

**A Comparative Study of Four Indigenous Cattle Breeds for
Tick Resistance and Tick- Borne Diseases in Ghibe Valley**

A Thesis submitted to The School of Graduate Studies, in partial fulfilment for
the Degree of Master of Science in Biology (Parasitology)

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Abstract

The purpose of the study was to obtain information on the resistance levels of four indigenous breeds of cattle (Abigar, Gurage, Horro and Sheko) to natural infestation with ticks in the Ghibe Valley. Monthly collection of ticks from randomly selected cohort of 15 heifers of each breed, in the period January through October 2002, showed the most common tick species to be: *Amblyomma variegatum* (39.5%), *Boophilus decoloratus* (39.1%), *A. cohaerens* (10.5%) and *Rhipicephalus evertsi evertsi* (6.7%). Species of least abundance include, *R. praetextatus*, *Haemaphysalis aciculifer*, *Hyalomma marginatum rufipes*, *R. bergeoni*, *R. lunulatus*, *R. muhsame* and *R. pravus*, altogether comprising 4.2% of the overall tick species. The monthly abundance of adult and immature ticks, their feeding sites on the hosts and the male to female sex ratio were determined. Resistance to ticks between breeds was evaluated based on the cumulative log transformed counts of total ticks, fully engorged females and adult ticks and was analysed by the method of least squares. The results revealed that the count of total ticks in Horro and Gurage (mean count of 5 ticks per month per breed) was lower than those of Abigar and Sheko (7 ticks per month per breed) ($P < 0.001$). The mean count of fully engorged females in Horro and Gurage cattle breeds was also lower (2 ticks per month per breed) than Abigar and Sheko (3 ticks per month per breed) ($P < 0.05$). The mean count of adult ticks was the lowest in Horro (4 ticks per month per breed), followed by Gurage (5 ticks per month per breed), Abigar and Sheko (6 ticks per month per breed) ($P < 0.01$). Based on the three parameters, Horro and Gurage breeds were found to exhibit significantly higher resistance to ticks than Abigar and Sheko breeds. Packed Cell Volume (PCV) of the study animals was monitored monthly during the study period and negative correlation was found between tick burden and PCV levels in all the animals ($P < 0.05$). Infection with tick-transmitted parasites/pathogens: *Anaplasma marginale* (84.2%), *Babesia bigemina* (87.1%) and *Theileria mutans* (54.2%) was confirmed serologically by indirect

ELISA. Sera were also screened using indirect ELISA to investigate the animals for anti-*Amblyomma variegatum* antibody responses by using larval homogenate. Comparisons between antibody responses of each breed showed the presence of variations in IgG responses between the cattle breeds. Higher IgG response was found in Horro than the other three breeds ($P < 0.05$). The relationships between infestation with *A. variegatum* and the level of IgG were positive in Abigar ($r = 0.3$, $P > 0.05$), Gurage ($r = 0.4$, $P < 0.05$) and Sheko ($r = 0.5$, $P < 0.05$) breeds, while weak negative correlation ($r = - 0.2$, $P > 0.05$) was observed in Horro breed, indicating the possible role of IgG in the acquisition of host resistance to ticks.

1.Introduction

1.1. Ticks and Their Life Cycles

Ticks are among the most important ectoparasites and vectors of animal and human diseases on global scale, particularly in tropical and sub tropical parts of the world. Because of the direct and indirect effects on their hosts, they are considered to be a significant threat to successful livestock production and seriously interfere with the economy of a country. Approximately 80% of the world's cattle population is at risk from ticks and tick- borne diseases. Annual worldwide losses due to tick infestation and diseases transmitted by ticks have been estimated to be 18 billion US dollars (de Castro, 1997). Furthermore, the costs associated with maintaining chemical control of ticks in tropical and subtropical regions of the world have been estimated at US\$25.00 per head of cattle per year (Pegram, 2001).

Ticks are arthropods, which are classified in the subclass Acari and Order Parasitiformes of the class Arachnida. There are at least 840 species of ticks in three major families, namely the Ixodidae, Argasidae and Nuttalliellidae. The family Ixodidae is relatively large and comprises thirteen genera and 650 species. Seven of these genera contain species of veterinary and medical importance. These are *Amblyomma*, *Boophilus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* (Arthur, 1961; Jongejan and Uilenberg, 1994).

