, that the importance of predictor factors for seroconversion to *B. bigemina* infection differed with the phase of seroconversion. The level of tick challenge, staring coat, weight loss, lacrymation and anaemia were important factors during the entire episode, while the level of tick challenge, weight loss, staring coat and lacrymation were important for the acute phase and the level of tick challenge, lacrymation and anaemia were important for the convalescent phase.

For *T. parva* infections, the level of tick challenge at village level, *R. appendiculatus* intensity on individual animals, lymph node enlargement, rectal temperature and PCV were significant predictors for seroconversion to *T. parva* infection in cattle. Enlarged lymph nodes and fever

are important clinical signs associated with *T. parva* infection in cattle (Shannon, 1977; Omuse, 1978; Kiptoon *et al.*, 1983: Mbassa *et al.*, 1998; Maloo *et al.*, 2001b) and *R. appendiculatus* is an important vector of *T. parva* infection in Uganda (Okello-Onen *et al.*, 1998). Anaemia has been reported in chronic cases of East Coast fever in more resistant breeds of cattle such as Zebu (Omuse, 1978; Mbassa *et al.*, 1994) and in animals with concurrent disease involving theileriosis and haemotropic parasite infections (Kiptoon *et al.*, 1983).

On the other hand, the long duration of lymph node enlargement, fever (rectal temperature) and anaemia (PCV) during the process of East Coast fever in cattle could have made these clinical signs to be important predictors of seroconversion to *T. parva* infection. Following primary infection after *R. appendiculatus* tick bites, the incubation period of *T. parva* infection takes on average 13 days and the duration of the diseases usually takes about 14 days (Bruce *et al.*, 1910; McKeever and Morrison, 1990). The disease commences with fever that stays on until death or recovery (about 7 days). Fever is often followed by swelling of superficial lymph nodes, on recovery of calves, both fever and the lymph nodes enlargement subside but lymph nodes never regain their normal size, instead remain permanently enlarged throughout life (Bruce *et al.*, 1910). Anaemia is associated with chronic forms of the East Coast fever; therefore it exhibits a long duration.

It was noted that *Rhipicephalus* spp. intensity on individual animals, lymph node enlargement and PCV were important predictors for seroconversion to *T. parva* infection during the entire seroconversion episode. The level of tick challenge at village level, lymph node enlargement and rectal temperature were important predictors during the acute phase, while the level of tick challenge and lymph node enlargement were important predictors during the convalescent phase.

In conclusion, anaemia (pallor), staring coat, weight loss and enlarged lymph nodes are good indicators of endemic diseases in cattle under the mixed crop-livestock production system in

South East Uganda. In addition, presence and intensity of the respective tick vectors for tick-borne diseases and generally clinical features whose duration is long such as anaemia, weight loss, staring coat and enlarged lymph nodes are good indicators of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infections in cattle. These good disease indicators could be exploited in the development of decision support tools for differential diagnosis of endemic bovine diseases.

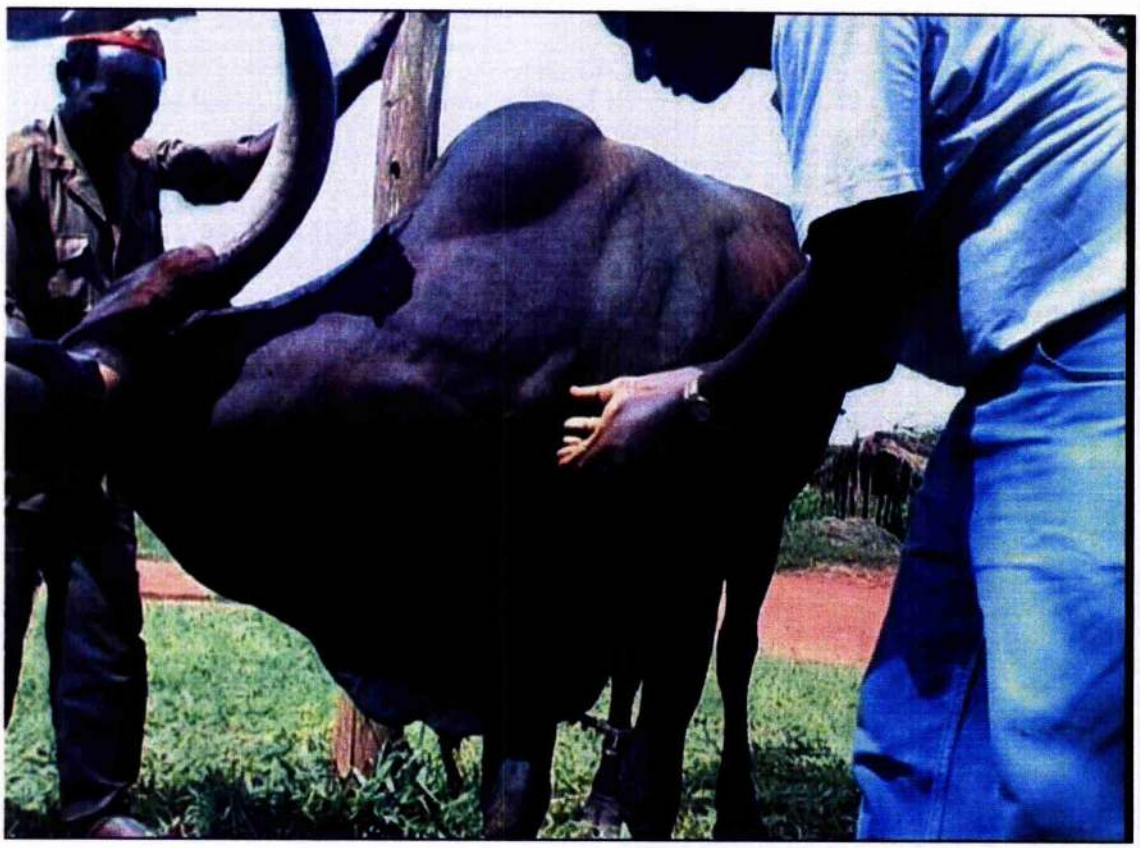


Figure 8.1: A Ugandan Ankole cow with an enlarged prescapular lymph node - an important clinical feature for diagnosis of trypanosomosis and East Coast fever

Chapter 8 Development of a low technology decision support system for diagnosis of endemic bovine diseases

8.1 Introduction

Serological, molecular and parasitological diagnostic tests for endemic bovine diseases in sub-Saharan Africa, such as trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, PGE, schistosomosis and fasciolosis described in Chapter 2 detect specific organisms or agents in animals that may or may not have evidence of clinical disease. Moreover, these tests are unavailable to groups such farmers, extension workers and agro-veterinary traders who make diagnosis and treatment decisions of endemic bovine diseases in Africa in the modern scenario (Bossche *et al.*, 2000b; Machila *et al.*, 2003), given the current reduction in state funding for veterinary services and under-utilisation of veterinary diagnostic laboratories by fee-paying farmers (Kenyon and Nour, 1996). Detection of clinical disease may be facilitated by use of a combination of clinical examination, simple diagnostic tests and decision support tools. Hence, there is an urgent need for development of low technology diagnostic decision support tools to facilitate detection of clinical livestock disease in rural areas of Africa.

Diagnostic decision support tools are useful when used by non-experts (Cockcroft, 1999a). These tools would therefore be suitable in developing countries, especially in Africa, where there is shortage of qualified veterinarians and thus animal health auxiliaries are used in the recognition, treatment and control of animal diseases (FAO, 1992). Decision support tools are commonly applied to disease diagnosis where they incorporate a set of rules for solving problems, details of clinical signs, lesions, laboratory results and opinions of experts (Thrusfield, 1995). Development of practical diagnostic decision support tools relies on the availability of comprehensive datasets (Blood *et al.*, 1990; Knox *et al.*, 1998). However, such datasets containing quantitative information on the frequency of occurrence of individual

clinical signs, haematological diagnostic features and epidemiological associations of livestock diseases are rare. In their absence, designers of such tools have had to rely on subjective expert opinions (Dewey *et al.*, 1992; McKendrick *et al.*, 2000). Quantitative information on the most important clinical signs and risk factors for clinical diagnosis of endemic bovine diseases was elicited from veterinary experts through a Delphi survey and was used to develop a decision support card. The entire process of this undertaking is described in this Chapter.

8.2 Materials and methods

8.2.1 Delphi survey

A questionnaire was developed with a list of thirty-four (Table 8.1) clinical signs and risk factors reported to be associated with various diseases under consideration, based on review of literature. It was self-administered according to the Delphi method (Linstone and Turoff, 1975). This method involved participants answering a short series of questions by mail. Once the answers from all or a reasonable number of participants were received, a summary of the collective results was sent back to each participant, who then had the opportunity to modify his or her original response in light of the general opinion. The process may involve several rounds and ceases once consensus has been attained among participants on the issue at hand.

Potential participants were identified through published literature and others in various fields were identified through professional contacts. Expertise was defined as extensive diagnostic or research experience with bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis with emphasis on sub-Saharan Africa. One hundred and twenty-eight experts from Africa (111), Europe (9), America (3) and Australia (5) were identified and asked to participate in the Delphi survey, concentrating on the diseases of their specialization. The participants consisted of 64 international scientists with extensive research experience on the selected diseases and 64 experts with extensive experience in clinical diagnosis and treatment of the diseases under

consideration. Of the 128 participants, 93, 75, 76, 75, 75, 82, 81 and 81 had expertise on bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis, respectively.

An explanatory covering letter and a copy of the questionnaire on the different diseases were mailed to relevant experts. In the first round, veterinary scientists were sent questionnaires only on diseases of their specialization, while veterinary practitioners were sent questionnaires on all the eight diseases under consideration. Panel members were asked to choose between five and ten of the clinical signs and risk factors listed that they thought were the most useful in clinical diagnosis of the diseases of their specialization. They were then asked to score the relative importance of the selected 5 to 10 clinical signs and risk factors using a scale comprising the values 1, 2, 3, 4 and 5, where 1 indicated lowest importance, while 5 indicated highest importance. Participants were further requested to base their scores on the their experience rather than textbook knowledge.

A second-round questionnaire was mailed to each respondent after compiling the responses from the first survey. Participants were sent a questionnaire with all the original clinical signs and risk factors together with consensus and individual results from the first round. In addition, questions in the second-round questionnaire were rephrased to make them clearer and to avoid confusion that led to some participants reversing the scores during the first round. Consensus results consisted of a list of clinical signs and risk factors chosen by all participants specialising on a given disease with the mean scores arranged in a descending manner. Individual results consisted of a list of clinical signs and risk factors chosen by each individual for the various diseases of his or her interest with mean scores arranged in a descending fashion.

After the completed questionnaires from the second round were received, the final mean scores for the chosen clinical signs and risk factors for each of the diseases: trypanosomosis,

theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis, were calculated. In cases where participants had not returned the second-round questionnaires, their first-round answers were used. Visual inspection of frequency plots of each clinical sign and risk factor chosen for each disease for bimodal distribution was done to assess whether the panel had reached consensus after the second round (Brunceau *et al.*, 1999).

8.3 Results

8.3.1 Delphi survey results

Forty-six of the 128 people asked to participate returned the first-round questionnaire. They consisted of 32 veterinary scientists and 14 veterinary practitioners. Twenty-six, 21, 23, 23, 20, 23, 22 and 12 answered questions regarding trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis, respectively. Twenty-seven of the 46 original participants returned the second-round questionnaire. These included 15 veterinary scientists and 12 veterinary practitioners. Nineteen, 17, 16, 16, 16, 14, 15 and 6 responded to questions on trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis, respectively. The non-responsive rate during the first round was high (64 %), but was low (41 %) during the second round. The interval between mailing the first- and second-round questionnaires was approximately six months. Bimodal distribution occurred in some factors during the second round and a change of scores was witnessed within groups between the successive survey rounds. The overall scores for individual clinical signs and risk factors are shown in Table 8.2 and a summary of the top clinical signs (risk factors) for the various diseases are shown in Table 8.3. These top clinical signs (risk factors) were those chosen by at least 30% (an arbitrarily threshold) of the participants in each disease group.

