CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The sedentary mixed crop-livestock smallholding system encompasses >50% of poor people resident in East Africa. In Kenya, a total of 67.7% of the total population of 38.6 million people live in rural areas (KNBS 2010). Livestock are one of the principle capital assets of these resource-poor farmers. Indigenous breeds, of which the small East African Short-horn Zebu is the most common, are regionally very important and constitute up to 77% of the total Kenyan cattle population of 17,4 million (Rege, Kahi, Okomo-Adhiambo, Mwacharo & Hanotte 2001; Mwacharo, Okeyo, Kamande & Rege 2006; KNBS 2010). Farmers prefer indigenous breeds because of their adaptability in terms of disease resistance, heat tolerance, and water and food requirements (Mwacharo & Drucker 2005).

The tropical climate in western Kenya is conducive for the survival of many infectious pathogens and vectors. The most economically important diseases of livestock in sub-Saharan Africa are tick-borne diseases, especially East Coast fever (ECF), heartwater, anaplasmosis and babesiosis, and also trypanosomosis (Uilenberg 1995; Minjauw & McLeod 2003; Maudlin 2006) and helminthosis (Gray, Connell & Phimphachanhvongsod 2012). Small-holder farmers are particularly vulnerable to the economic impact of infectious diseases of livestock. Generally tick-borne infections do not affect trade in livestock, but they are a significant cause of production losses (Perry & Young 1995). Losses include lowered production rates, mortalities, decreased reproduction rates and costs of treatment and control measures. These diseases also indirectly constrain livestock production through limiting the use of the highly susceptible improved breeds of livestock which are used in other countries to improve livestock productivity (Perry & Young 1995).

2. LITERATURE REVIEW

Anaemia is a common clinical sign of many of the tick-borne diseases, trypanosomosis and helminthosis. Anaemia is defined as an erythrocyte count, haemoglobin concentration or a packed cell volume (PCV) that is below what is considered as reference values for the species (Jain 1993). Anaemia is the most common abnormality of blood (Anon. 2009a) and

can be grouped into three classes, namely *haemolysis* (erythrocyte destruction); *haemorrhage* (loss of erythrocytes) and *dyshaemopoiesis* (ineffective erythrocyte production) (Jain 1993; Anon. 2009a). It is not unexpected that haemoparasitic infections can cause anaemia, since their life cycles are closely linked to the circulatory system of their hosts. Apart from their effect on erythrocytes, these infections can also bring about changes in the white blood cells and thrombocytes. The cells are affected either directly by the infecting parasites, or indirectly by the host's response to the infection.

2.1 Infectious causes of anaemia

2.1.1 Tick-borne diseases
Anaplasmosis
Anaplasmosis is predominantly caused by *Anaplasma marginale*, an arthropod-borne parasite. Also known as gall-sickness, clinical disease is generally associated with fever, progressive anaemia and icterus (Potgieter & Stoltsz 2004). Another species, *Anaplasma centrale*, is less virulent and seldom causes clinical disease. The principal vectors of this parasite are Ixodid ticks, in particular *Rhipicephalus* (*Boophilus*) *decoloratus*, but other ticks that can potentially act as vectors are *Rhipicephalus* (*Boophilus*) *microplus, Rhipicephalus simus, Rhipicephalus evertsi evertsi* and *Hyalomma marginatum rufipes* (Potgieter & Stoltsz 2004). Mechanical transmission of anaplasmosis by blood-sucking arthropods has also been described (Potgieter & Stoltsz 2004), as well as transplacental transfer (Aubry & Geale 2010).

Anaplasma spp. are intra-erythrocytic parasites. Acute disease is associated predominantly with anaemia, which is caused by extensive erythrophagocytosis. The animal presents with pallor of mucous membranes, inappetance, general weakness and fever. As the disease progresses clinical signs such as constipation, rumen stasis, icterus and weight loss become apparent. As the parasite damages erythrocytes, the cells are removed by the reticuloendothelial system in the spleen, lungs, liver and associated lymph nodes. Anti-erythrocytic antibodies also damage the non-parasitised red blood cells. Macroscopic pathology is typical for erythrophagocytosis and includes severe anaemia, icterus, splenomegaly and hepatomegaly (Potgieter & Stoltsz 2004).

Calves have an age-related resistance to severe clinical disease up to the age of 6 months (Potgieter & Stoltsz 2004). Calves that are born in endemic areas and are exposed during this period of resistance will develop immunity to the disease before the age when clinical effects become severe. Clinical disease usually develops only in older animals that have not

been exposed during calfhood (Gale, Leatch, De Vos & Jorgensen 1996b). Older animals infected with *A. marginale* develop more severe reactions with higher parasitaemias and percentage reductions in PCV than younger animals (Parker, Shephard, Trueman, Jones, Kent & Polkinghorne 1985; Aubry & Geale 2010). Although the prepatent period in anaplasmosis is inversely related to the infective dose, the clinical outcome, namely anaemia, does not appear to be correlated to the infective dose (Gale *et al.* 1996b). Once animals have recovered from anaplasmosis, they remain life-long carriers of the parasite (Potgieter & Stoltsz 2004; Aubry & Geale 2010).

Babesiosis

Bovine babesiosis is caused by *Babesia bovis* and *Babesia bigemina*, respectively known as Asiatic and African redwater. The only known vector of *B. bovis* in southern Africa is *R. microplus*. Transmission in the vector is transovarial (De Vos, De Waal & Jackson 2004). *Babesia bigemina* has several tick vectors, including *R. microplus, R. decoloratus* and *R. evertsi evertsi*. Transovarial transmission as well as transstadial transmission of the parasite occurs in *Rhipicephalus* (*Boophilus*) spp. Both nymphal and adult stages thus transmit the parasite to the host. Only the nymphal stages of *R. evertsi evertsi* can infect the host (De Vos *et al.* 2004). *Babesia* spp. are intracellular organisms but only erythrocytes are involved in the development of the parasite. Once the infective sporozoites enter the erythrocyte, they develop into trophozoites which in turn develop into two new merozoites which are infective to the tick vector (De Vos *et al.* 2004).

During the first two months of life, calves are protected from clinical babesiosis by passive transfer of immunity from resistant dams (Bock, Jackson, De Vos & Jorgensen 2004; De Vos *et al.* 2004; Magona, Walubengo, Olaho-Mukani, Jonsson, Welburn & Eisler 2008a). After two months of age calves have a natural resistance to babesiosis which lasts for 6 to 9 months, irrespective of the immune status of their dams (De Vos *et al.* 2004; Mahoney, Wright & Mirre 1973). Animals that have recovered from infection remain latent carriers for variable periods, after which they lose the infection (De Vos *et al.* 2004). Recovery is followed by a solid, long-lasting immunity, even in the absence of re-infection. In cattle that have recovered from *B. bigemina* there is a level of cross-immunity against *B. bovis* (Callow, McGregor, Parker & Dalgliesh 1974; Bock *et al.* 2004). The reverse is not true, however.

The clinical signs associated with *B. bigemina* infections are the result of severe intravascular haemolysis. Animals present with high fever, pallor, haemoglobinuria, icterus, inappetance and lethargy. Clinical signs associated with *B. bovis* infections are similar but more severe with the development of a hypotensive shock syndrome. Disseminated

intravascular coagulation results in generalized organ failure. Cerebral involvement is manifested by clinical signs of hyperaesthesia, circling, nystagmus, head pressing and aggression, and without intervention is almost invariably fatal. As in anaplasmosis, the initial infective dose in babesiosis does not correlate with the clinical outcome or time to recovery (Allred 2007; Gale *et al.* 1996b).