There are four stages in the life cycle of ixodid ticks: egg, larva, nymph and adult. The female drops off its vertebrate host and seeks a sheltered locality in which to develop and lay a single large batch of eggs, after which she dies while males usually remain much longer on

the host, where they may mate repeatedly. Typically a batch of eggs contains several thousand brown globular eggs, and oviposition continues for many days or in some cases,

months. Depending upon the climatic conditions, eggs hatch in two weeks to several months, giving rise to hexapod larvae. The larvae climb into a host and suck blood for several days and moult to octopod nymphs. The nymphs then feed for on the host and moult to adult male and female ticks. The process of moulting can take place either on the ground or on the host depending on the pattern of life cycle of the species. There are three types of life cycles that the different species follow (Arthur, 1961; Jongejan and Uilenberg, 1994):

- A. **One-host ticks:** In this life cycle, larvae hatch from eggs, climb and attach onto a host and engorge, moult on the host to nymphs, which attach and continue to feed and engorge, then moult on the host to males and females. The adults attach, partially engorge, mate and the females then fully engorge. After engorgement the females detach to the ground and lay a single large batch of eggs in a sheltered locality and die. The next generation of larvae hatches from these eggs. The genus *Boophilus* has species with this lifecycle.

- B. **Two-host ticks:** In this life cycle, the larvae hatch from eggs, climb and attach onto a host. After engorgement, moulting to nymphs will take place on the same host. The nymphs then engorge and drop to the ground and moult to adult male and females in a sheltered locality. The adults then climb onto a second host and engorged females drop and lay eggs as in the case of the one- host ticks. Species that adopt this two-host cycle include *Rhipicephalus evertsi evertsi* and *Hyalomma marginatum rufipes*.

- C. **Three-host ticks:** the larvae of these tick species hatch from eggs, climb onto the first host and attach to engorge. The engorged larvae detach and drop to the ground and moult to nymphs in a sheltered locality. The nymphs then climb to a second host to attach and engorge. After full engorgement, the nymphs detach, drop to the ground and

moult to males and females. Then the adults climb onto the third host and partially engorge. They then mate and the females, after fully engorging, drop to the ground and lay a single large batch of eggs in a sheltered locality and die. *Rhipicephalus appendiculatus* and most species of the genus *Amblyomma* belong to this pattern of life cycle.

1.2. Effect of Ticks on Animal Health and Production

The impact of ticks on livestock health and production can be divided into two. These are effects of tick burden as ectoparasites and the effect of diseases transmitted by ticks serving as vectors. As ectoparasites, ticks are responsible for blood loss, irritations that result in ‘tick worry’ and interrupt the grazing habits of cattle. Damage and loss of udders and damage to hides are also caused by the attachment and feeding activities of ticks, which provide portals of entry for secondary bacterial infections and for larvae that induce myiasis and tick paralysis due to the toxins they secrete into the blood. The secreted toxins may even disseminate to the respiratory organs and cause death of the animal (Sere, 1979; Soulsby, 1982).

In determining the direct effects of ticks on livestock, early studies indicated a large difference in mean annual weight gain (48 kg) between heavily tick-infested and tick-free animals (Springell, 1974, cited in Sere, 1979). It was also reported that in heavily tick infested European cattle, 65% of the body weight reduction was due to loss of appetite while the remaining 35% was due to interference with the growth process (Seebeck *et al.*, 1971). The latter is supported by the observation that tick infested animals had relatively more fat and less muscle than tick-free controls, suggesting interference in protein synthesis caused by tick bites (Norval *et al.*, 1988). High tick numbers cause proportionally greater live weight losses in tick susceptible Boran cattle than in tick resistant animals of the same breed (Sere,

1979). These factors also contribute to losses in milk production, calving interval and weaning performance.

The pathogens transmitted by ticks can cause high morbidity and mortality in livestock (Table 1). Some of the most important tick-borne diseases are East Coast Fever, Redwater, anaplasmosis and heartwater. Many other fatal and benign babesiosis, theileriosis and anaplasmosis are also transmitted by various tick species (Jongejan and Uilenberg, 1994). These diseases generally affect the blood and/or lymphatic system and cause fever, anaemia, jaundice, anorexia, weight loss, milk drop, malaise, swelling of lymph node, dyspnoea, diarrhoea, nervous disorders and even death.