8.3.2 Incorporation of data into a decision support tool

The overall scores of clinical signs of the targeted diseases were incorporated into a decision support tool, which was designed based on a combination of the pattern-matching and colour-banding techniques (Cockcroft, 1999a; Middleton, 2001).

8.3.2.1 Standardization of data

Because the scores in Table 8.2 were based on different numbers of experts in each disease category: anaplasmosis (23), babesiosis (23), cowdriosis (20), fasciolosis (23), PGE (22), schistosomosis (12), theileriosis (21) and trypanosomosis (26), they had to be scaled by a factor (each score was divided by the number of experts in the respective disease category) to equalise all disease categories before removal of the least important clinical signs. This step made all scores for clinical signs in each disease category to add up to 30. After standardization, clinical signs were then sorted in descending order of the their totals across disease categories to allow for selection of the most important clinical signs (Table 8.4).

8.3.2.2 Selection of clinical signs according to their diagnostic value

Selection of key clinical signs for each disease to be included into the diagnostic decision support tool was undertaken. For this purpose dendrograms displaying diseases in the sign space (Figure 8.2) and clinical signs in the disease space (Figure 8.3) were used. The dendrogram of diseases in the sign space illustrated how similar the target diseases are in terms of the clinical signs they manifest, while the dendrogram of clinical signs in the disease space illustrated the power of the clinical signs for the differential diagnosis of the target diseases. Figure 8.2 demonstrated that it is easy to distinguish among tick-borne disease and between tick-borne diseases from trypanosomosis and helminthoses using clinical signs under consideration. But it was less easy to distinguish between helminthoses and trypanosomosis and very difficult to distinguish between parasitic gastroenteritis and fasciolosis.

Figure 8.3 allowed identification of clinical signs, which were similar in terms of their power for the differential diagnosis of the diseases under consideration. Such signs, which were treated as sign pairs (sign X or Y), included, ataxia or abnormal behaviour (nervous signs), anaemia or pallor of mucous membranes, anorexia or depression, dyspnoea or coughing, and stunted growth or potbelly. Combination of clinical signs into pairs ultimately led to reduction of the number of clinical signs from 34 to 16 signs and sign-pairs, which were reasonable for inclusion into the decision support card.

8.3.2.3 Final set of data

The final set of data (Table 8.5) used to build the decision support card was attained after data standardization, elimination of least important clinical signs and combination of signs into sign-pairs. Least important clinical signs were removed because they provided little information to aid differential diagnosis. The data was then transformed into a prototype decision support card (Figure 8.4) using a scoring and colour-coding system: 0 to 4, in which 0, 1, 2, 3 and 4 represent values (colours), 0% (black); >0% (grey); >9% (yellow); >14% (orange) and >21% (red), respectively. The colours were selected arbitrarily.

8.3.2.4 Prototype decision support card

The prototype decision support card shown in Figure 8.4 is composed of a grid on top in which diseases included are listed. The clinical signs associated with these diseases are listed on the far left column. The colour band and the score reflect the weight of a sign state in the event that a disease is present. The basis of this card is the comparison of clinical signs observed with disease profiles. A list of differential diagnoses is constructed ranked in the order in which the disease profiles match the clinical signs observed. To make differential diagnosis, scores of the various sign states of each disease are added up and overall scores of the possible differentials are ranked. The disease with the highest total is considered the leading differential diagnosis. A

tie for the top rank is considered to signify a case of concurrent disease involving more than one disease.

Table 8.1: A list of eight bovine diseases and thirty-four clinical signs and risk factors

Diseases	Clinical signs/risk factors
Anaplasmosis	Abnormal behaviour/consciousness
Babesiosis	Abortion
Cowdriosis	Age
Fasciolosis	Anaemia
PGE	Anorexia
Schistosomosis	Ataxia/locomotor dysfunction
Theileriosis	Breed
Trypanosomosis	Constipation
	Coughing
	Dehydration
	Depression
	Diarrhoea
	Dysentery
	Dyspnoea
	Haemogloburia
	Icterus
	Lacrymation
	Lymph node enlargement
	Muscle tremors
	Nasal discharge
	Pallor of mucous membranes
	Petechial haemorrhages
	Pica
	Pot belly (calves)
	Pyrexia
	Reduced milk yield
	Reproductive status
	Sex
	Staring hair coat
	Stunted growth
	Submandibular/ventral oedema
	Thirst (excessive)
	Weakness
	Weight loss

Table 8.2: Overall scores of all 34 clinical signs of endemic bovine diseases obtained through the Delphi survey

Clinical signs	Anaplasmosi	Babesiosi	Cowdriosi	Fasciolosi	PGE	Schistosomosis	Theileriosi	Trypanosomosis
Abnormal behaviour	14.1	5.3	60.9	0.0	0.0	0.0	2.4	0.0
Abortion	11.2	8.9	11.8	0.0	0.0	0.0	1.9	58.7
Age	24.4	24.0	19.6	23.0	62.3	23.3	44.8	19.4
Anaemia	104.0	65.2	6.8	62.1	59.0	42.2	17.9	135.3
Anorexia	55.1	44.0	74.4	4.8	13.5	6.5	44.7	4.7
Ataxia	4.5	6.7	97.7	0.0	0.0	0.0	0.0	0.0
Breed	21.3	28.4	13.1	1.6	0.0	0.0	43.2	17.1
Constipation	97.1	1.4	0.0	0.0	0.0	0.0	6.2	0.0
Coughing	0.0	0.0	2.8	2.0	12.7	1.0	40.8	2.6
Dehydration	12.7	0.0	0.0	5.6	3.3	2.6	4.8	12.9
Depression	45.7	57.5	48.9	7.7	6.1	0.0	20.9	12.9
Diarrhoea	0.0	2.8	10.2	41.4	75.4	20.4	0.0	18.1
Dysentery	0.0	0.0	6.0	8.8	18.4	25.4	15.0	0.0
Dyspnoea	0.0	2.5	10.0	0.0	0.0	0.0	47.2	2.7
Excessive thirst	1.7	3.8	0.0	1.4	6.8	1.7	0.0	0.0
Haemoglobinuria	4.4	143.3	7.3	0.0	0.0	8.2	0.0	0.0
Icterus	37.5	70.3	0.0	20.4	0.0	8.5	0.0	0.0
Lacrymation	0.0	2.5	7.8	0.0	0.0	0.0	35.6	8.3
Lymph node enlargement	4.6	3.3	7.3	0.0	0.0	0.0	98.8	66.2
Muscle tremors	1.8	2.9	18.1	0.0	0.0	0.0	1.0	0.0
Nasal discharge	0.0	0.0	12.1	0.0	0.0	0.0	18.6	1.7
Pallor	69.8	36.3	12.0	70.9	51.9	58.6	5.1	63.4
Petechial haemorrhage	8.2	2.7	6.9	4.0	0.0	0.0	32.0	1.4
Pica	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Pot belly (calves)	4.4	0.7	0.0	14.2	72.9	8.3	0.0	4.8
Pyrexia	74.1	94.6	93.7	0.0	9.9	0.0	86.2	20.9
Reduced milk yield	27.2	23.0	26.6	26.9	10.6	0.0	44.4	46.0
Reproductive status	4.3	0.0	1.4	7.1	10.5	0.0	0.9	6.1
Sex	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
Staring coat	11.0	4.1	0.0	64.8	45.0	16.9	4.8	95.0
Stunted growth	0.0	0.0	0.0	64.1	72.8	28.2	0.0	9.6
Submandibular oedema	1.8	2.5	0.5	83.5	51.2	9.4	1.8	1.3
Weakness	23.3	49.5	40.1	78.5	21.3	47.0	10.2	58.4
Weight loss	25.8	3.7	3.8	97.0	55.0	51.8	1.0	112.5

*Shaded figures indicates clinical signs of high diagnostic value for individual diseases

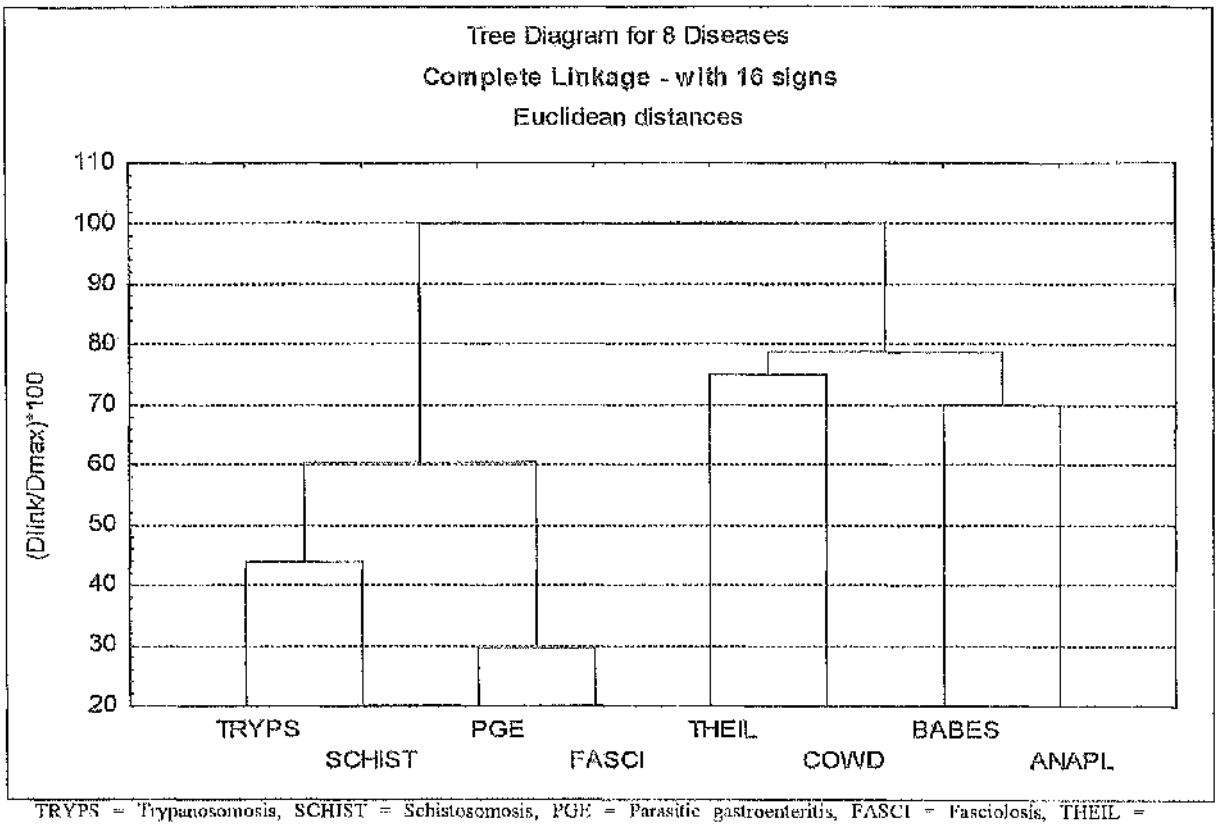
Table 8.3: Clinical signs (risk factors) with the highest scores as assessed by experts in the Delphi survey