Ehrlichiosis

Ehrlichia ruminantium causes heartwater in cattle, small stock and some wild ruminants. Heartwater is characterised by fever, neurological signs, hydropericardium, hydrothorax and lung oedema. The vector for this parasite in Kenya is *Amblyomma variegatum*. Transstadial transmission occurs in this three-host tick. Young calves have an age-related resistance to disease which lasts about four weeks from birth and is independent of the immune status of the dam. As in other tick-borne disease, there is also a difference in susceptibility between breeds. Zebu cattle are regarded as more resistant, and although infection can still be established, severe clinical signs are lesssevere (Allsopp, Bezuidenhout & Prozesky 2004).

After infection of the host, the parasite invades the endothelial cells of blood vessels where they multiply. Effusion into body cavities and tissue oedema develops due to increased vascular permeability. The extensive effusion leads to a reduction in blood volume. *Ehrlichia ruminantium* parasites can be demonstrated in the cytoplasm of endothelial cells of blood vessels of the brain by light microscopy during a post mortem examination. Infection of the endothelial cells in the brain and the development of brain oedema cause neurological signs (Allsopp *et al.* 2004).

Bovine ehrlichiosis, caused by *Anaplasma* (*Ehrlichia) bovis*, and Ondiri disease, caused by *Cytoecetes ondiri*, are lesser known rickettsial diseases but have been associated with disease in cattle in Kenya (Sumption & Scott 2004).

Although it is expected that the distribution of *A. bovis* is widespread, few studies have been done to confirm this (Sumption & Scott 2004). In a study done in Uganda close to Lake Victoria, 63% of cross-bred cattle and 23% of indigenous cattle sampled were positive for *A. bovis*. This suggests that *A. bovis* may potentially be a significant cause of disease in the area (Oura, Bishop, Wampande, Lubega & Tait 2004). The epidemiology of bovine ehrlichiosis has not been fully described yet, but it is suggested that *R. appendiculatus* is the vector in East Africa. Although rare, overt disease presents with clinical signs of fever, lymphadenopathy, depression and weight loss (Sumption & Scott 2004). Neurological signs are sometimes seen in acute primary infection and can readily be confused with heartwater

(Stewart 1992). Exotic breeds or naïve indigenous cattle are particularly susceptible to disease. In contrast to heartwater, haematological changes on bloodsmears can aid in diagnoses. Monocytes typically show vacuolisation which may contain some rickettsias. The nuclear membrane is also distorted. Monocytosis with eosinopenia is suggestive of bovine ehrlichiosis (Stewart 1992). Thrombocytopenia is also present (Sumption & Scott 2004).

Ondiri disease, also known as bovine petechial fever, has a very restricted distribution in the East African highlands. The vector is still unknown. Clinical signs associated with Ondiri disease include fluctuating fever, lowered milk yield, and generalized petechiation of mucous membranes. Case-mortality rates of up to 50% have been described. Infected granulocytes on blood smears can aid in diagnosis. Ondiri disease is not regarded as an important disease in Kenya (Sumption & Scott 2004).

Theileriosis

Four *Theileria* species have been reported from Kenya, namely *Theileria parva, Theileria mutans, Theileria taurotragi* and *Theileria velifera* (Kariuki 1990). Two types of *T. parva* are found in Kenya. Buffalo-derived *T. parva* strains are maintained within African buffalo populations and are the cause of Corridor disease in cattle. The disease is acute and usually fatal and cattle are regarded as dead-end hosts for the parasite (Lawrence, Perry & Williamson 2004b).

Cattle-derived *Theileria parva* strains cause ECF in cattle, which is regarded as a major disease of cattle in East Africa (Young & Mutugi 1990b). Classical ECF (Lawrence, Perry & Williamson 2004a) is fatal in European cattle breeds, but Zebu cattle raised in endemic areas suffer a less severe form of the disease or remain unaffected. The principal vector is the brown ear tick, *Rhipicephalus appendiculatus* (Young & Mutugi 1990b). This is a three-host tick. Transmission of *T. parva* is trans-stadially between larval and nymphal stages, as well as between the nymphal and adult stages (Young, Leitch, Newson & Cunningham 1986). In East Africa all the instars of the tick occur on cattle throughout the year (Young & Mutugi 1990b).

Two cell lines of the host are intimately involved in the intracellular proliferation of *Theileria* spp., namely lymphocytes and erythrocytes. After infection the sporozoites enter the lymphocytes of the host and develop into schizonts, which stimulate the lymphocytes to transform into lymphoblasts after which the schizonts divide. These infected lymphoblasts proliferate exponentially and spread throughout the lymphoid tissue as well as metastasising to non-lymphoid tissues. Later still, merozoites are liberated from a generation of

microschizonts, which then enter erythrocytes and thus complete their life cycle (Lawrence *et al.* 2004a).

East Coast fever is characterised by lymphoid proliferation followed by lymphoid destruction. Animals present with fever, enlargement of superficial lymphnodes and severe pulmonary oedema. Panleukopenia coincides with the onset of pyrexia (Lawrence *et al.* 2004a). A non-regenerative anaemia has been described but is not a consistent feature of the disease (Mbassa, Balemba, Maselle & Mwaga 1994; Fanduma, Marcotty, Brandt, Duchaleau, Speybroeck, Dolan & Berkvens 2007). As opposed to babesiosis and anaplasmosis, the initial infective dose does affect the clinical outcome and severity of disease (Koch, Kambeva, Norval, Ocama, Masaka, Munatswa, Honhold & Irvin 1990).

Zebu cattle that are raised in ECF-endemic areas exhibit a low innate susceptibility (Perry & Young 1995). Mortalities in these Zebu calves are well below 10% (Moll, Lohding, Young & Leitch 1986). Indigenous breeds outside endemic areas and introduced breeds are highly susceptible and ECF is frequently fatal.

When calves recover after infection they can become asymptomatic carriers of the parasite and thus remain a source of infection to ticks. The persistence of infection in the host is due to division and persistence of the schizont stage of the parasite (Young, Mutugi, Kariuki, Maritim, Linyonyi, Mining, Kwena, Ngumi, Ndungu, Lesan, Lampard, Awich, Stagg, Leitch, Williamson & Grootenhuis 1990a). There is, however, limited cross-protection between different strains of *T. parva* (Morrison 2009). It is postulated that for endemic stability to develop, calves in endemic areas must become sequentially infected with several field strains (antigenic variants) of *T. parva* and thus become immune but also carriers of all the different strains in the field (Moll *et al.* 1986; Young *et al.* 1990a). In the Trans-Mara division of the Narok district, 100% of the calf population became infected by three months of age with at least three incidents of clinical ECF (Moll, Lohding & Young 1984; Moll *et al.* 1986). Where calves do not become infected during calfhood, either due to the unsuitability of the environment for the tick vector or due to too rigorous tick control, the population immunity against *T. parva* will be low and high mortality rates can occur (Perry & Young 1995).

Theileria mutans, T. taurotragi and *T. velifera* are considered less pathogenic than *T. parva*. *Theileria mutans* has been described as a cause of low growth rates in calves (Moll *et al.* 1984) and clinical cases are typically associated with mild transient fever and anaemia (Kariuki 1990). *Theileria mutans* and *T. velifera* are transmitted by *Amblyomma* ticks, whereas *T. taurotragi* is also transmitted by *R. appendiculatus* (Moll *et al.* 1984). Mixed

infections of different *Theileria* species occur and can complicate the clinical presentation of disease as well as diagnostic test results.

2.1.2 Tsetse-borne pathogens

Trypanosomosis

Trypanosomosis, caused by protozoan parasites of the genus *Trypanosoma,* has been one of the most important diseases in livestock in sub-Saharan cattle since before colonial times. It remains a serious constraint to economic development in the region (Maudlin 2006). The main vector is tsetse flies, *Glossina* spp., although other biting flies and mechanical transmission are implicated in the transmission of certain species (Connor & Van den Bossche 2004).