Cattle play a significant role in the socio-economic life of the people of Ethiopia and livestock industry represents the second largest national income contributing to the GDP of the country which accounts for nearly 15 % of the total GDP and about 40 % of the agricultural GDP (Sendros and Tesfaye, 1998). Export of livestock and livestock by-products also have appreciable contribution to foreign exchange earnings of the country amounting to about 15 % and 70 % of all export earnings and earnings from agricultural exports. Hides and skins are important components of the agricultural sector in generating foreign export earnings. Besides, animals provide draught power for cultivating the agricultural holdings of almost all peasants.

Several tick species of the genera *Amblyomma*, *Boophilus*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* have been identified in Ethiopia (Hoogstraal, 1956; Morel, 1980). Tick surveys have been carried out in different regions by different researchers (Pegram *et al.*, 1981; de Castro, 1994; Solomon, 1994; Yilma *et al.*, 1995; Seyoum, 2001). More than 60 species of ticks infesting both domestic and wild animals have been recorded (Morel, 1980)

and 33 of these are known to be most common and are important parasites of livestock (Pegram *et al.*, 1981). Tick-borne diseases transmitted by the major tick species in Ethiopia are anaplasmosis, babesiosis, cowdriosis and benign theileriosis (Solomon, 1994; Mekonnen, 2000). At present the direct losses caused by tick infestations tend to be more economically important than losses due to tick-borne diseases. This is mainly because the most important tick-borne diseases, East Coast Fever and acute theileriosis which are caused by *Theileria parva* and *Theileria annulata*, respectively and their vectors are not so far reported in Ethiopia. Nevertheless, the impact of ticks in the improvement of production of the indigenous stock cannot be ignored and long-lasting control strategies should be designed.

Table 1 Summary of known protozoan and rickettsian tick-borne pathogens and diseases affecting cattle (Source: Andrews *et al.*, 1992; Soulsby, 1982; Camus *et al.*, 1996).

Pathogen	Vector	Disease	Distribution
<i>Theileria annulata</i>	<i>Hyalomma spp.</i>	Mediterranean coast infection (tropical theileriosis)	Northern Africa and the Sudan
<i>T. lawerenci</i>	<i>R. appendiculatus</i>	Corridor disease	East, Central and Southern Africa
<i>T. mutans</i>	<i>R. appendiculatus</i> , <i>R. evertsi</i> , <i>A. variegatum</i>	Benign bovine theileriosis	South and East Africa, Australia, Asia
<i>T. parva</i>	<i>R. appendiculatus</i>	East Coast Fever	East, central and parts of Southern Africa
<i>Babesia bovis</i>	<i>I. ricinus</i> , <i>I. presulcatus</i> , <i>B. microplus</i> , <i>B. calcaratus</i> <i>R. bursa</i>	cerebral babesiosis	S. Europe, Africa, Asia, Central and South America
<i>B. divergens</i>	<i>I. ricinus</i>	Babesiosis ¹	North and South Europe
<i>B. bigemina</i>	<i>B. annulatus</i> , <i>B. calcaratus</i> , <i>B. decoloratus</i> , <i>B. microplus</i> , <i>R. e. evertsi</i> , <i>R. bursa</i> , <i>R. appendiculatus</i> , <i>Haemaphysalis punctata</i>	Cattle tick fever, red water fever, piroplasmosis	N & S Africa, Panama, Europe, Eurasia, N America
<i>B. major</i>	<i>B. calcaratus</i>	Babesiosis ²	N & W Africa, S. America, Russia, Europe
<i>Anaplasma marginale</i>	<i>Boophilus spp.</i>	Anaplasmosis (Gall sickness)	East central and southern parts of Africa, Europe, Asia and N & central America
<i>A. centrale</i>	<i>Boophilus spp.</i>	Anaplasmosis ³	Africa, S.E.Asia, N, S & central America
<i>Cowdria ruminantium</i>	<i>Amblyomma spp.</i>	Heartwater, brainwater	East, central and southern parts of Africa Caribbean islands

¹The disease is milder than the one caused by *B. bovis*

²The disease is milder than the one caused by *B. bigemina*

³The disease is milder than the one caused by *A. marginale*

1.3. Tick Control Strategies

The different tick control strategies can basically be divided into two groups. These are: off host control and on host control.