Rank	Anaplasmosi	Babesiosis	Cowdriosi	Fasciolosis	PGE	Schistosomosis	Theileriosis	Trypanosomosis
1	Anaemia	Haemoglobinuria	Ataxia	Weight loss	Diarrhoea	Pallor	Lymph node enlargement	Anaemia
2	Constipation	Pyrexia	Pyrexia	Submandibular oedema	Pot belly	Weight loss	Pyrexia	Weight loss
3	Pyrexia	Icterus	Anorexia	Weakness	Stunted growth	Weakness	Dyspnoea	Staring coat
4	Pallor	Anaemia	Abnormal behaviour	Pallor	Age	Anaemia	Age	Lymph node enlargement
5	Anorexia	Depression	Depression	Staring coat	Anaemia	Stunted growth	Anorexia	Pallor
6	Depression	Weakness	Weakness	Stunted growth	Weight loss	Dysentery	Red. milk yield	Abortion
7	Icterus	Anorexia	Red. milk yield	Anaemia	Pallor	Age	Breed	Weakness
8	Red. milk yield	Pallor		Diarrhoea	Submandibula oedema	Diarrhoea	Coughing	Red. milk yield
9					Staring coat	Staring coat	Lacrymation	
10							Petechial haemorrhage	

Table 8.4: Scores of clinical signs standardized and sorted in descending order

Clinical signs	Anaplasmosis	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosomosis	Theileriosis	Trypanosomosis	Total
Anaemia	4.52	2.84	0.34	2.70	2.68	3.52	0.85	5.20	22.7
Pallor mucous membrane	3.04	1.58	0.60	3.08	2.36	4.88	0.24	2.44	18.2
Pyrexia	3.22	4.11	4.68	0.00	0.45	0.00	4.10	0.80	17.4
Weight loss	1.12	0.16	0.19	4.22	2.50	4.32	0.05	4.33	16.9
Weakness	1.01	2.15	2.01	1.41	0.97	3.92	0.48	2.25	16.2
Age	1.06	1.04	0.98	1.00	2.83	1.94	2.13	0.75	11.7
Anorexia	2.40	1.91	3.72	0.21	0.61	0.55	2.13	0.18	11.7
Staring coat	0.48	0.18	0.00	2.82	2.05	1.41	0.23	3.65	10.8
Reduced milk yield	1.18	1.00	1.33	1.17	0.48	0.00	2.11	1.77	9.0
Depression	1.99	2.50	2.45	0.34	0.28	0.00	0.99	0.50	9.0
Stunted growth	0.00	0.00	0.00	2.79	3.31	2.35	0.00	0.37	8.8
Diarrhoea	0.00	0.12	0.51	1.80	3.43	1.70	0.00	0.70	8.3
Lymph node enlargement	0.20	0.14	0.36	0.00	0.00	0.00	4.70	2.54	8.0
Haemoglobinuria	0.19	6.23	0.37	0.00	0.00	0.68	0.00	0.00	7.5
Submandibular oedema	0.08	0.11	0.02	3.63	2.33	0.78	0.09	0.05	7.1
Icterus	1.63	3.06	0.00	0.89	0.00	0.70	0.00	0.00	6.3
Breed	0.92	1.24	0.66	0.07	0.00	0.00	2.06	0.66	5.6
Ataxia	0.20	0.29	4.88	0.00	0.00	0.00	0.00	0.00	5.4
Pot belly (calves)	0.19	0.03	0.00	0.62	3.31	0.69	0.00	0.18	5.0
Constipation	4.22	0.06	0.00	0.00	0.00	0.00	0.30	0.00	4.6
Dysentery	0.00	0.00	0.30	0.38	0.84	2.12	0.71	0.00	4.4
Abnormal behaviour	0.61	0.23	3.05	0.00	0.00	0.00	0.11	0.00	4.0
Abortion	0.48	0.39	0.59	0.00	0.00	0.00	0.09	2.26	3.8
Dyspnoea	0.00	0.11	0.50	0.00	0.00	0.00	2.25	0.10	3.0
Coughing	0.00	0.00	0.14	0.09	0.58	0.08	1.94	0.10	2.9
Petechial haemorrhages	0.36	0.12	0.35	0.17	0.00	0.00	1.52	0.05	2.6
Lacrymation	0.00	0.11	0.39	0.00	0.00	0.00	1.69	0.32	2.5
Dehydration	0.55	0.00	0.00	0.24	0.15	0.22	0.23	0.50	1.9
Nasal discharge	0.00	0.00	0.61	0.00	0.00	0.00	0.89	0.07	1.6
Reproductive status	0.19	0.00	0.07	0.31	0.48	0.00	0.04	0.23	1.3
Muscle tremors	0.08	0.13	0.91	0.00	0.00	0.00	0.05	0.00	1.2
Excessive thirst	0.08	0.16	0.00	0.06	0.31	0.14	0.00	0.00	0.7
Pica	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.0
Sex	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.0

*Shaded figures indicate important clinical signs for diagnosis of individual diseases



TRYPS = Trypanosomosis, SCHIST = Schistosomosis, PGE = Parasitic gastroenteritis, FASCI = Fasciolosis, THEIL = Theileriosis, COWD = Cowdriosis, BABES = Babesiosis, ANAP = Anaplasmosis

Figure 8.2: A dendrogram showing diseases in the sign space

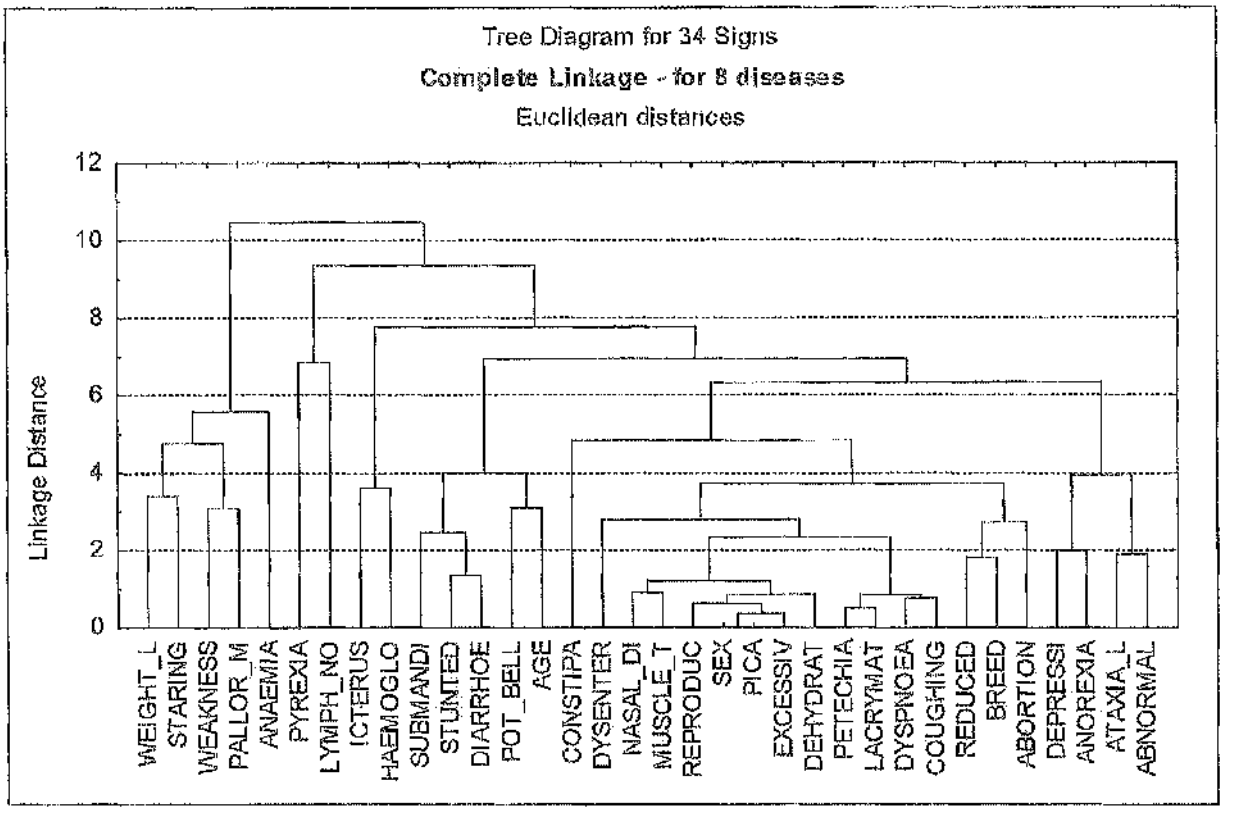


Figure 8.3: A dendrogram showing a full set of clinical signs in the disease space

Table 8.5: Final set of scores of clinical signs and sign-pairs included in the decision support card

	Anaplasmosi	Babesiosi	Cowdriosi	Fasciolosis	PG	Schistosomosis	Theileriosi	Trypanosomosis
Anaemia or Pallor	25%	14%		14%	16%	24%	6%	28%
Anorexia or Depression	13%	12%	24%				14%	
Ataxia or Abnorm. behaviour			32%					
Constipation	23%							
Diarrhoea				8%	20%	8%		
Dysentery						10%	5%	
Dyspnoea or Coughing							15%	
Haemoglobinuria		30%						
Icterus	9%	15%						
Lymph node enlargement							32%	14%
Pyrexia	18%	20%	31%				28%	4%
Staring coat				13%	12%	7%		19%
Stunted growth/pot belly				13%	19%	11%		
Submandibular oedema				17%	13%			
Weakness	6%	10%	13%	16%	6%	19%		12%
Weight loss	6%			19%	14%	21%		23%

	Anaplasmosis	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosomosis	Theileriosis	Trypanosomosis
Anaemia or Pallor	4	2		2	3	4	1	4
Anorexia or Depression	2	2	4				3	
Ataxia or Abnormal behaviour			4					
Constipation	4							
Diarrhoea				1	3	1		
Dysentery						2	1	
Dyspnoea or Coughing							3	
Haemoglobinuria		4						
Icterus	1	2						
Lymph node enlargement							4	2
Pyrexia	3	4	4				4	1
Staring coat				2	2	1		3
Stunted growth or pot belly				2	3	2		
Submandibular/ventral oedema				3	2			
Weakness	1	2	3	3	1	3		2
Weight loss	1			3	2	3		4

Figure 8.4: A prototype decision support card for differential diagnosis of endemic bovine diseases

8.3.2.5 Evaluation of the decision support card

Preliminary evaluation of the decision support card was conducted using 50 cases. Among them were field cases of anaplasmosis, fasciolosis, parasitic gastroenteritis, theileriosis and trypanosomosis found during the cross-sectional study and others obtained from literature: babesiosis (Ajayi *et al.*, 1982; Flach *et al.*, 1990), cowdriosis (van Amstel *et al.*, 1988; Flach *et al.*, 1990) and schistosomosis (McCauley *et al.*, 1983b). Results for the preliminary testing of the decision support card are shown in Table 8.6. The intended diagnoses were placed in the top rank in 38% of cases, and in second and third ranks in 26% and 30% of the cases, respectively. Thus if only top rank scores are considered, 38% of the diagnoses would be true, but by including the second and third place scores there was a substantial improvement in the detection rate of the true diagnoses to 64% and 94%, respectively. For the majority of the cases, the diagnostic hierarchy was different from the one intended for. Out of 50 cases, 31 top rank diagnoses were different from the true diagnoses. For examples, though in all cases of fasciolosis, fluke eggs had been detected and all cases of anaplasmosis were parasitologically confirmed, none of cases had fasciolosis and anaplasmosis placed on the top rank by the decision support card based on the clinical signs observed. However, the clarity of the diagnoses was good, for there were only 3 multiple first choice diagnoses out of 50 placed on the top rank. In the majority of the cases, trypanosomosis masked other diseases just because weight loss, for which trypanosomosis has a high weighting, was a common clinical sign among the cases. A further evaluation was conducted using 16 cases of known aetiological and clinical diagnosis whereby diagnoses by 15 pairs of experts were compared to that of the decision support card. The decision support card detected 15 out of 16 (93.8%) cases, while experts detected 12 out of 16 (75%) cases (Table 8.7).