Of the tsetse-transmitted trypanosomes, *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei* are of particular importance in cattle. *Trypanosoma theileri*, a stercorarian parasite, is transmitted by tabanid flies. It is regarded as non-pathogenic, but has been associated with disease in certain circumstances (Connor & Van den Bossche 2004). These trypanosomes are blood-borne, with *T. congolense* confined to the circulatory system of the host. However, *T. vivax* and *T. brucei* also invade the host's tissues (Murray & Dexter 1988).

Anaemia is a well recognized consequence of trypanosomosis. In fact, PCV is used as an indicator for trypanosomosis on a herd-level, even when trypanosomes are not detected by parasitological diagnostics (Van den Bossche, Mudenge, Mubanga & Norval 1999; Van den Bossche & Rowlands 2001b). Murray & Dexter (1988) divided the progression of anaemia in trypanosomosis into two phases. During the first, acute phase of anaemia the PCV falls at the same time that the first wave of parasitaemia develops. Anaemia is indirectly caused by infection through the activation of mononuclear phagocytosis which results in massive erythrophagocytosis. At the same time, trypanosome haemolysins and proteases also induce haemolysis (Murray & Dexter 1988). There is an indication of increased haemopoiesis to compensate for the massive erythrophagocytosis. The second, chronic phase can overlap with the first phase, but is associated with low transient parasitaemias. Erythrophagocytosis is ongoing but there are now indications of dyshaemopoiesis. The synthesis of red blood cells is insufficient to compensate for the degree of anaemia seen (Dargie, Murray, Murray, Grimshaw & McIntyre 1979; Murray & Dexter 1988). This indicates a degree of bone marrow dysfunction. The affected animal can show either spontaneous recovery, survive this phase with persistent low-grade anaemia or die. The response to treatment during the acute phase is rapid, compared to the chronic phase where treatment is

often ineffective (Urquhart & Holmes 1987). An acute haemorrhagic syndrome has been described in *T. vivax* (Magona, Walubengo & Odimin 2008b).

Pancytopenia develops in the host during the first wave of the parasitaemia. Apart from anaemia, thrombocytopenia and leukopenia are also constant findings in trypanosomosis (Wellde, Kovatch, Chumo & Wykoff 1978; Murray & Dexter 1988). The decrease in thrombocytes, lymphocytes and neutrophils correlate with the onset, severity and prevalence of trypanosomes in the host's blood. The development of disseminated intravascular coagulation in end-stage trypanosomosis adds to the loss of thrombocytes and is reflected by generalized petechiae and ecchymoses seen at post mortem examinations (Murray & Dexter 1988).

Immunosuppression in trypanosomosis is also well recognized (Holmes, Mammo, Thomson, Knight, Lucken, Murray, Murray, Jennings & Urquhart 1974; Mackenzie, Boyt, Emslie, Lander & Swanepoel 1975; Askonas 1984; Urquhart & Holmes 1987, Uilenberg 1998). Although the host exhibits an active lymphoid response, an immune response to other antigens is almost completely lacking, in particular antigens that stimulate an antibody response (Urquhart & Holmes 1987).

The long-term effects of trypanosomosis in cattle are emaciation, reduced growth rates, reduced reproductive rates, abortions and reduced milk yields (Trail, D'Ieteren, Murray, Ordner, Yangari, Collardelle, Sauveroche, Maille & Viviani 1993). Exotic breeds of cattle are more susceptible than local breeds, such as N'dama cattle (Connor & Van den Bossche 2004).

2.1.3 Gastrointestinal parasites

The most significant impact of helminthosis in cattle in Africa is through the erosive effect of reduced weight gain. Often these sub-clinical production losses largely go unnoticed (Waller 1997; Waruiru, Weda, Otieno & Ngotho 2002). Gastrointestinal helminths can be a significant cause of anaemia in ruminants, however, in particular haemonchosis and fasciolosis (Kaufmann, Dwinger, Hallebeek, Van Dijk & Pfister 1992). *Haemonchus* is considered as one of the most pathogenic parasites of ruminants (Kaufmann1996) and is consistently reported as the most prevalent helminth species in cattle in Kenya (Moll *et al.* 1984; Latif, Rowlands, Punyua, Hassan & Capstick 1995; Waruiru, Thamsborg, Nansen, Kyvsgaard, Bogh, Munyua & Gathuma 2001; Waruiru *et al.* 2002). The pathogenesis is that of a haemorrhagic anaemia (Kaufmann *et al.* 1992). During extremely high parasite burdens the animal will die due to haemorrhage from the abomasum. In chronic cases animals

develop a steady drop in PCV and serum albumin which results in emaciation of the animal. If the animal survives, the compensatory erythropoiesis will eventually deplete iron reserves.

Fasciolosis is an important disease in areas where the fluke's intermediate host, *Lymnaea* spp. snails, occur (Kaufmann 1996). Acute death due to liver fluke occurs rarely in cattle. Chronic infestation is more common and the animal presents with gradual wasting, severe anaemia, ascites and oedema and high faecal fluke egg counts. In massive infections, the fluke's migration through the liver of the host can cause acute anaemia. However, anaemia is mainly caused by the consumption of blood by the flukes when they arrive in the bile ducts. Initially the host exhibits compensatory erythropoiesis but eventually an iron deficiency develops which contributes to the level of anaemia. There is also a marked eosinophilia (Kaufmann 1996).

Prevalence of helminth infections appears to have a seasonal pattern, with faecal egg outputs reported to follow rainfall patterns (Fall, Diack, Diaté, Seye & d'Ieteren 1999; Waruiru *et al.* 2002). Where climatic conditions in parts of Kenya are favourable, however, hypobiosis does not appear to be important in the epidemiology of certain nematode species and animals remain at risk of infection throughout the year, be that at a lower level during the dry season (Waruiru *et al.* 2001). There also appears to be an age-related susceptibility, with calves harbouring higher burdens than adult cattle (Latif *et al.* 1995; Fall *et al.* 1999). This is possibly due to an acquired resistance that develops within five to eight months after weaning (Latif *et al.* 1995).

Coccidiosis is another potential cause of haemorrhagic diarrhoea and anaemia in cattle (Stewart & Penzhorn 2004). It primarily affects calves from three weeks to six months of age. Clinical coccidiosis in cattle is most commonly caused by *Eimeria zuernii* and *Eimeria bovis* (Kaufmann 1996). The clinical course of infection is dose-dependent, with overt disease only developing in animals that have ingested a large number of oocysts (Stewart & Penzhorn 2004).

2.1.4 Non-infectious causes of anaemia

There are also non-infectious causes of anaemia, such as nutritional deficiencies, traumatic blood loss and physiological anaemias in neonates. Nutritional deficiencies that result in anaemia include mineral deficiencies of iron, copper, cobalt and selenium, and certain vitamins (Jain 1993). Nutritional deficiencies are generally associated with dyshaematoopoiesis and cause normocytic, normochromic non-regenerative anaemia, suggesting that the bone marrow response is ineffective (Jain 1993). Iron deficiencies cause

microcytic hypochromic anaemia due to impaired synthesis of haemoglobin (Duncan, Prasse & Mahaffey 1994). Poor nutrition and resultant nutritional deficiencies will exacerbate anaemia caused by infectious agents and inhibit the host's response to treatment (Waller 1997; Waruiru *et al.* 2002; Swai, Karimuribo, Kambarage & Moshy 2009).