Off host control: - It includes pasture spelling and the use of anti-tick plants. Pasture spelling involves keeping stock off the area for as long as possible to allow starvation and death of all the ticks. This method works best where the ticks are short-lived as in warm arid conditions. It is best performed by treating pastures in rotation. The effectiveness of pasture spelling depends on whether alternative hosts, either domestic or wild, are available to the ticks. Traditional burning of pastures and transhumance are also related with such control measures. However, under conditions of extensive production such as those in most of tropical Africa these approaches are of little practical relevance. The use of tick-repellent grasses is another possible means of control of tick population in the field. Plants that have been investigated and reported so far to have anti-tick properties include *Melinis minutiflora* (Beauv.) or molasses grass, *Stylosanthes spp.* and *Gynandropsis gynandra* (L.) (Thompson *et al.*, 1978; Suthrest *et al.*, 1982; Malonza *et al.*, 1992).

On host control: This involves the application of chemicals (acaricides) and herbal preparations on the host. Acaricides are usually applied by spraying, hand dressing or dipping. Spraying involves the use of spray-races or hand spraying. Spray-races require expensive initial installation and qualified maintenance service and hence have very limited applicability to large parts of Africa. Hand spraying and dressing involve a large physical labour input. Nevertheless, they are frequently the only feasible methods available for smallholders where a scattered settlement structure inhibits the use of communal facilities. Dipping is the most frequently selected method of application for communal tick-control facilities. The frequency

of acaricide application varies widely ranging from three days to several months according to the type of tick to be controlled, seasonal dynamics of the tick population, efficacy of the acaricide used and the type of control strategy pursued (de Castro and Newson, 1993).

Several constraints have arisen in the animal health industry from the heavy dependency on chemical control. These include the development of acaricide resistance, environmental and health concerns and the high cost (Pegram, 2001). The earlier acaricides used belong to the arsenic compounds (As_2O_3) and chlorinated hydrocarbons (lindane and toxaphene). These compounds are currently of limited use due to the residual toxicity they induce in the environment and the emergence of resistant tick strains. Resistance to arsenical acaricides in *B. decoloratus* was detected in South Africa in 1941 (Baker, 1978, cited in Assefa and de Castro, 1993). Toxaphene resistance was also reported from Kenya, Malawi, South Africa, Uganda, Zambia and Zimbabwe (Drummond, 1982). Despite this, toxaphene was widely used in many parts of the world (FAO, 1984). To date, the compounds frequently used are organophosphates and synthetic pyrethroids. Organophosphorous dips are effective but have limited residual activity and can be toxic especially when used by unskilled labour. Resistance of *B. microplus* to most of the organophosphate compounds had been reported from several parts of the world (Drummond, 1982). Synthetic pyrethroids are good and relatively non-toxic, but some resistance to them is developing especially in tick strains that are DDT resistant (Drummond, 1982; Andrews *et al.*, 1992). The cost of developing new acaricides, in relation to the economic return expected before resistance occurs, has discouraged research leading to new chemicals for tick control (Jongejan and Uilenberg, 1994). Therefore, it is very essential that new methods for tick control be explored.

In many tropical and subtropical areas, active restriction in acaricide use may not be feasible, as intensive tick control will still be required where improved livestock is exposed to ticks.

Current market trends in some countries for more productive beef will encourage producers to satisfy this demand by raising the proportion of European blood in their cattle herd. This inevitably leads to a greater requirement for tick control. Any breakdown in tick-borne disease control measures will require at least a temporary re-introduction of intensive acaricide use, especially in improved livestock (de Castro and Newson, 1993; Jongejan and Uilenberg, 1994).

Other control methods are acaricide application in combination with tick-derived pheromones, which are naturally produced chemicals serving as a communication system within the tick population. Applications of traditional herbal preparations and biological control by predators and pathogens of ticks are also used by various pastoralists and small-scale farmers as repellents and control methods, respectively (Malonza *et al.*, 1992; Dreyer *et al.*, 1997; Assefa, 2000). New treatment practices of acaricides like impregnated ear tags, slow-release rumen boluses, subcutaneous implants and intramuscular injections have been promoted but have not yet been widely applied for tick control (de Castro and Newson, 1993).