Table 8.6: Results of the evaluation of the decision support card

Disease diagnosed	Observed clinical signs							Colour-coded chart results (options)		
								1st (score)	2nd (score)	3rd (score)
Fasciolosis	Pallor	Fever						A (7)	B (6)	T/E (5)
Fasciolosis	Weight loss	Pallor						T (8)	S (7)	A/F/P (5)
Fasciolosis	Weight loss	Pallor	Enlarged Ln	Diarrhoea				T (10)	P/S/A (8)	F (6)
Fasciolosis	Diarrhoea	Fever						E (4)	P/A (3)	F/S/T (1)
Fasciolosis	Weight loss	Staring coat						T (7)	F (5)	P/S (4)
Fasciolosis	Weight loss	Icterus	Diarrhoea					P (5)	S/F/T (4)	A/B (2)
Fasciolosis	Weight loss	Pallor	Anaemia					T (8)	S (7)	A/F/ P (5)
Fasciolosis	Weight loss	Enlarged Ln						T (6)	S/F(3)	A/P (2)
Fasciolosis	Pallor	Enlarged Ln						T (6)	E (5)	S/A (4)
Fasciolosis	Anaemia	Enlarged Ln						T (6)	E (5)	S/A (4)
Trypanosomosis	Weight loss	Pallor						T (8)	S (7)	A/F/P (5)
Trypanosomosis	Weight loss	Staring coat						T (7)	F (5)	P/S (4)
Trypanosomosis	Pallor	Fever						A (5)	B (6)	E/T (5)
Trypanosomosis	Pallor	Fever						A (7)	B (6)	E/T (5)
Trypanosomosis	Pallor	Enlarged Ln	Anaemia					T (6)	E (5)	A/S (4)
Trypanosomosis	Fever	Anaemia						A (7)	B (6)	E/T (5)
Trypanosomosis	Pallor	Enlarged Ln	Fever					E (9)	A/T (7)	B (6)
Trypanosomosis	Weight loss	Pallor	Diarrhoea	Fever	Anaemia			T (9)	P/S/A (8)	B/F (6)
Trypanosomosis	Weight loss	Pallor	Enlarged Ln	Diarrhoea	Fever			T (11)	E (9)	P/S/A (8)
Trypanosomosis	Weight loss	Pallor	Icterus	Staring coat	Enlarged Ln	Diarrhoea	Anaemia	T (10)	S (9)	F/P (8)
PGE	Weight loss	Pallor	Enlarged Ln	Diarrhoea				T (10)	P/S/A (8)	F (6)
PGE	Weight loss	Pallor						T (8)	S (7)	A/F/P (5)
PGE	Weight loss	Icterus	Diarrhoea					P (5)	F/S/T (4)	A/B (2)
East Coast fever	Pallor	Enlarged Ln						T (6)	E (5)	A/S (4)
East Coast fever	Pallor	Enlarged Ln						T (6)	E (5)	A/S (4)
East Coast fever	Pallor	Enlarged Ln						T (6)	E (5)	A/S (4)

Table 8.6: Results of the evaluation of the decision support card (Continued)

Disease diagnosed	Observed clinical signs							Colour-coded chart results (options)		
East Coast fever	Pallor	Enlarged Ln						T (6)	E (5)	A/S (4)
East Coast fever	Weightloss	Enlarged Ln	Fever					E (7)	T (7)	A/B/C (4)
East Coast fever	Enlarged Ln	Fever	Anaemia					E (9)	A (7)	B (6)
East Coast fever	Staring coat	Enlarged Ln	Diarrhoea	Fever				E (8)	T(6)	P (5)
Anaplasmosis	Weight loss	Icterus	Staring coat					T (7)	F (5)	P/S (4)
Anaplasmosis	Weight loss	Pallor	Staring coat	Enlarged Ln	Fever	Anaemia		T (14)	E (9)	A/S (8)
Anaplasmosis	Weight loss	Diarrhoea						P (5)	F/S/T (4)	A (1)
Anaplasmosis	Weight loss	Pallor	Icterus	Anaemia				T (8)	S (7)	A (6)
Anaplasmosis	Weight loss	Staring coat	Anaemia					T (11)	S (8)	F/P (7)
Anaplasmosis	Weight loss	Pallor	Icterus	Staring coat	Anaemia			T (11)	S (8)	F/P (7)
Anaplasmosis	Weight loss	Pallor	Enlarged Ln	Fever				T (11)	E (9)	A (8)
Anaplasmosis	Weight loss	Pallor	Fever					T (9)	A (8)	S (7)
Anaplasmosis	Weight loss	Pallor	Staring coat	Fever	Anaemia			T (12)	A/S (8)	F/P (7)
Babesiosis	Fever	Anaemia	Haemogloburia	Anorexia	Weakness			B (14)	C (11)	A (10)
Babesiosis	Fever	Anaemia	Haemogloburia	Anorexia	Weakness	Ataxia		B/C (14)	A (10)	T (7)
Babesiosis	Fever	Anaemia	Haemogloburia	Anorexia	Weakness			B (14)	C (11)	A (10)
Babesiosis	Pallor	Icterus	Haemogloburia	Depression	Anaemia	Anorexia	Fever	B (14)	A (10)	C (8)
Cowdriosis	Fever	Depression						C (8)	E (7)	B (6)
Cowdriosis	Diarrhoea	Abnormal behav.						C (4)	P (3)	F/S (1)
Cowdriosis	Fever	Abnormal behav.						C (8)	B/E (4)	A (3)
Cowdriosis	Fever	Anaemia						A/B (6)	E/T (5)	C/S (4
Schistosomosis	Weight loss	Staring coat	Diarrhoea	Anaemia				T (11)	P (10)	S (9)
Schistosomosis	Weight loss	Staring coat	Dysentery	Anaemia	Anorexia			T (11)	S (10)	A/F/P (7)
Schistosomosis	Weight loss	Staring coat	Dysentery	Anaemia				T (11)	S (10)	F/P (7)

A-anaplasmosis, B-babesiosis, C-cowdriosis, E-East Coast fever, F-fasciolosis, P-parasitic gastroenteritis (PGE), S- schistosomosis, T- trypanosomosis

Table 8.7. Comparison of diagnoses by experts and the decision support card

Case presentation	Field diagnosis	Experts' diagnosis	DS Card
Anaemia, Icterus, Pyrexia, Weight loss	Anaplasmosis	A (67%); S/T (17%)	A/T; B, S
Ataxia, Pyrexia, Weakness	Cowdriosis	C (79%); E (13%)	C; B
Anaemia, Anorexia, Haemoglobinuria, Pyrexia	Babesiosis	B (63%); A (29%)	B; A; C/T
Depression, Dyspnoea, Lymph node enlargement	East Coast fever	E (83%); T (17%)	E
Anaemia, Lymph node enlargement, Staring coat, Weight loss	Trypanosomosis	T (58%); A (17%); F (17%)	T; S; F/P
Abnormal behaviour, Anaemia, Pyrexia	Cowdriosis	C (79%); E (13%)	C; A; B
Anaemia, Constipation, Pyrexia, Weight loss	Anaplasmosis	A (75%); B (21%)	A; T; S
Anaemia, Lymph node enlargement, Weight loss	Trypanosomosis	T (67%); E (17%)	T; S
Anaemia, Anorexia, Lymph node enlargement	East Coast fever	E (63%); T (33%)	E; A/T
Anorexia, Depression, Haemoglobinuria, Weakness	Babesiosis	B (83%)	B; C
Anaemia, Diarrhoea, Weight loss	Fasciolosis	P (50%); T (33%) ; S (17%)	P; S; T; F
Anaemia, Dysentery, Weight loss	Schistosomosis	P (58%); E (25%)	S; T
Diarrhoea, Stunted growth, Weight loss	Parasitic gastroenteritis	P (67%); F (17%); S (13%)	P; F/S
Anaemia, Dysentery, Staring coat, Weakness	Schistosomosis	S (33%); T (33%); F (17%)	S; T; F; P
Staring coat, Submandibular/ventral oedema, Weakness, Weight loss	Fasciolosis	T (38%) ; P (33%); F (13%)	F; T; P/S
Anaemia, Pot belly, Staring coat	Parasitic gastroenteritis	P (58%); F (33%)	P; S/T; F

A = Anaplasmosis, B = Babesiosis, C = Cowdriosis, E = East Coast fever, F = Fasciolosis, P = Parasitic gastroenteritis, S = Schistosomosis, T = Trypanosomosis

8.4 Discussion

The Delphi method was employed to explore the available wealth of veterinary expertise on clinical diagnosis of endemic bovine diseases under consideration to elicit quantitative information on the most important clinical signs and risk factors for designing decision support systems.

Responses were received from 46 experts regarding important clinical signs and risk factors for clinical diagnosis of bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, fasciolosis and schistosomosis. Despite the high non-response rate during the first round, the 46 participants by the end of the second round were sufficient, given that even minimal expertise can have a significant impact on the accuracy of forecasts (Kirigia, 1997). Unfortunately, literature on the Delphi methodology does not give clear recommendations on the appropriate number of people to include in a panel, but it is recommended that the panel should be sufficient to accommodate attrition between rounds (Bruneau *et al.*, 1999).

The common criterion to stop is when agreement among the respondents has been attained (Bruneau *et al.*, 1999). Though some factors portrayed bimodal distribution after the second round, giving a clue of divergence of opinion, the iterative process was stopped after the second round to avoid the relatively high dropout rate between rounds. Ideally, the iterative process should continue for several rounds, but studies have shown that usually there is no meaningful change after the second round of evaluation (Bruneau *et al.*, 1999). The interval between mailing the first- and second-round questionnaires was long (approximately six months) because of the wide coverage of participants from the whole world i.e. Africa, America, Europe and Australia.

The participants' lists of chosen clinical signs and risk factors were consistent with existing literature on the various diseases assessed. For clinical diagnosis of bovine trypanosomosis,

anaemia, followed by weight loss, staring coat, enlarged lymph nodes, pallor of mucous membranes, abortion, weakness and reduced milk yield had the highest scores. This finding concurred with existing literature (Fiennes, 1970; Morrison *et al.*, 1981; Molyneux and Ashford, 1983; Murray and Trail, 1986; Stephen, 1986; Holmes *et al.*, 2000).

Enlarged lymph nodes, followed by pyrexia, dyspnoea, age, anorexia, reduced milk yield, breed, coughing, lacrymation and petechial haemorrhages had the highest scores for clinical diagnosis of bovine theileriosis. Expert opinions were consistent with findings of other studies (Bruce *et al.*, 1910; Shannon, 1977; Omuse, 1978; Kiptoon *et al.*, 1983; Mbassa *et al.*, 1994; Mbassa *et al.*, 1998).

The clinical signs with the highest scores for diagnosis of bovine anaplasmosis included anaemia, followed by constipation, pyrexia, pallor of mucous membranes, anorexia, depression, icterus and reduced milk yield. Studies on natural cases of bovine anaplasmosis conducted in Nigeria (Ajayi *et al.*, 1987; Egbe-Nwiyi *et al.*, 1997) and in Kenya (Omuse, 1978), similarly recorded these clinical signs. These signs have also been reported elsewhere (Theiler, 1910; Potgieter and Stoltz, 1994).

Haemoglobinuria, followed by pyrexia, icterus, anaemia, depression, weakness, anorexia and pallor of mucous membranes had the highest score for clinical diagnosis of bovine babesiosis. Consistent with expert judgement, haemoglobinuria, pyrexia, jaundice and anaemia have been observed to be the major clinical signs manifested by cases of bovine babesiosis (Omuse, 1978; Ristic, 1981; Losos, 1986; Urquhart *et al.*, 1996). However, icterus is never a prominent clinical sign associated with babesiosis, as is the case with anaplasmosis (Potgieter and Stoltz, 1994).