In several ruminant species the red cell parameters in neonates has been shown to be below reference ranges described for adult animals (Karesh, Janssen & Oosterhuis 1986; Harvey 1989; Parsons, Penzhorn, Reyers, Steyl & Becker 2006). Physiological changes soon after birth generally cause a significant decrease in haematocrit which is followed by a gradual increase to adult levels (Harvey 1989; Roberts 2011). Factors thought to contribute to these changes include: 1) an increase in plasma volume due to the osmotic effect of absorbed colostral proteins; 2) a decrease in the production of red blood cell early in the neonatal period; 3) red blood cells formed *in utero* have a decreased life span; 4) total plasma volume expansion occurring more rapidly than red cell numbers resulting in haemodilution; and 5) a temporary decrease in erythropoeitin production in certain species (Harvey 1989). A delayed switching from foetal haemoglobin to adult haemoglobin has been described in several ruminant species, including Mouflon sheep (Hawkey, Hart & Fitzgerald 1984) and roan antelope (*Hippotragus equinus*) (Parsons *et al.* 2006). This delay results in a decrease in total haemoglobin, and thus causes anaemia in neonatal animals. A possible link between this neonatal anaemia in roan antelope and an increased susceptibility to theileriosis has been suggested (Parsons *et al.* 2006).

2.2 From infection to disease

2.2.1 Concepts and definitions
Clinical disease does not invariably result when a pathogen infects a host. Several factors associated with the pathogen, the vector, the host, as well as the environment interact and as a whole determine the outcome of infection. A few relevant concepts and definitions will be briefly discussed.

Definitions
- *Immunodepression* is the lowering of an immune response or its complete abrogation (Cox 1987).
- *Non-specific immunity* is the protective immune response against subsequent infections not directed at a specific antigen (Cox 1987).

- *Heterologous immunity* is the immunity induced by a related or unrelated infectious agent, also called *cross-protection* (Cox 1987; Clark 2001).
- *Concomitant immunity:* The host has acquired immunity against reinfecting or clinical disease, yet remain persistently infected (Cox 1993; Brown, Norimine, Knowles & Goff 2006).
- *Premunity:* recovered animals remain latently infected without showing clinical signs (De Vos *et al.* 2004).

Endemic stability

There are situations where, without human interventions, the pathogen, vector, and host co-exist in an environment without any significant effect on animal production and few if any clinical cases are reported. Such a situation is referred to as endemic stability (Perry & Young 1995; De Vos *et al.* 2004).

Endemic stability of ECF is reported from calves in areas of Kenya (Young *et al.* 1986) and Uganda (Okello-Onen, Heuer, Perry, Tukahirwa, Senyonga, Heinonen & Bode 1995). According to Perry and Young (1995) the low incidence of clinical ECF in endemically stable areas can be attributed to: 1) the Zebu cattle that are raised in endemic ECF areas that show a *high innate resistance* to ECF; 2) the acquired immunity to infection develops rapidly and effectively due to a *low but continuous exposure to ticks*; and 3) because of low parasitaemias in immune carrier animals there are *low infection rates in ticks*. In these situations, there are few clinical cases of ECF and mortality is limited to young animals. There is a strong correlation between endemic stability and high antibody prevalence (Perry & Young 1995). Typically, high antibody prevalence rates for *T. parva* (70%) occur in animals over six months of age (Okello-Onen *et al.* 1995). The high antibody prevalence is possibly due to persistence of infection, re-infection or superinfection (Young *et al.* 1986). The age of first exposure is also an important factor in establishment of endemic stability of *Babesia* (De Vos *et al.* 2004). If calves are infected with *Babesia* during the period of age-related resistance early in life, a solid, long-lasting immunity will develop. In *Babesia* infections premunity is not considered to be important. After recovery from infection, cattle develop a lasting immunity even in the absence of infection (De Vos *et al.* 2004).

High antigenic diversity of *T. parva* as well as a lack of cross-immunity between strains is well-described. The implication of this is that for endemic stability to occur calves must be infected with the whole spectrum of prevailing antigenic strains over time (6 months) and thus undergo several clinical episodes of *T. parva* infections (Young *et al.* 1986). This period

coincides with the period of passively derived maternal antibody protection (Okello-Onen *et al.* 1995).

Endemic stability can develop for babesiosis as well. During the first two months calves are protected after birth by colostral antibodies. An innate immunity against babesiosis exists in calves from three to nine months of age. When calves are infected during this period, they rarely develop clinical disease but they do develop a solid long-lasting immunity. When primary infection only occurs after this innate immunity has passed, animals will develop severe clinical signs, which is typical under endemically unstable conditions (Bock *et al.* 2004).

The frequency at which transmission of the pathogen occurs is also important. *Babesia bigemina* has higher transmission rates in its tick vector compared to *B. bovis.* Because of this, *B. bigemina* has a higher prevalence than *B. bovis* in areas where both species occur. Endemic stability is therefore more likely to develop to *B. bigemina* than to *B. bovis* (De Vos *et al.* 2004).

In endemic *Trypanosoma* areas where cattle serve as the main reservoir of trypanosomes a stable situation can also potentially develop. In these areas there is a selection pressure against strains of trypanosomes that are very pathogenic or lethal, thus these strains become rare in the herd of cattle. Under these circumstances cattle are able to develop a non-sterile immunity which contributes to the development of an endemic state of trypanosomosis where its effect on herd production is relatively low, such as in Petauke District of eastern Zambia (Van den Bossche 2001a). In areas where tsetse flies rely heavily on wildlife as a source of blood meals, there is no selection pressure against the low pathogenic strains of trypanosomes and disease occurs in epidemics that are unpredictable. In these situations the impact of trypanosomosis on the production of cattle can be considerable (Van den Bossche 2001a).
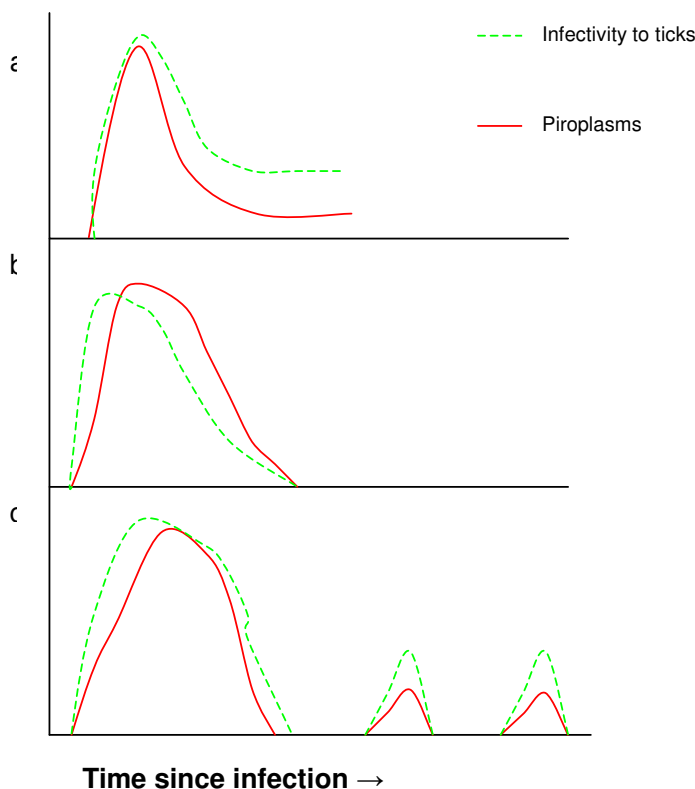
Carrier state
A carrier state has been defined as "the ability of parasites present in an infected and recovered (and therefore asymptomatic) host to infect ticks which are then able to transmit the parasites to a susceptible host" (Bishop, Sohanpal, Kariuki, Young, Nene, Baylis, Allsopp, Spooner, Dolan & Morzaria 1992).

Three types of carrier states in *T. parva*-infected hosts are described by Young, Mutugi, Maritim & Linyonyi (1990c). Cattle can act as initial carriers after which they clear the

infection, or they can act as either sporadic or continual carriers. This will depend on whether there are gametocytes infective to ticks present in the blood intermittently or continuously (Figure 1.1).

Figure 1.1 Types of carrier states (from Young *et al.* 1990c): a. Persistent carrier; b. Initial carrier; and c. Sporadic carrier

- - - - Infectivity to ticks

——— Piroplasms

Time since infection →

Carrier state in *Babesia* is seldom life long. Cattle infected with *B. bigemina* remain infective for ticks for a few weeks only and lose the infection in less than a year. Zebu cattle will lose an infection of *B. bovis* within two years. European breeds can remain infected with *B. bovis* for life, but only remain infective for ticks for up to two years (De Vos *et al.* 2004). Animals recovered from *Anaplasma* infections, however, remain infected for life (Potgieter & Stoltsz 2004).

The carrier state in animals can revert to clinical disease if the immune system of the animal is compromised to the extent that it cannot contain the infection. An example would be animals that are latently infected with *Anaplasma* that develop anaemia after they are superinfected with *T. parva* which causes immunosuppression (Moll *et al.* 1984).

Breed tolerance and susceptibility

The potential exploitation of possible genetic resistance to disease has been the subject of much investigation, especially with regard to resistance to trypanosomosis (Murray, Morrison & Whitelaw 1982). The term "trypanotolerant" is used in literature in different ways. Naessens, Teale & Sileghem (2002) used the term to describe animals that can remain productive while infected with trypanosomes. Murray *et al.* (1982) feel it more appropriate to use tolerance to describe animals that exhibit a greater degree of resistance to the disease. Trypanotolerance has also been associated with traits such as weight gain, an ability to control anaemia and efficient clearance of parasites (Van der Waaij, Hanotte, van Arendonk, Kemp, Kennedy, Gibson & Teale 2003).

The N'Dama breed of West Africa and West African shorthorn cattle are considered to be trypanotolerant. Zebu cattle in East Africa are more susceptible, but in some areas have developed a degree of trypanotolerance compared to European exotic cattle breeds (Murray *et al.* 1982). Indigenous wildlife are highly resistant or completely refractory to infection by trypanosomes and often serve as reservoirs of disease in certain areas (Murray *et al.* 1982, Van den Bossche 2001a).

Trypanotolerant cattle have a superior immune response against infection, thus develop lower parasitaemias, and also exhibit an ability to control the level of anaemia better, as compared to less tolerant breeds (Naessens *et al.* 2002). Tolerance is relative, however. The severity of clinical symptoms is related to infective dose, and when the challenge is high enough, even trypanotolerant breeds will develop disease (Murray *et al.* 1982).

Differences in breed susceptibility have also been described for other diseases. Taurine breeds are also considered to be more susceptible to a variety of tick species, ECF, babesiosis and anaplasmosis compared to Zebu and Sanga breeds (Norval, Perry & Young 1992; Perry & Young 1995; De Vos *et al.* 2004; Potgieter & Stoltsz 2004). However, one should bear in mind that even indigenous breeds that are immunologically naive will potentially develop severe disease when introduced into endemic areas (Perry & Young 1995).

2.2.2 Epidemiological states of vector-borne diseases in Kenya

Several studies have investigated the prevalence and epidemiological states of tick-borne diseases and trypanosomosis in cattle populations at a range of study sites throughout Kenya, including in the Coastal Province (Deem, Perry, Matende, McDermott, Mahan, Maloo, Marzaria, Musoke & Rowlands 1993; Maloo, Thorpe, Kioo, Ngumi, Rowlands & Perry

2001b), the Eastern Province (Gachohi, Ngumi, Kitala & Skilton 2010), in the Rift Valley (Moll *et al.* 1984; Moll *et al.* 1986); western Kenya (Okuthe & Buyu 2006); and the Central Province (Gitau, Perry, Katende, McDermott, Morzaria & Young 1997; Gitau, Perry & McDermott 1999; Gitau, McDermott, Katende, O'Callaghan, Brown & Perry 2000), as well as the neighbouring countries Tanzania (Swai, French, Beauchamp, Fitzpatrick, Bryant, Kambarage & Ogden 2005a; Swai, French, Karimuribo, Fitzpatrick, Bryant, Browne & Ogden 2005b) and Uganda (Rubaire-Akiiki, Okello-Onen, Musunga, Kabagambe, Vaarst, Okello, Opolot, Bisagaya, Okori, Bisagati, Ongyera & Mwayi 2006). In almost all of these areas ECF was recognized as the most important infectious disease in terms of morbidity, mortality and low production in cattle populations in Kenya (Uilenberg 1995; Phiri, Benschop & French 2010). Other vector-borne diseases that contributed considerably to morbidity in cattle populations, depending on endemic state of the causative pathogen, were anaplasmosis and trypanosomosis. Several risk factors have been identified by these studies that are associated with both spatial and temporal variation in the epidemiological states of vector-borne pathogens, and thus their association with disease.

Spatial distribution of pathogens

The endemic stability of the various pathogens varied considerably over the various study sites. The main attribute that affected the spatial variation in seroprevalence was agro-ecological zone (AEZ). Agro-ecological zone is a classification of smaller units of land based on soil type, landform, climatic conditions and land cover (FAO 1996). These characteristics determine the main land-use practices, e.g. mixed crop-livestock farming or dairy, and thus the predominant breed of cattle farmed and grazing systems used by farmers in such a zone (Gitau *et al.* 1997). These geophysical characteristics, together with the management practices employed by farmers in turn determine the ecological suitability for the vectors of these diseases (Deem *et al.* 1993).

Altitude is an important determinant of vector distribution. The prevalence and disease incidence of ECF in Uganda was reportedly higher at lower elevation (Rubaire-Akiiki *et al.* 2006). The calves in the lowland zone had a 2.6 times higher risk of seroconversion to ECF than calves in the upland zone due to a higher number of *R. appendiculatus* found in the lowland AEZ (altitude of 428-1275m). The highest number of *R. (B.) decoloratus* was in the upland AEZ (altitude of 1575-4368m). A similar pattern was described in the Murang'a District in Central Kenya (Gitau *et al.* 1997) where seroprevalence of *T. parva* was higher in lowland zones, compared to higher seroprevalence of *B. bigemina* in highland zones, which correlated with the abundance of the respective tick vectors in the two AEZ.

Different grazing systems affect the prevalence of TBD in cattle by influencing the levels of exposure cattle have to ticks. Calves in the same lowland AEZ in Uganda in a communal grazing system had a 10 times higher risk of seroconversion to ECF than zero-grazed calves (Rubaire-Akiiki *et al.* 2006). Cattle in an open-grazing, communal system are at a higher risk of exposure to ticks and contracting TBD (Gitau *et al.* 1999). It is expected that livestock in zero-grazing systems, especially with apparent stringent acaricide use would have no tick challenge, and thus a low prevalence or incidence of TBD (Maloo, Rowlands, Thorpe, Gettinby & Perry 2001a; Maloo *et al.* 2001b). However, cut forage is often a source of ticks, which are maintained by pastoral livestock. Thus, zero-grazing reduces, but does not prevent exposure to ticks (Swai *et al.* 2005b). Anaplasmosis was not necessarily found to be associated with grazing practices as stall-fed animals had similar incidence rates of anaplasmosis as grazed animals (Gitau *et al.* 1997). This is because *A. marginale* is also transmitted by biting flies (Gitau *et al.* 1997; Aubry & Geale 2010). Swai *et al.* (2009) on the other hand found that nutritional and environmental stress in pastoral grazing systems in Tanzania caused a higher mortality due to anaplasmosis.