Ataxia or locomotor dysfunction, followed by pyrexia, anorexia, abnormal behaviour or consciousness, depression, weakness and reduced milk yield had the highest scores for clinical

diagnosis of cowdriosis. Bovine cowdriosis is often fatal especially in susceptible populations of cattle introduced into endemic areas, particularly in imported exotic breeds and hence clinical diagnosis is rarely made. However, in line with the above ranking of clinical signs, cowdriosis in cattle is characterised by prolonged pyrexia and nervous symptoms before death (Cowdry, 1926; Losos, 1986; Bezuidenhout *et al.*, 1994).

Weight loss, followed by submandibular oedema, weakness, pallor of mucous membranes, staring coat, stunted growth, anaemia and diarrhoea had the highest scores for clinical diagnosis of bovine fasciolosis. Expert opinion concurred with existing literature, in that bovine fasciolosis is reported to present with gradual wasting, progressive weakness, anaemia, dehydration and oedema of the submandibular space and ventral abdomen (Egbe-Nwiyi and Chaudrai, 1996; Bowman and Lynn, 1999; Waruiru *et al.*, 2000).

Diarrhoea, followed by pot belly in calves, stunted growth, age, anaemia, weight loss, pallor of mucous membranes, submandibular oedema and staring coat had the highest scores for clinical diagnosis of parasitic gastroenteritis in cattle. This concurs with other studies (Kaufmann *et al.*, 1989; Kaufmann and Pfister, 1990; Waruiru *et al.*, 1993; Waruiru *et al.*, 1998; Eysker and Ploeger, 2000; Ganaba *et al.*, 2002).

For clinical diagnosis of bovine schistosomosis, the signs and risk factors that had the highest scores included pallor, followed by weight loss, weakness, anaemia, stunted growth, dysentery, age, diarrhoea and staring coat. Clinical signs of this disease are reported to be rare because the disease generally occurs at subclinical levels (de Bont and Vercruysse, 1998). Retarded growth of animals on a herd basis is the major sign for the chronic form of bovine schistosomosis and severe bloody or mucoid diarrhoea, marked anorexia, excessive thirst, anaemia and emaciation are the main clinical signs of the acute form of disease (de Bont and Vercruysse, 1998; McCauley *et al.*, 1983a). Other signs considered highly diagnostic for the acute form of bovine schistosomosis include, excessive thirst due to severe dehydration and the resulting sunken-eye

appearance (McCauley *et al.*, 1983a). Unfortunately, many experts did not consider many of these clinical signs important. Instead they mentioned clinical signs often associated with the chronic form of the disease, which probably implied this is the most prevalent form. Given the fact that clinical signs are quite rare and the cases are quite focalised (de Bont and Vercruysse, 1998), many experts probably had little experience with this disease. Indeed, several experts asked to respond to questions on this disease declined to participate because of lack of experience. Such areas where participants have little information tend not to be evaluated in Delphi surveys as being of primary importance (Bruneau *et al.*, 1999).

The degree of importance attached to the various clinical signs and risk factors chosen by the participants for the diseases under consideration makes this study unique. Nevertheless, it is possible that these results were biased by "received wisdom" i.e. what participants had been taught by their peers and teachers, which undoubtedly constitutes conventional veterinary medicine. This could have made the basis for participants making diagnoses. In completing the survey, they may have looked back and decided that these signs they were taught were indeed important in diagnosing the conditions. This situation arises because there are relatively few veterinary practitioners in the target production system with regular access to confirmatory techniques such as microscopy. It was hoped, however, that with their overwhelming field experience accumulated over time, many veterinary practitioners had been exposed to many classical cases of these diseases and hence had had chance to confirm the important clinical signs and risk factors associated with them.

On the other hand, normally veterinary scientists working on these diseases and who have access to confirmatory techniques are not regularly exposed to cases of these diseases under field conditions. They tend instead to work on experimental infections. Moreover, with emphasis over the last two decades on immunological and molecular diagnoses, clinical parameters are often overlooked in experimental studies. However, ensuring that veterinary

scientists selected had some background field experience with diseases under consideration mitigated this problem.

Bimodal distribution occurred in some factors, probably indicating lack of stability of these factors or represented an important and apparently unresolved difference in opinion within the panel (Dewey *et al.*, 1992). Lack of stability between the scores is often attributed to diversity in background among panel experts (Dewey *et al.*, 1992), as was the case with the veterinary scientist and practitioners. This diversity of background may lead to differences in the interpretation of the questionnaire.

Some change of scores was witnessed within groups between the successive survey rounds. Giving consensus results, an underlying property of the Delphi method, and clarification of the questions in the questionnaires after the first round, could have triggered this change of scores. It has been demonstrated in other studies that giving relevant facts on return questionnaires improves the accuracy of subsequent responses (Dewey *et al.*, 1992). The propensity for individuals to change their scores is thought to be a function of their distance from the average score and their strength of belief on the issue, hence individuals whose judgement is far from the mean score are most likely to change their score than those close to the mean (Dewey *et al.*, 1992).

Unlike in many textbooks of veterinary medicine, whereby clinical signs of bovine diseases considered in this study are described as a sequence of events during the disease process, this study has yielded quantitative information indicating the degree of importance of various clinical signs and risk factors associated with bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, fasciolosis, parasitic gastroenteritis and schistosomosis that are useful for their clinical diagnosis.

Using the quantitative information on clinical signs/risk factors gathered from the Delphi survey, a decision support card was developed. The decision support card was intended for people involved in field diagnosis of endemic bovine diseases in rural areas of Africa. Unlike CaDDiS, an expert system that better analyses diagnosis of diseases by utilising Bayesian probabilistic reasoning to tackle conjunction occurrence of various disease signs in the same animal (McKendrick *et al.*, 2000) and thus is more accurate, the decision support card is a low technology decision support system that utilises pattern-matching and a scoring system to make differential diagnosis. It was purely designed to conform to conditions in rural areas of Africa where there is no electricity and to allow for easy mobility. Computer-independent systems, such as this are usually limited to identifying differential diagnoses for a single sign or a limited number of combinations (Cockcroft, 1999a). It is recognised that such decision support systems need to be simple and credible to the users, however, their major setback is inability to allow for uncertainty of observations, which may lead to misinterpretation or missing of subtle signs (Cockcroft, 1999a).

As regards the preliminary evaluation of the decision support card, if only top rank scores are considered, 38% of the diagnoses would be true but by including the second and third place scores there is substantial improvement in the detection rate of the true diagnoses to 64% and 94%, respectively. Middleton (2001) also suggested the inclusion of the second and third choice scores to improve the detection rate of a diagnostic decision support tool.

It was observed that in the majority of the cases the diagnostic hierarchy was different from the one intended. This though largely was attributed to diseases under consideration manifesting similar clinical signs, also pointed out the fact that there is need to refine the decision support card to allow for clear differentiation of diseases. For cases of fasciolosis, fluke eggs had been detected, but many of them manifested weight loss and pallor of mucous membranes, signs that are also manifested by trypanosomosis. A sign like submandibular oedema that would have distinguished fasciolosis from trypanosomosis was not manifested by any of the field cases of

fasciolosis. For cases of anaplasmosis that were parasitologically confirmed, many of them presented with weight loss and anaemia, which are similarly manifested by trypanosomosis. None of the field cases of anaplasmosis manifested constipation, a sign that would have distinguished anaplasmosis from trypanosomosis.

Trypanosomosis masked other diseases in the majority of the cases just because weight loss, for which trypanosomosis has a high weighting, was a common clinical sign among the cases used. Recognising the fact that they were field cases, chances of intercurrent infection are quite high. There was a possibility of undetected underlying trypanosomosis, since weight loss and enlarged lymph nodes observed in most of the cases diagnosed to have trypanosomosis are common signs associated with trypanosomosis (Fiennes, 1970; Morrison *et al.*, 1981; Molyneux and Ashford, 1983; Murray and Trail, 1986; Stephen, 1986).

In a comparative evaluation of the decision support card using cases of known aetiological and clinical diagnosis, the card detected 15 out of 16 (93.8%) cases, while experts detected 12 out of 16 (75%) cases. Whereas the card misdiagnosed only one case of fasciolosis that presented with anaemia, diarrhoea and weight loss for parasitic gastroenteritis, the experts misdiagnosed four cases: two cases of fasciolosis and two cases of schistosomosis for parasitic gastroenteritis and trypanosomosis. Distinction of parasitic gastroenteritis from fasciolosis and schistosomosis, and trypanosomosis from schistosomosis and fasciolosis is difficult since these diseases present with similar signs. The decision support card was better than the experts in distinguishing parasitic gastroenteritis from schistosomosis and, schistosomosis and fasciolosis from trypanosomosis. However, the decision support card still needs refinements to allow it to distinguish parasitic gastroenteritis from fasciolosis, especially when cases of fasciolosis present with diarrhoea in addition to anaemia and weight loss.

Weight loss is an important clinical sign of chronic gastrointestinal nematode infections in older cattle, while anaemia, oedema, diarrhoea and anorexia are associated with severe cases of

parasitic gastroenteritis observed in younger cattle, with diarrhoea being the main clinical sign (Eysker and Ploeger, 2000; Ganaba *et al.*, 2002). Weight loss and anaemia are also associated with mild or chronic fasciolosis in cattle (Urquhart *et al.*, 1996). Presence of anaemia and diarrhoea has been assigned higher diagnostic scores for parasitic gastroenteritis than for fasciolosis, while presence of weight loss and submandibular edema have been assigned higher diagnostic scores for fasciolosis than for parasitic gastroenteritis in the decision support card. This leads to misdiagnosis of cases of fasciolosis that present with anaemia, diarrhoea and weight loss, but without submandibular oedema. However, such cases are unusual. Veterinarians could deal with such situations by applying broad-spectrum anthelmintics that clear both gastrointestinal nematodes and flukes.

There is also need for a threshold score for inclusion of diseases as the top rank. This would probably capture the second and third place scores for most cases thus allowing for detection of intercurrent diseases and also ensure correct diagnoses in up to 94% of the cases.

The decision support card is simple to use, however, it is only useful in cases with at least two clinical signs. One clinical sign does not provide enough information to differentiate diseases. In addition, this tool is meant for people with some arithmetic proficiency and background veterinary training. Veterinary training would empower one to understand the epidemiology of the diseases under consideration: anaplasmosis, babesiosis, cowdriosis, fasciolosis, parasitic gastroenteritis, schistosomosis, theileriosis and trypanosomosis, particularly their presence or absence and distribution in the area where one is using the tool. It should also be noted that people with colour blindness might find some difficulty in using the tool. However, the inclusion of the scoring system in addition to the colour coding system was aimed at eliminating this problem.

To ensure durability and inexpensive production of the decision support card, it will preferably be made of a lightweight plastic or laminated cardboard, which would make it possible to re-use by writing on it with a wipe-clean marker or pencil.

Before the decision support card is released for routine use, there is need for further evaluation under controlled conditions to assess its diagnostic performance in terms of its sensitivity and specificity, and thereafter evaluation by a larger group of independent end-users.