Okuthe & Buyu (2006) reported a higher incidence and prevalence of theileriosis, anaplasmosis and babesiosis in peri-urban areas than in rural areas in the highlands of western Kenya. They attributed this to three reasons: a) more irregular tick control in peri-urban areas; b) rural people prioritising livestock rearing more highly; and c) the difference in grazing practices. Cattle in rural areas were kept in smaller groups and had more pasture available to them compared to peri-urban areas. Swai *et al.* (2005b) on the other hand found that cattle in rural areas were up to four times more likely to seroconvert to *T. parva* than cattle kept around town areas.

Temporal distribution of pathogens and incidence of disease
Seasonal variation in vector-borne parasites correlates with the seasonal occurrence of their respective vectors (Latif *et al.* 1995). In ECF endemically stable areas, the favourable climatic conditions allow for all instars of *R. appendiculatus* to occur simultaneously throughout the year (Deem *et al.* 1993). Therefore a good level of herd immunity in calves develops due to sufficient challenge by *T. parva* parasites. Seroprevalence in the calves is typically lower in areas where climatic conditions are less favourable and tick seasonality is more pronounced. In these endemically unstable conditions, herd immunity against *T. parva* is insufficient, which renders many adult cattle susceptible to ECF.

Seasonal fluctuations in rainfall also dictate the availability of forage to livestock. In Nigeria, nutritional stress during the dry season was thought to compound the effect of haemoparasites (Kamani, Sannusi, Egwu, Dogo, Tanko, Kemza, Tafarki & Gbise 2010).

Seasonal patterns in Western Kenya are associated with rainfall rather than daily temperature. The annual rainfall in western Kenya is bimodal, with two rainy seasons from April-July (long rains) and September-November (short rains) (Anon. 2009b). The climate is equatorial with little difference in hours of daylight between months of the year. An increase in cattle diseases was observed during the long rainy season in Busia, but this seasonal pattern was not consistent between years (Thuranira-McKeever, Shaw, Machila, Eisler, Welburn & Maudlin 2010).

2.2.3 Host immune mechanisms against pathogens

There are two arms to the immune cascade with one represented by the Th1-cell cytokine secretion profile and the other by the Th2-cell profile. During infectious disease, depending on the antigen present, polarization towards either a Th1-cell response or a Th2-cell response occurs (Figure 1.2) (Jankovic, Liu & Gause 2001).

Figure 1.2 The polarization of immune responses towards Th1-cell or Th2-cell responses (from Jankovic, Liu & Gause 2001)
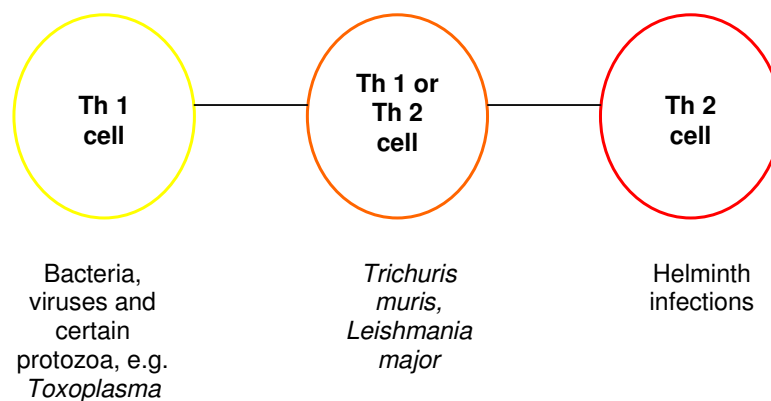
The Th1- and Th2 cell have common T-helper (Th) cell precursors and the direction taken by the immune response is only determined at the level where the antigen interacts with antigen-presenting cells (APC) and dendritic cells (DCs). In the spleen and the T-cell-rich regions of lymphnodes antigen is presented to naïve T-helper cells by DCs. Dendritic cells express recognition receptors that will bind to molecular structures which are common to a

group of pathogens. From here the DCs express signals that will direct the effector Th cells towards either the Th1- or the Th2-cell cascade, depending on the antigen encountered. In both the Th1- and the Th2-cell cascades humoral and cell-mediated immune responses play a role, but different types of effector cells and antibody isotypes are involved (Jankovic *et al.* 2001).

Very briefly, the Th1-cells produce interferon-gamma and tumor necrosis factor which will activate macrophages and induce the production of immunoglobulin G (IgG) that supports opsonisation and phagocytosis. Also, Th-1 cells support antiviral effector T-cells. Th2-cells secrete interleukin-4 (IL-4), IL-5, IL-9, IL-10 and IL-13, which stimulate eosinophils and mast cells are stimulated to differentiate, aided by IgE (Jankovic *et al.* 2001).

2.2.3a Immunity against bovine protozoan infections
Babesiosis
Immune responses against *Babesia* infections involve both humoral and cell-mediated mechanisms. Calves have a natural resistance to babesiosis after two months of age, which lasts for 6 to 9 months, irrespective of the immune status of their dams (De Vos *et al.* 2004; Mahoney *et al.* 1973). This innate immunity is mainly cell mediated and the spleen has been shown to play a major role in this non-specific immunity (Bock *et al*. 2004). Monocytes, macrophages and neutrophils, together with the resultant cascade of antimicrobial agents, cytokines and enzymes, including nitrous oxide (NO), are responsible for phagocytosis of the pathogens.

Acquired immunity against re-infection in recovered animals or in vaccinated animals is strong and potentially life-long. Some bovines can become latent carriers of the parasite after recovery from disease without the development of clinical symptoms. Premunity is not required to maintain protection against re-infection with *Babesia* spp. (Callow & Dalgliesh 1982). The involvement of antibodies in immunity against babesiosis is through their role as opsonins which mediate phagocytosis during cell-mediated cytotoxicity. In response to re-infection, Th-1 (mainly CD4+) cells proliferate, which in turn promotes phagocytic cells, as well as promoting immunoglobulin production by autologues B- lymphocytes. The strain-specific antibodies are of mostly IgM and also IgG1 and IgG2 type and are directed against antigens on the surface coat of infected erythrocytes as well as the parasites (Bock *et al*. 2004; De Vos, Dalgliesh & Callow 1987, De Vos *et al*. 2004). Immunity against the sporozoite stage is an antibody response, but it is not known if this response is effective against re-infection. The response against the erythrocyte stage appears, in part, to be

antibody mediated, and prevents the invasion of red blood cells (Cox 1993). The antibody levels are, however, not correlated with the level of immunity in the animal.

Theileriosis

Immunity against *Theileria* infection is thought to be primarily cell-mediated and directed against the schizont stage. Cytotoxic T-lymphocytes (CD8+) recognize parasite- and strain-specific surface antigens on schizont-infected lymphoblasts in a classical MHC (major histocompatability complex) class I restricted reaction (Cox 1993; McKeever 2006; Morrison 2009). Other cellular responses, such as provided by CD4+ helper T cells, are secondary to and play a supportive role in the cytotoxic T-lymphocyte reaction (Morrison, Taracha & McKeever 1995). Memory T-cells are activated in re-exposed animals, which in turn stimulate the activation of the cytotoxic T-lymphocytes. The cytotoxic T lymphocytes target the schizont-infected lymphocytes, which allow the persistence of piroplasma-infected red blood cells (McKeever 2006). Recovered cattle have immunity against re-infection by homologous *T. parva* strains but cross-immunity against heterologous strains is poor (Irvin & Morrison 1987).

Infected animals elicit a humoral response, with antibody against all stages of *Theileria* parasites (Norval *et al.* 1992). The antibody response against sporozoites is directed against a cluster of surface antigens which neutralize infectivity *in vitro*, is less strain-specific than antibody against schizont stages, and is thought to play a protective role in subsequent infections. Antibody responses against schizonts are not considered to be important in acquired immunity (Lawrence *et al.* 2004a), but are exploited in the field of diagnostics. In haemoproliferative *Theileria* species, such as *T. mutans*, antibody against the piroplasm stage has proved to be protective (Irvin 1985).

Trypanosomosis

Trypanosomes are extracellular parasites. In contrast to *Babesia* and *Theileria* species, it appears that immunity to trypanosomes is primarily antibody mediated. The main target for IgM antibodies is the surface coat of trypanosomes which consists of glycoprotein. The antibody response agglutinates trypanosomes and initiates complement-mediated lysis. Before the infection is completely cleared, however, some of the parasites switch to a gene that codes for a different protein, namely a variable surface glycoprotein (VSG), which is not recognized by the initial antibody response. Infection with trypanosomes is characterized by successive waves of parasitaemia. Each parasitaemic peak consists of parasites in which a different VSG predominates. This VSG has enormous capacity for antigenic variation, called

variable antigen types (VAT), and is a very effective mechanism for evasion of the host's immune responses (Connor & Van den Bossche 2004; Mansfield & Paulnock 2005),.

Eventually, if the animal survives, it will have built up a degree of immunity against all major variants prevalent in the field. However, repeated immune responses to changing targets (VSGs) leads to gross immunopathological changes, such as auto-anitbodies against the host red blood cells and platelets, and prolonged immunosupression (Cox 1993).

Immunosupression due to trypanosome infections is well-known, although the aetiology is not yet fully understood. Several factors have been implicated, including blocking of the release of antibody by plasma cells, impaired T-helper function, B-cell mitogens and depletion of lymphoid cells. Disintegrating trypanosomes initiate the release of toxic products, as well as the release of cytokines by cells of the mononuclear phagocytic system (MPS). Both probably contribute to the immunosupression (Connor & Van den Bossche 2004).

2.2.3b Host immune response against helminth infections

Helminths are typically long-lived and are associated with chronic infections. In order to survive, these parasites need to modulate the immune response by their host, be that by suppressing the parasite-antigen-specific response or general immunosuppression. Helminth infections are typically associated with polarization towards a Th2-type response, as indicated by increased peripheral eosinophil levels and mast cells, as well as parasite-specific IgE levels (Maizels & Yazdanbakhsh 2003). Peripheral eosinophilia can be of value clinically as a marker for parasitic infection (Bejon, Mwangi, Lowe, Peshu, Hill & Marsh 2008). Experimental trials have shown that the peak of peripheral eosinophilia is associated with expulsion of worms, as indicated by a drop in faecal output of worm eggs in infected lambs (Buddle, Jowett, Green, Douch & Risdon 1992) and adult sheep (Doligalska, Moskwa & Stear 1999). Eosinophil counts can be used as a measure of the Th-2 immune-mediated response to infestation (Dawkins, Windon & Eaglesons 1989; Bejon *et al.* 2008; Jolles, Ezenwa, Etienne, Turner & Olff 2008) and has been used to monitor patients' recovery from helminth infection (Loutfy, Wilson, Keystone & Kain 2002).

Cytokines associated with Th2-response down-regulate potentially pathogenic Th1-cell-mediated inflammation, thus ensuring the host's own survival and ultimately the parasites' survival. This suppression is dependent on high parasite intensities (Maizels & Yazdankakhsh 2003) with a stimulatory effect seen in low infestation burdens (Kamal & El Sayed Khalifa 2006). These modulations affect immune responses against co-infecting

pathogens and the outcome can be either beneficial or detrimental for the host. The beneficial effect of the modulatory effect in allergic conditions in persons infected with helminths is well documented (Maizels & Yazdanbakhsh 2003; Kamal & El Sayed Khalifa 2006). On the other hand, helminth-infected hosts might have an impaired immune response to other pathogens where Th1-cell responses are required to limit the progression of infection.

2.3 Interactions between concomitant pathogens

Traditionally studies on infectious diseases have focused on single pathogens, often based on experimental conditions. In the field, however, it is impossible to study a disease complex in isolation without reference to other causes of disease (Moll *et al.* 1984). This is because animals in the field are exposed to a variety of pathogens that occur in the same environment (Petney & Andrews 1998). In fact, humans and animals living under field conditions are more likely to suffer from concomitant infections than single infections (Cox 2001; Telfer, Birtles, Bennett, Lambin, Paterson & Begon 2008). The higher the prevalence of each pathogen, the more likely a host will harbour co-infections of the various pathogens (Petney & Andrews 1998). The same risk factors that predispose a host to become infected with one pathogen might also increase the risk of infection with another pathogen. Swai *et al.* (2005b) found a correlation between tick-borne diseases in Tanzania in that cattle that were seropositive to one tick-borne pathogen were more likely to be seropositive to another tick-borne pathogen.

Several studies have reported concomitant infections of trypanosomes, *Anaplasma, Babesia, Theileria* and helminth species in cattle in Africa (Magona & Mayende 2002; Swai *et al.* 2005b; Kamani *et al.* 2010; Marufu, Chimonyo, Dzama & Mapiye 2010), and particularly Kenya (Moll *et al.* 1984; Maloo *et al.* 2001b; Muraguri, McLeod, McDermott & Taylor 2005).

2.3.1 Pathogen interactions

It is easy to conceive that pathogens that concomitantly infect a host would directly interact with each other, particularly pathogens that occupy the same niche in the host, e.g. the abomasum or red blood cells. These pathogens compete for the same resources, such as nutrients and attachment sites. The outcome is often a limitation on population size in either or both of the implicated pathogens (Petney & Andrews 1998).

Pathogens can also interact indirectly, by modifying the host's resistance or susceptibility to other infections. The immune modulation by helminth parasites has already been discussed

(see 3.3.2). Through polarization of the T-cell response towards the Th2- type response, the helminth parasite facilitates the establishment of the protozoal parasite, for instance by impairing the host's response against the super-infecting protozoal parasite.

Many pathogens are associated with immunosuppression of the host, e.g. trypanosomes (Holmes *et al.* 1974; Mackenzie *et al.* 1975; Askonas 1984; Urquhart & Holmes 1987); and ECF (Moll *et al.* 1986). When one pathogen causes immunosuppression in the host, it will lead to the recrudescence of other infections. Immunosupression can be caused directly by the implicated pathogen by depleting the host's lymphoid cells, or by impairing its cellular functions. By-products of ongoing protective immune or allergic reactions in the host might also result in immunodepression.

The outcome and type of interaction between pathogens are in part determined by the timing of each infection. Non-specific immune reactions against the first pathogen might impair the establishment of the second pathogen. Heterologous immunity, or cross-protection, induced by the first pathogen can in some cases be either partly or completely protective against a second pathogen (Clark 2001). This, in turn, will alter the clinical course of infection of the second pathogen.

The sequence in which the host acquires different infections also affects the clinical outcome and prognosis of disease. The interaction between *Trypanosoma congolense* and *Haemonchus contortus* in Djallonké sheep (Goossens, Osaer, Kora, Jaitner, Ndao & Geerts 1997) and in N'Dama cattle (Kaufmann *et al.* 1992) was investigated. Both breeds are regarded as *Trypanosoma* resistant. Sheep that were initially infected with *Haemonchus* larvae and subsequently super-infected with *T. congolense* developed less severe chronic anaemia. When sheep were first infected with *T. congolense* and subsequently infected with *Haemonchus* larvae, however, the sheep developed acute signs of trypanosomosis with severe drops in PCV (Goossens *et al.* 1997). Cattle with a primary *T. congolense* infection that were super-infected with *H. contortus* had a reduced prepatent period of *H. contortus* infection and showed increased pathogenicity as compared to controls. When a patent *H. contortus* infection was followed by a *T. congolense* infection, the disease process was even more aggravated (Kaufmann *et al.* 1992).