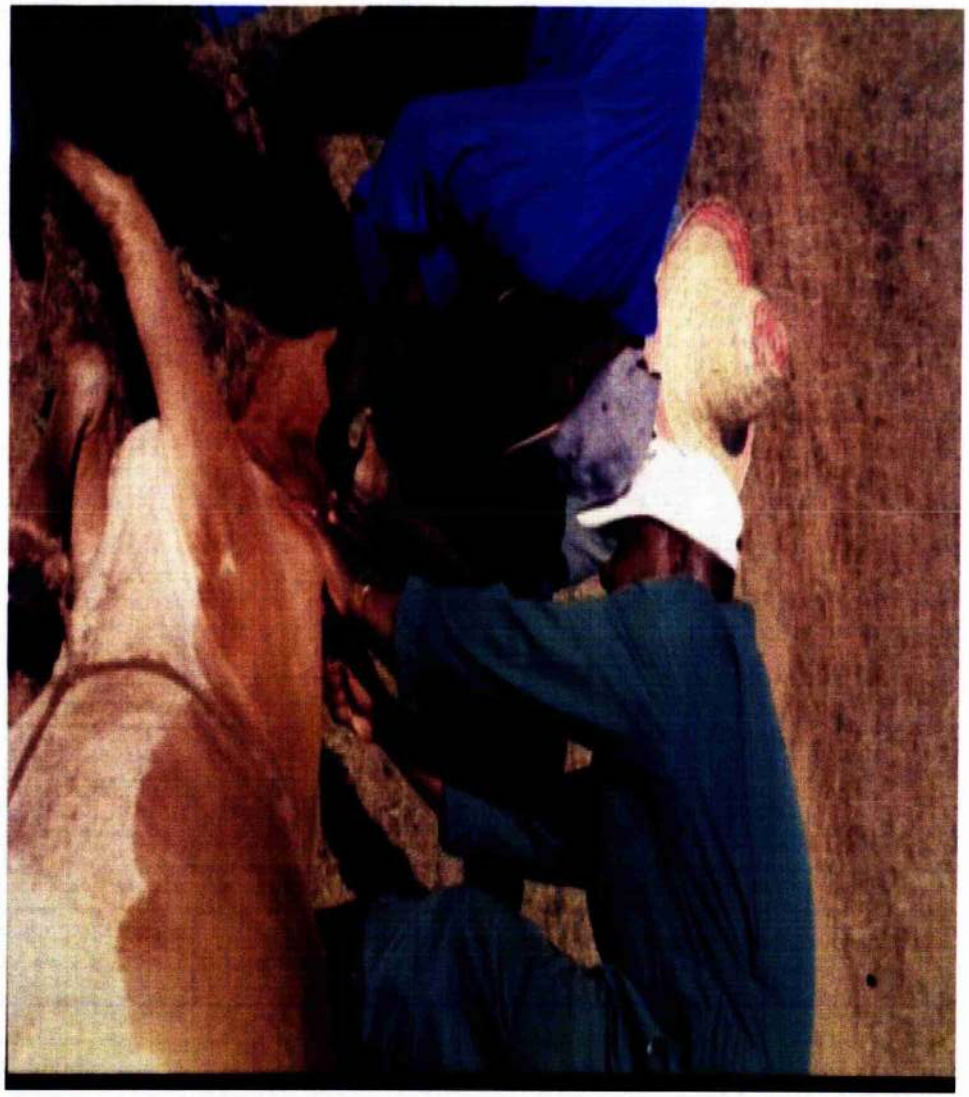


Figure 9.1: Clinical examination of a cow and taking of a blood sample in the field in Uganda - given the unavailability of field-level diagnostic tests and dwindling veterinary diagnostic facilities in sub-Saharan Africa, detection of clinical disease and effective management of endemic bovine diseases by field veterinarians, animal health assistants and community animal health workers may be facilitated by a combination of clinical examination and guiding decision support tools

Chapter 9 General Discussion

9.1 Introduction

Trypanosomosis, tick-borne diseases and helminthoses are the major endemic diseases affecting cattle health and productivity in sub-Saharan Africa (Wamae and Ihiga, 1991: de Castro, 1997; Pandey and Ahmadu, 1998; de Bont and Vercruysse, 1998; Kristjanson *et al.*, 1999; Eysker and Ploeger, 2000). Endemic diseases of cattle are a major constraint to the use of livestock by poor people in sub-Saharan Africa (Holden *et al.*, 1997).

Losses due to major endemic diseases may not be as dramatic as those occurring at the height of outbreaks of epidemic diseases, but their continuous presence, relentless attrition and negative impact on livestock productivity makes them economically important (OIE, 2003). Major epidemic diseases of cattle such as rinderpest, foot and mouth disease (FMD) and contagious bovine pleuropneumonia (CBPP) usually cause large-scale mortality, morbidity and severe economic losses during outbreaks, but they cause minimal economic losses during their long inter-epidemic periods when they are either absent or present at low prevalence. In their control, national governments and international organisations are obliged to act because of their immense negative impact on international trade on animals and animal products, and their infectious nature that allows them easily spread across national boundaries (OIE, 2003). Whereas national governments or international organisations take responsibility for epidemic disease control, the costs of endemic disease control are met by individual farmers due to the localised nature of these diseases (OIE, 2003). Major endemic bovine diseases in sub-Saharan Africa include trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, parasitic gastroenteritis, schistosomosis and fasciolosis.

Until recently, control of major endemic bovine diseases in sub-Saharan Africa has been the responsibility of state veterinary services, which have been reduced as a result of privatisation

(Armbruster, 1994). Moreover, in many African countries veterinary diagnostic laboratories have fallen into disuse due to under-utilisation by fee-paying farmers and absence of public support (Kenyon and Nour, 1996). With the advent of privatisation of veterinary services, diagnosis and treatment of livestock diseases in many African countries is now carried out by farmers, field veterinarians, animal health assistants, community animal health workers and agro-veterinary traders (Bossche *et al.*, 2000b; Machila *et al.*, 2003). Worse still, existing laboratory-based techniques for diagnosis of these diseases are too costly and unavailable to these groups making treatment decisions (Machila *et al.*, 2003). To improve field-level disease diagnosis, there is a necessity for development of simple, reliable and cheap diagnostic tests and guiding decision support tools to enable these groups render prompt diagnosis and treatment of endemic bovine diseases in rural areas of Africa.

9.2 Major Achievements

The goal of this thesis was to evaluate simple diagnostic tools and develop guiding decision support tools to facilitate the diagnosis and treatment of endemic bovine diseases in rural areas of Africa by field veterinarians, animal health assistants and community animal health workers, with particular emphasis on the mixed crop-livestock production system in South East Uganda. In this chapter the salient findings with respect to the objectives of the study and the future application of the decision support tools are reviewed.

The economic importance of major endemic bovine diseases in terms of losses to livestock productivity is highlighted in Chapter 1. In addition, the specific objectives and the thesis hypothesis are outlined. The thesis hypothesis was: farmer-based interventions utilizing low technology decision support systems including haemoglobin measurements might help improve animal health in the mixed crop-livestock farming systems in South East Uganda. Indeed as shown in Chapters 4 and 8, portable haemoglobinometers could facilitate the haemoglobin

measurement in the detection of anaemia associated with endemic bovine diseases and the decision support card could aid in differential diagnosis of endemic diseases in this production system (see Sections 9.3 and 9.4).

In Chapter 2, major endemic bovine diseases under consideration were reviewed in relationship to the key clinical signs, pathological features, epidemiological features and available definitive diagnostic tests. The role of anaemia in the pathogenesis of the various endemic diseases was also elucidated. Furthermore, the diagnostic procedure often undertaken in clinical diagnosis, and available parasitological, serological and molecular diagnostic tests for the various endemic diseases were reviewed in Chapter 2, looking at their advantages and disadvantages in relationship to detection of clinical disease. Chapter 2 ended with reviewing the role of decision support tools in improving the detection of clinical disease in light of the inadequacies of the available diagnostic tests. The study areas, production system and general methods used in the collection and analysis of samples are described in Chapter 3.

In Chapter 4, portable haemoglobinometers such as the Haemoglobin Colour Scale (HCS), HemoCue (HCU) and DHT-haemoglobinometer (DHT), commonly used in human medicine, were evaluated for their suitability in the detection of anaemia in cattle. These haemoglobinometers were found to be potentially useful for penside detection of anaemia, however each had its drawbacks. The Haemoglobin Colour Scale is cheap, easy to use and reliable when instructions are followed, but has the element of subjective judgement of results. Its low cost property makes it convenient for the target end-users. The HemoCue is easy to use, reliable and has no inter-observer error, but is expensive due to the considerable cost of microcuvettes (USD 0.5 (€ 0.44) per microcuvette), making it unaffordable to the target end-users. The DHT-haemoglobinometer is simple, cheap because cuvettes are re-usable, but is imprecise due to the dilution step involved. The dilution step makes it unsuitable for use by

field veterinarian, animal assistants and community animal health workers because this step involves pipetting, which is difficult and inconvenient for these end-users to perform under field conditions.

In Chapter 5, the influence of time of day and coat colour of Zebu cattle on rectal temperature was assessed. This study revealed that time of the day and coat colour had a highly significant influence on rectal temperature ($P < 0.001$). The period between 13.00 and 17.00 hours could be the most suitable time of the day for veterinarians to detect pyrexia, however there is the likelihood of picking healthy cattle (false positives) that have raised rectal temperature. Since veterinarians are usually presented with sick rather than healthy animals by farmers, it is unlikely that picking healthy animals would be a problem. Taking into consideration the influence of time of day and coat colour of cattle on rectal temperature measurements could improve the assessment of pyrexia and detection of clinical disease during clinical diagnosis of endemic diseases.

Furthermore, diurnal variations of the sensitivity of the common parasitological diagnostic tests for trypanosomosis were investigated in Chapter 5. Despite the small sample size ($n = 2$), the highest detection rate was observed at 21.00 hr and the lowest at 13.00 hr. Neither time of the day nor day-to-day variation had a significant influence on the sensitivity of the tests. Nevertheless, these results suggested optimal detection rate of trypanosomosis with microscopy was achieved when cattle blood samples were taken and examined between 17.00 and 09.00 hours under tropical conditions. Apart from optimising the detection rate of parasitological tests by taking blood samples at a point of time of the day when trypanosome parasitaemia is expected to be highest, there is need to supplement these techniques with more sensitive penside diagnostic tests, clinical diagnosis and decision support tools to enable field veterinarians to effectively diagnose and treat cases of trypanosomosis.

In Chapter 6, epidemiological studies conducted on endemic diseases in Zebu cattle kept under the mixed crop-livestock production system in South East Uganda are described. Parasitological, clinical and serological data were obtained from these studies. Parasitological data was analysed to determine the parasitological prevalence of various diseases under consideration. Clinical data provided means of determining disease morbidity rates. Serological data on major tick-borne diseases: anaplasmosis, babesiosis and theileriosis were used to calculate seroprevalences and seroconversion rates, and plot serological profiles. Parasitological prevalences and morbidity rates gave an indication to which diseases were most important in the mixed crop-livestock system in South East Uganda. These included anaplasmosis, theileriosis, fasciolosis, PGE, trypanosomosis and babesiosis. Overt cases of bovine schistosomosis were not found, although a few cattle secreted *Schistosoma* eggs. Cases of cowdriosis were not encountered either, despite the abundance of the tick vector *A. variegatum*. Neither clinical nor pathological signs attributable to *Cowdria ruminantium* organism were found.

Medium to high seroprevalences of 68.9-85.8%, 56.2-85.6% and 54.9-76.9% for *Theileria parva*, *Anaplasma marginale* and *Babesia bigemina* infections, respectively, were found in indigenous breeds of cattle under the mixed crop-livestock production system in South East Uganda, indicating that the cattle populations studied were extensively exposed to these organisms. A state of endemic stability probably existed for anaplasmosis and babesiosis (Callow, 1977; Norval *et al.*, 1992; Gitau *et al.*, 2000; Maloo *et al.*, 2001b). Local differences were observed among villages in terms of *T. parva* seroconversion rates. A significant difference in the abundance of *Rhipicephalus* spp. and *Boophilus* spp. ticks was found among the villages, which fell into two distinct categories: high and low tick challenge.

In Chapter 6, serological investigations took into account the fact that calves are born seronegative and then they may acquire passive immunity from maternal antibodies received through colostrum (Burridge and Kimber, 1973; De Vos, 1979; Hildebrandt, 1981; Losos, 1986). Later the maternal antibodies wane and calves seroconvert on first exposure to tick-borne diseases. In adult cattle, antibody titre may decline or rise depending on the tick challenge (Burridge and Kimber, 1973; De Vos, 1979; Hildebrandt, 1981; Losos, 1986).

Serological investigations revealed that seroconversion rates to *A. marginale* infection of cattle of all age groups: 0-6m, 7-12m, 13-24m and >24m under low tick challenge were higher than those of their counterparts under high tick challenge, but not significantly so. Seroconversion rates decreased with age up to 24m as expected, since as animals grew older more of them were exposed to continuous tick challenge and *A. marginale* infections under this extensive production system, hence more seroconverted, leading subsequently to fewer susceptible individuals and a lower seroconversion rate.

Unlike *A. marginale* infections, seroconversion rates of *B. bigemina* infection in cattle of different age groups greater than 6 months under high tick challenge were higher than those of their counterparts under low tick challenge, but the reverse was true for 6 months old calves. A significant difference was observed between seroconversion rates of older cattle (>24m) in the two zones. This was expected because there is age-related susceptibility of cattle to *B. bigemina* infection which increases with age (Losos, 1986). Whereas under low tick challenge the seroconversion rates decreased with age, under high tick challenge the seroconversion rates increased with age up to 24m. The implication of this trend is that a higher incidence of cases of babesiosis would be expected under high tick challenge than under low tick challenge.

For *T. parva* infections, seroconversion rates of cattle older than 6 months under low tick challenge were significantly higher than those of their counterparts under high tick challenge. Seroconversion rates were similar in all age groups under low tick challenge, but decreased with age under high tick challenge. This implies that a higher incidence of cases of East Coast fever is expected under low tick challenge than under high tick challenge. While cases of East Coast fever are expected in all age categories: 0-6m, 7-12m, 13-24m and >24m under low tick challenge, cases are expected only among calves of up to 6 months old under high tick challenge.

By and large, seroconversion rates to *A. marginale*, *B. bigemina* and *T. parva* infections varied among cattle of different age groups depending on the level of tick challenge, however, significant differences between cattle under different levels of tick challenge were only observed with *T. parva* infections.

Analysis of field cases of trypanosomosis, anaplasmosis, theileriosis, babesiosis, fasciolosis, PGE and schistosomosis to identify clinical signs that had significant association with presence of aetiological agents was undertaken in Chapter 7. For trypanosomosis, anaemia, pallor, fever, enlarged lymph nodes, lacrymation, staring coat and weight loss had significant associations. The clinical signs that were significantly associated with anaplasmosis included anaemia, pallor, fever, staring coat and weight loss. For theileriosis, they included fever, enlarged lymph nodes, lacrymation, anaemia, pallor, staring coat and diarrhoea. For babesiosis, they included anaemia, pallor and diarrhoea. For fasciolosis, they included anaemia, pallor, staring coat, weight loss and diarrhoea, while for PGE, they included weight loss, staring coat, anaemia and pallor. For schistosomosis, no clinical signs had any significant association to presence of *Schistosoma* eggs. These results suggested that generally anaemia (pallor), staring coat, weight loss and enlarged lymph nodes are good indicators of endemic diseases in cattle under the

mixed crop-livestock production system in South East Uganda. However, most of these clinical signs were non-specific. The field study revealed very few specific signs for individual diseases that could be used for differentiation of endemic bovine diseases under consideration. Enlarged lymph nodes had a significant association with trypanosomosis and theileriosis, thus could be used to differentiate these two diseases from anaplasmosis, babesiosis and helminthoses generally. Lacrymation had a significant association to theileriosis and thus could be used to differentiate theileriosis from the rest of the diseases.

Furthermore, in Chapter 7 important clinical signs and risk factors that could best be used to predict the probability of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infections in cattle were assessed. The significant predictors for seroconversion to *A. marginale* infection in cattle included the level of tick challenge at village level, *Rhipicephalus* spp. and *Boophilus* spp. intensity on individual animals, rectal temperature and packed cell volume. For *B. bigemina* infection, they included the level of tick challenge at village level, *Boophilus* species intensity on individual animals, anaemia, weight loss, staring coat, lacrymation and age. While for *T. parva* infection, they included the level of tick challenge at village level, *R. appendiculatus* intensity on individual animals, lymph node enlargement, rectal temperature and PCV. This analysis revealed that presence and intensity of the respective tick vectors for the tick-borne diseases and generally clinical features whose duration is long such as anaemia, weight loss, staring coat and enlarged lymph nodes were good indicators of seroconversion to *A. marginale*, *B. bigemina* and *T. parva* infections in cattle.

In Chapter 8, a Delphi survey, involving 46 veterinary experts consisting of 32 international and 14 local Ugandan experts, was conducted. Experts responded to questions regarding trypanosomosis (26), theileriosis (21), anaplasmosis (23), babesiosis (23), cowdriosis (20), PGE (23), fasciolosis (22) and schistosomosis (12). Veterinary expert opinion was sought on

the most important clinical signs and risk factors for clinical diagnosis of bovine trypanosomosis, theileriosis, anaplasmosis, babesiosis, cowdriosis, PGE, fasciolosis and schistosomosis.

In a descending order of overall scores, for diagnosis of trypanosomosis the important clinical signs included anaemia, weight loss, staring coat, enlarged lymph nodes, pallor of mucous membranes, abortion, weakness and reduced milk yield. For theileriosis, the most important clinical signs (risk factors) were enlarged lymph nodes, pyrexia, dyspnoea, age, anorexia, reduced milk yield, breed, coughing, lacrymation and petechial haemorrhages. While for anaplasmosis, they included anaemia, constipation, pyrexia, pallor, anorexia, depression, icterus and reduced milk yield. For babesiosis they included haemoglobinuria, pyrexia, icterus, anaemia, depression, weakness, anorexia and pallor. For cowdriosis they included ataxia, pyrexia, anorexia, abnormal behaviour, depression, weakness and reduced milk yield. For PGE, they included diarrhoea, potbelly, stunted growth, age, anaemia, weight loss, pallor, submandibular oedema and staring coat. For fasciolosis, they included weight loss, submandibular oedema, weakness, pallor, staring coat, stunted growth, anaemia and diarrhoea. While for schistosomosis, they included pallor, weight loss, weakness, anaemia, stunted growth, dysentery, age, diarrhoea and staring coat. These results demonstrated that there were several common clinical signs among trypanosomosis, tick-borne diseases and helminthoses.

The major differences between the results of the field cases (Chapter 7) and those of the Delphi survey (Chapter 8) were:

1. More clinical signs and diseases were obtained from the Delphi study than from the field study.
2. Clinical signs obtained in the field study were mainly common visible ones i.e. anaemia, pallor, staring coat, weight loss, lacrymation, fever, but those obtained from

the Delphi study included both common visible signs and rare or sporadic or signs that are noticeable when in continuous contact with cases and are usually obtained from animal owners through case history i.e. abortion, stunted growth, coughing, reduced milk yield.

3. While results of the field study had a strong element of confirmatory laboratory diagnosis, the Delphi results had a strong element of international expertise, wider coverage of disease distribution and represented accumulated experience in disease diagnosis of participants over many years.
4. While clinical signs obtained from the field study were statistically demonstrated to have significant association to the respective diseases, results of the Delphi study indicated the degree of importance of the various clinical signs and risk factors for clinical diagnosis of the respective diseases.

The major similarity between the field and the Delphi study of practical importance in terms of development of decision support tools was that both studies highlighted clinical features common to several diseases and rare but specific clinical signs that are pathognomonic to particular diseases. As recommended by others (Cockcroft, 1999b; Middleton, 2001), clinical signs common to several diseases such as anaemia, pallor, weight loss, staring coat, enlarged lymph nodes and fever increase the sensitivity of the decision support tool i.e. ability to detect true cases. A good diagnostic sensitivity of a decision support tool improves chances of clearly determining what the disease problem is during diagnosis. The rare but pathognomonic signs for individual diseases such as submandibular oedema, petechial haemorrhages, icterus, abortion, ataxia increase the specificity of the decision support tool i.e. ability to detect true negative animals and eliminate false positives. A good diagnostic specificity of a decision support tool improves chances of eliminating healthy animals during diagnosis.

In Chapter 8, quantitative information on clinical signs obtained from the Delphi survey was used to develop a decision support card that utilizes a combination of pattern-matching and colour-banding scoring systems to execute differential diagnosis. The decision support card was designed for field veterinarians, animal health assistants and community animal health workers for use in the rural areas of Africa. An evaluation under controlled conditions to assess its diagnostic performance in terms of its sensitivity and specificity in the target production system i.e. mixed-crop livestock is underway, before it is released for further evaluation and routine use by a larger group of independent end-users.

9.3 Application of the decision support card and portable haemoglobinometers

The field scenario at hand under which field veterinarians, animal health assistants and community animal health workers operate in many rural areas of Africa, such as in the mixed crop-livestock production system in South East Uganda, is a situation where cattle are frequently affected by a combination of intestinal and haemoparasites, which mutually aggravate each other's pathogenic effects (Dwinger *et al.*, 1994). For example, occurrence of mixed infections of *B. bigemina*, *T. parva*, *A. marginale* and trypanosomes in cattle in Uganda were long reported by Bruce and others (1910) and more recent reports (Magona and Mayende, 2002) still reflect a similar situation. Occurrence of concurrent infections of *T. parva*, *B. bigemina*, *T. congolense* and *T. vivax* (Kambarage, 1985) and of concurrent trypanosomosis and anaplasmosis (Fox *et al.*, 1993) has also been observed in cattle in Tanzania. Concurrent infections of *T. vivax*, *A. marginale* and gastrointestinal nematode infections have also been found during an outbreak of acute trypanosomosis in sedentary herds of Friesian cattle in Nigeria (Kalu, 1996).

Anaemia has been shown in Chapters 7 and 8 to be a feature common to trypanosomosis, anaplasmosis, babesiosis, theileriosis, parasitic gastroenteritis and fasciolosis, and has also been

reported in other studies (Kambarage, 1985; Fox *et al.*, 1993; Dwinger *et al.*, 1994; Kalu, 1996; Magona and Mayende, 2002). Anaemia is an important visible sign for diagnosis of endemic diseases. It can be detected by examination of mucous membranes but this is insensitive. Therefore there is a need for a quantitative measure of anaemia.

The occurrence of multiple disease conditions undoubtedly complicates diagnosis. Such a situation may require decision support tools to facilitate differential diagnosis. A low technology decision support tool such as the decision support card could be a useful aid for field veterinarians, animal health assistants and community animal health workers in the differential diagnosis of endemic bovine diseases in rural areas of Africa. Portable haemoglobinometers could provide a quantitative and more sensitive means of detecting anaemia than clinical examination of mucous membranes.

It is envisaged that the decision support card would largely be used as a guide for differential diagnosis. In addition, it is also a potential tool for training veterinary students, animal health workers and farmers. When used as intended, the decision support card could potentially lead to a number of benefits. Its use requires clinical examination of individual cases before treatment is made. This would ensure only infected animals are treated, thereby reducing the cost of treatment to the farmer and preventing drug misuse that leads to drug resistance, drug toxicity or reduction in livestock productivity (Stevenson *et al.*, 1993; Eisler *et al.*, 1997; Geerts and Holmes, 1998; ICPTV, 1999; Mdachi, 1999). Similar low technology decision support tools such as the FAMACHA have been used to tackle the problem of anthelmintic resistance in sheep through reduction in anthelmintic use (Van Wyk *et al.*, 1997). On a positive note, the decision support card is envisaged to facilitate disease management. Such diagnostic aids that increase the ability of the human mind to interpret information correctly are said to be helpful

to clinicians (Morley, 1991). Effective disease management and control is anticipated to lead to improved cattle health and productivity in the rural areas of Africa.

Portable haemoglobinometers could facilitate point of care haemoglobin measurement especially under remote field conditions during detection of anaemia for disease diagnosis and treatment. Portable haemoglobinometers could also be useful for measurement of haemoglobin in studies for evaluation of the efficacy of chemotherapy and on trypanotolerance or disease resistance. An ideal field haemoglobinometer would be simple, reliable and cheap. Of the portable haemoglobinometers tested in Chapter 4, only the Haemoglobin Colour Scale and the HemoCue are easy to use and reliable, and only the Haemoglobin Colour Scale and the DHT-haemoglobinometer are cheap.