2.3.2 Clinical implications of concurrent infections
Each pathogen contributes to the clinical outcome and prognosis of infection in the host. Interactions between co-infecting pathogens can influence the course of the resultant infection (Petney & Andrews 1998). These interactions can potentially be synergistic,

neutral, as well as antagonistic from the point of view of the host (Cox 1987; Petney & Andrews 1998). Petney & Andrews (1998) summarise how the interaction between two pathogens can affect the pathogenicity to the host (see Table 1). The more pathogens that concurrently infect a host, the more outcomes are possible.

Table 1 Possible outcomes of a two-species parasite infection on the pathogenicity to the host (from Petney & Andrews 1998)

Influence of parasite 1 on parasite 2	Influence of parasite 2 on parasite 1	Relationship to pathogenicity
+	+	Each species increases the pathogenicity of the other
+	0	One species shows increased pathogenicity, the other its usual pathogenicity
+	-	One species shows increased pathogenicity, the other reduced pathogenicity
0	0	Neither species affects the other
0	-	One species shows its usual pathogenicity, the other reduced pathogenicity
-	-	Both species show reduced pathogenicity

Interaction between pathogens can result in reduced prepatent periods (Kaufmann *et al.* 1992), increased pathogenicity (Kaufmann *et al.* 1992; Goossens *et al.* 1997; Petney & Andrews 1998), reduced immune reactions (Holmes *et al.* 1974; Mackenzie *et al.* 1975; Urquhart & Holmes 1987; Kaufmann *et al.* 1992) and increased susceptibility (Holmes *et al.* 1974; Mackenzie *et al.* 1975).

Immunosuppression that is caused by certain pathogens is an important sequel to infection. Anaemia in field cases of ECF is thought to develop from co-infections with latent pathogens such as *T. mutans* or *A. marginale* in immunocompromised ECF cases (Moll *et al.* 1986). Immunosuppression in animals that suffer from trypanosomosis predisposes the host to secondary infections (Holmes *et al.* 1974; Mackenzie *et al.* 1975). This immunosupression also interferes with the effectiveness of vaccines against other diseases. In cattle infected with *T. congolense* that were vaccinated with mixed clostridial vaccines, foot-and-mouth disease vaccines and *Brucella abortus* vaccines showed reduced antibody responses (Urquhart & Holmes 1987). This interaction is of particular importance when designing control programmes for the various diseases (Howard, Donnelly & Chan 2001).

Interactions between pathogens that are of benefit to the host also occur. Earlier infection with *Oestrus ovis* decreased the level of eggs in faeces, worm fecundity and burdens of *Trichostrongylus colubriformis* in sheep compared to control groups (Yacob, Terefe, Jacquiet, Hoste, Grisez, Prevot, Bergeaud & Dorchies 2006). An increased resistance against *A. marginale* in *Theileria buffeli*-carrier cattle has been reported and is ascribed to a non-specific cell-mediated immunity. The *Theileria*-carrier animals had lower parasitaemias and increased time to maximum parasitaemias compared to controls (Gale, Leatch, Dimmock & Gartside 1997).

It is clear that co-infection of pathogens and interactions between them do occur. The relationship between pathogens is complicated and the level and type of interaction are determined by many factors, such as timing and order of infection. Pathogen interactions alter the outcome of infection (Cox 1987) and are of practical significance when clinical presentation of disease is unusual. This in turn can complicate diagnoses and treatment efforts. When designing control programmes, such as vaccination campaigns, one should take note of the diseases prevalent in the population (Cox 1987; Petney & Andrews 1998; Howard *et al.* 2001).

3. STUDY OBJECTIVES

This project formed part of the IDEAL (Infectious Diseases of East African Livestock) project, which is a collaborative effort between the Department of Veterinary Tropical Diseases, University of Pretoria (DVTD), the University of Edinburgh, the University of Nottingham and the International Livestock Research Institute (ILRI), Nairobi. The focus of the IDEAL project was on the sedentary mixed crop-livestock smallholding system. The study was set in Western Kenya which was considered representative of smallholder livestock farmers in East Africa. The main aim of the IDEAL project was to investigate the total disease burden of endemic cattle in the study area during their first year of life.

The main objectives of this particular study revolved around anaemia as a syndrome in the indigenous calves in the study area. Aspects investigated include diagnostic methods to identify animals with anaemia; longitudinal trends in baseline values of the haematological parameters of the calves; as well as the main infectious causes of anaemia and their contribution, either singly or in combination with other pathogens, towards the level of anaemia in the calves.

The general study design, including site selection, population description, sampling procedures, and general statistical methods used, are discussed in Chapter 2. Under each subsequent chapter, the methodology used to investigate the specific objectives of that chapter is discussed in more detail.

Anaemia in animals can be provisionally diagnosed based on clinical signs, such as pallor of mucous membranes, but a confirmatory diagnosis is based on measuring of red blood cell parameters, such as PCV, red cell counts (RCC) or haemoglobin (HGB). The FAMACHA® score card is a field diagnostic test developed to detect anaemia and haemonchosis in sheep. Its use and validity as a field diagnostic tool in East African short-horn Zebus, as measured against PCV, is evaluated and validated in Chapter 3. The red cell parameters and indices, as well as white cell and platelet parameters and indices of the East African short-horn Zebu were measured by a Sysmex® automated cell-analyzer. Prior to its use in the field, the Sysmex® performance was verified against an established automated cell-analyser with known precision and accuracy. These results are also presented in Chapter 3.

It is known that there are differences between breeds in the baseline ranges of haematological parameters (Jain 1993). There are no published values for these parameters in East African short-horn Zebu calves, thus, in Chapter 4, an attempt was made to define baseline values for this breed, as measured by the Sysmex® and manual PCV. The population means for each parameter were calculated at different ages and trends over time were then compared to published reference ranges for other breeds. In addition, in the absence of published reference ranges for this breed, a subgroup of calves was selected based on a set of criteria to identify relatively healthy calves. Using the data on healthy calves as a reference sample, the significance of anaemia in the general study population was further investigated.

In a tropical environment calves are exposed to a myriad of pathogens, even from early calfhood. Many of these pathogens potentially cause anaemia in the infected host. The prevalence and incidence of pathogens was investigated in Chapter 5 to get a better understanding of the disease burden of calves in the study area. The variation in prevalence with age has allowed the identification of high risk periods. The prevalence of co-infection of pathogens was also considered, with particular reference to pathogens known to cause anaemia.

The East African short-horn Zebu breed is considered to be less tolerant to trypanosomosis than West African breeds, yet more resistant against certain tick-borne disease, such as East Coast Fever, than European breeds and even West African breeds (Murray *et al.* 1982). In order to determine the relative contribution of different pathogens to the development of anaemia in the study population, the haematological profiles over age of calves infected by specific pathogens were investigated and the results are presented in Chapter 6. The development of other syndromes, such as thrombocytopenia and pancytopenia are also discussed.

Interaction between pathogens and its effect on the clinical course of disease has been investigated in several studies (Goossens *et al.* 1997; Kaufmann *et al.* 1992). These studies have focused mainly on single pairs of pathogens, often in controlled environments. However, controlled environments are not truly representative of the challenges cattle face in the field where cattle are exposed to multiple infections. It is very difficult to study animals in their natural environment due to the interplay of many risk factors that affect the incidence of infection and disease. Apart from a few studies on anaemia, little work has been done on how interactions between pathogens bring about changes in kinetics of white blood cells and thrombocytes. In Chapter 7 the cumulative impact of co-infecting pathogens and their interactions on several haematological parameters of calves were investigated.

An IDEAL calf at 51 weeks of age.
The calf is just over 1meter in length from snout to tail root.