9.4 Recommended use of the decision support card and portable haemoglobinometers

Figure 9.2 is a flow chart that illustrates the steps animal health workers might be expected to follow when dealing with suspected cases of endemic disease in rural areas of Africa and the recommended application of the decision support card and portable haemoglobinometers in disease management. In reference to Figure 9.1, a veterinarian is expected to conduct thorough clinical examination of cases that involves general inspection of the animal and its surroundings, obtaining case history and thorough physical examination as well as seeking laboratory confirmation. By conducting the entire diagnostic process, a veterinarian can increase chances of recognizing visible clinical signs such as pallor of the mucous membrane, submandibular oedema, pot belly, enlarged superficial lymph nodes, staring coat, abnormal behaviour, weakness, weight loss, petechial haemorrhages and inappetence, and gather enough history from the animal owner on other clinical signs such as reduced milk yield, abortion, haemoglobinuria, constipation and coughing. However, misdiagnoses have been attributed to omission of one or more parts of a clinical diagnosis or failure to consider relevant differential

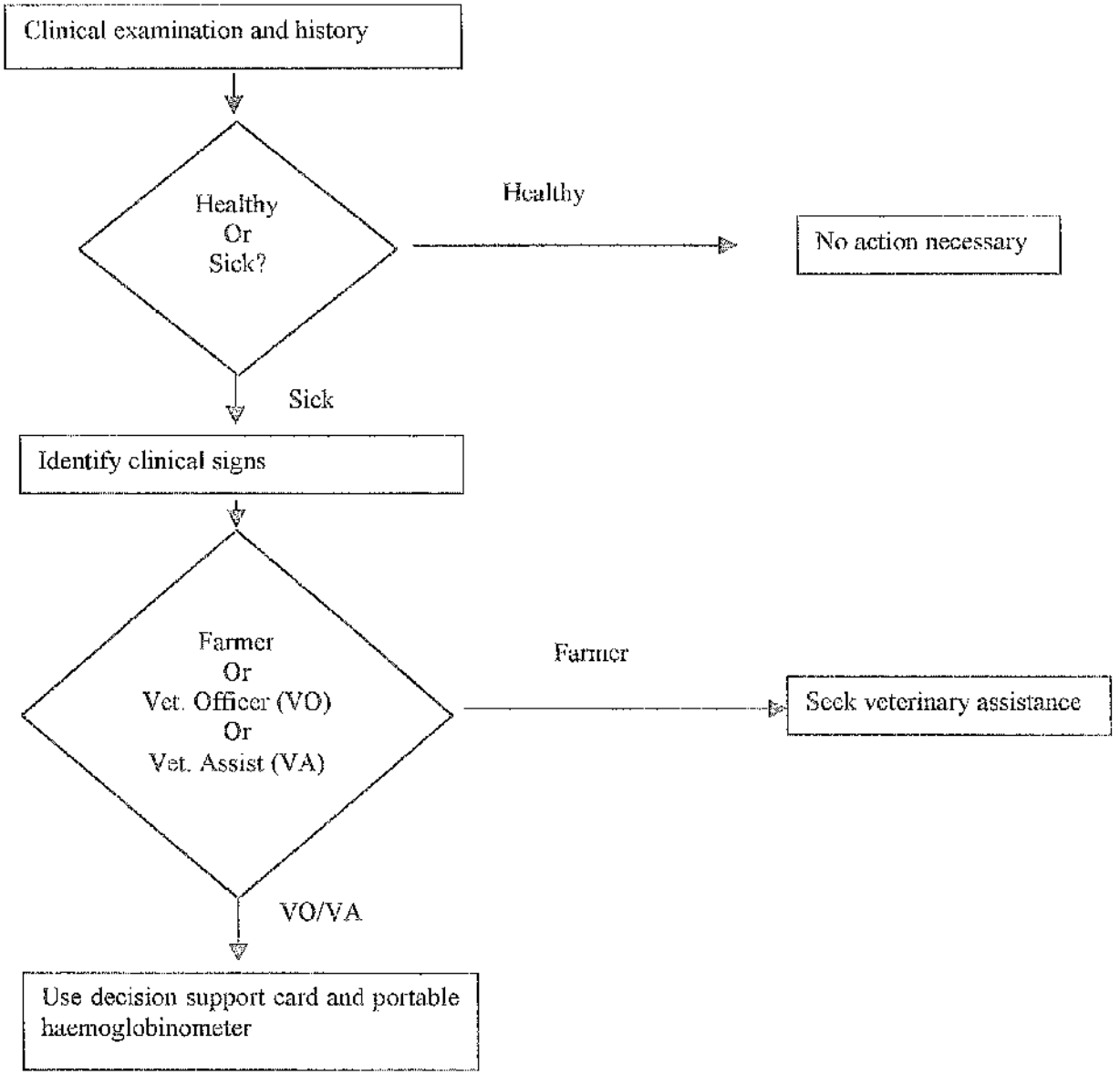
diagnoses and premature closure of the differential diagnosis (Kelly, 1984; Morley, 1991; Cockcroft, 1999a).

Veterinary assistants are expected to conduct clinical examination, taking note of key signs associated with common diseases in an area. They are expected to recognize visible clinical signs and also take history from animal owners. On the other hand, farmers can take note of clinically obvious observations such as abortions, weight loss, reduced milk yield, diarrhoea and coughing. Such observations could be an important component of case history for veterinarians or veterinary assistants that are called to attend to the cases. However, it has been shown that veterinary assistants and farmers are unskilled in differential diagnosis (Machila *et al.*, 2003). In addition, there is evidence that farmers' diagnoses are not always reliable (Machila *et al.*, 2003). These primary animal healthcare providers i.e. farmers and veterinary assistants have been reported to rely on visible clinical signs, history of sick animals, post-mortem observations and perceived causes of disease in making diagnoses, in absence of decision support tools (Delchanty, 1996; Hefferman *et al.*, 1996; Machila *et al.*, 2003).

The animal health auxilliaries i.e. animal health assistants and community animal health workers involved in the diagnosis of endemic diseases in sub-Saharan Africa have limited training (FAO, 1992). Limited knowledge on clinical signs of diseases may lead to inappropriate drug use, drug failure, incurring unnecessary costs of drugs and risking development of drug toxicity or drug resistance (Stevenson *et al.*, 1993; Eisler *et al.*, 1997; Geerts and Holmes, 1998). History taking and clinical examination by people involved in disease diagnosis are critical to avoid misdiagnosis. It is envisaged that these groups involved in diagnosis and treatment of bovine diseases in rural areas of South East Uganda in particular and sub-Saharan Africa in general, could benefit from the use of the decision support card once

validated and other tools with the ability of detecting anaemia such as the portable haemoglobinometers.

Fig. 9.2 Recommended application of the decision support card and haemoglobin measurement in farm-level management of endemic bovine diseases in rural areas of Africa



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Appendix

Table 1. List of veterinary experts who participated in the Delphi survey

Name	Type	Country/District	Anaplasmosis	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosomosis	Theileriosis	Trypanosomosis
Dr Bernard Otim	Field Vet	Uganda/Lira	x	x	x	x	x	x	x	x
Dr Charles Nohi	Field Vet	Uganda/Lira	x	x	x	x	x	x	x	x
Dr Francis Ajuga	Field Vet	Uganda/Arua	x	x	x	x	x	x	x	x
Dr Frederick Kagwa	Field Vet	Uganda/Kamuli	x	x	x	x	x		x	x
Dr James M. Tumwine	Field Vet	Uganda/Masindi	x	x	x	x	x	x	x	x
Dr Kiiza-Waako	Field Vet	Uganda/Kamuli	x	x	x	x	x		x	x
Dr Kizito Drazua	Field Vet	Uganda/Arua	x	x	x	x	x		x	x
Dr Munyambonera	Field Vet	Uganda/Kisoro	x	x	x	x	x		x	x
Dr Rashid Mubiru	Field Vet	Uganda/Masindi	x	x	x	x	x	x	x	x
Dr Richard Ssemirembe	Field Vet	Uganda/Mpigi	x	x	x	x	x		x	x
Dr Stephen Onzima	Field Vet	Uganda/Arua	x	x	x	x	x	x	x	x
Dr Thomas Anyuru	Field Vet	Uganda/Lira	x	x	x	x	x	x	x	x
Dr Virgil B. Ecoku	Field Vet	Uganda/Masindi	x	x	x	x	x		x	x
Dr Paul Opio Maloba	Field Vet	Uganda/Rakai	x	x	x	x	x	x	x	x
Dr A. Makundi	Scientist	Tanzania				x	x	x		
Dr A.S. Peregrine	Scientist	Canada								x
Dr A.U. Kalu	Scientist	Nigeria								x
Dr B. Bauer	Scientist	Kenya								x
Dr Bert de Vos	Scientist	Australia	x	x						
Dr C.K. Doku	Scientist	Ghana								x
Dr E. F. Redmond	Scientist	Australia	x	x						
Dr F.M. Mukendi	Scientist	Kenya							x	x
Dr G. Hendrickx	Scientist	Belgium								x
Dr G.K. Gitau	Scientist	Kenya							x	
Dr G.O. Matete	Scientist	Kenya	x	x	x	x	x		x	x
Dr H. W. Ploeger	Scientist	The Netherlands					x			

Table 1. Animal health experts who participated in the Delphi study (continued)

Name	Type	Country/District	Anaplasmosis	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosomosis	Theileriosis	Trypanosomosis
Dr H.A. Mbwambo	Scientist	Tanzania						x	x	
Dr J. Vercruysse	Scientist	Belgium				x	x	x		
Dr J. Mubanga	Scientist	Zambia							x	
Dr L. Lynen	Scientist	Tanzania	x	x	x			x		
Dr L.S.B. Mellau	Scientist	Tanzania						x		
Dr Maarten Eysker	Scientist	The Netherlands				x	x			
Dr R. Bock	Scientist	Australia	x	x						
Dr R. Dalgliesh	Scientist	Australia	x	x						
Dr R. Taylor	Scientist	South Africa	x	x	x					
Dr R. Waruiru	Scientist	Kenya				x	x	x		
Dr S. Mahan	Scientist	Zimbabwe			x					
Dr T.K. P-Motsu	Scientist	Botswana							x	
Dr W. Jorgensen	Scientist	Australia	x	x						
Prof. A.A. Ilemobade	Scientist	Nigeria			x				x	

Prototype Decision Support System for Endemic Diseases of Zebu Cattle in SE Uganda

	Anaplasm.	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosm.	Theileriosis	Trypanosm.
Anaemia or Pallor	4	2		2	3	4	1	4
Anorexia or Depression	2	2	4				3	
Ataxia or Abnormal behaviour			4					
Constipation	4							
Diarrhoea				1	3	1		
Dysentery						2	1	
Dyspnoea or Coughing							3	
Haemoglobinuria		4						
Icterus	1	2						
Lymph node enlargement							4	2
Pyrexia	3	4	4				4	1
Staring coat				2	2	1		3
Stunted growth or pot belly				2	3	2		
Submandibular/ventral oedema				3	2			
Weakness	1	2	3	3	1	3		2
Weight loss	1			3	2	3		4

Instructions for use

Identify the rows of the table showing the clinical signs present in the animal. Add up the numbers in the 'disease' columns for these rows only. Compare the totals for each column. The heading of the column with the highest total is the most likely diagnosis. Note that an animal may be suffering from more than one disease, which will complicate diagnosis. The system is intended to assist individuals with veterinary clinical training, and all other available information should be taken into consideration.

(This system is a prototype for research purposes only. The authors accept no responsibility for the consequences of its use in clinical situations.)