1.3.1. Tick Control in Ethiopia

Ninety nine percent of the cattle in Ethiopia are indigenous breeds (DAGRIS 2002). Crossbreed cattle comprising about one percent are concentrated in the central regions and some are scattered in other regions of small dairy farms. In rural farm areas of Ethiopia, tick control using chemicals is practised very rarely and if at all used, irregularly, depending on the proximity of veterinary clinics. This helped in maintaining the enzootic stability in the indigenous cattle for ticks and tick-borne diseases. Due to lower productivity of pure indigenous cattle breeds than the exotic *Bos taurus* breeds, large scale crossbreeding has been considered to be the best way to increase production of meat and milk. However, such attempt to improve animal husbandry through cross-breeding is highly affected by tick

infestations and tick-borne diseases due to the high level of susceptibility of exotic and cross breeds to prevailing ticks and tick-borne diseases, indicating the production of a high risk tick and tick borne disease susceptible stock (Ali and de Castro, 1993; Mekonnen *et al.*, 2001).

In most dairy farms and ranches, acaricides are frequently used due to the susceptibility of exotic and crossbred cattle for ticks and tick-borne diseases. As a result, resistance of ticks to frequently used acaricides have been reported in some dairy farms of western Ethiopia in Boran X Friesian crossbred cattle (Assefa and de Castro, 1993). In privately owned ranches and dairy farms, acaricides have been empirically applied both on the crossbreds and the indigenous cattle breeds which definitely leads to the loss of resistance of the indigenous breeds to ticks and loss of enzootic stability to tick-borne diseases. These findings suggest the need to pay attention and revise to tick control methods and cattle productivity improvement programmes that have been practised in Ethiopia.

1.4. The Immunology of Tick-Host Interactions and Host Resistance

Ticks induce a complex series of immunological responses in a variety of hosts. Immunological interactions at the tick-host interface involve innate and acquired host defences against infestation and immunomodulatory countermeasures by the tick. The primary response by a naïve animal consists of complement cleavage leading to mast cell degeneration, which induces the accumulation of basophils, neutrophils and eosinophils at the feeding lesion (Preston and Jongejan, 1999). Acquired resistance to tick infestation involves humoral and cellular immunoregulatory and effector pathways, involving reaction of antigenic components of tick saliva with sensitised epidermal langerhans cells and macrophages to promote T-cell sensitization that leads to either Th1 or Th2 responses

(Nithiuthai and Allen, 1985). The process of these immune effector mechanisms is repeated upon repeated feeding of the ectoparasites.

The patterns of innate immune responses to ixodid ticks have long been studied in laboratory animals. In Guinea pigs that are infested with *Dermacentor variabilis* larvae, large accumulations of polymorphonuclear leukocytes with few eosinophils have been observed at the attachment sites (Wikel and Whelen, 1986). Histological observations in guinea pigs that are infested with larvae of *Dermacentor andersoni* have also shown to contain intense accumulations of basophils at attachment sites (Allen, 1973, cited in Wikel and Whelen, 1986). Similar responses have also been observed in infestations with *Ixodes holocyclus*, *Amblyomma americanum*, *Ixodes ricinus* and *Rhipicephalus appendiculatus* (Brown and Askenase, 1981; Brossard and Fivaz, 1982).

In cattle, the innate response is expressed by immediate hypersensitivity reactions with higher levels of histamine in the circulation and at the bite site. This reaction leads to increased vaso-permeability and local irritation, and stimulates the grooming activities of the host that damage or dislodge feeding ticks (de Castro and Newson, 1993).

Langerhans cells have been shown to be involved in presenting tick salivary antigens to T-lymphocytes. In a study on the fate of *Dermacentor andersoni* antigen in the skin of infested guinea pigs, tick salivary gland antigens have been associated with langerhans cells at the attachment sites. It is also observed that during repeated exposure to infestations, the number of langerhans cells increased at sites of tick feeding (Nithiuthai and Allen, 1984). Depletion of these cells from resistant guinea pigs by ultraviolet irradiation has resulted in impairing the acquisition and expression of resistance to *Dermacentor andersoni* (Nithiuthai and Allen, 1984), suggesting the efficient antigen-presenting role of these cells.

A wide range of acquired humoral and cell mediated immune responses have been observed in laboratory animals as well as in cattle. The scenario of humoral immunity starts from langerhans cells trapping tick salivary molecules in the epidermis and migrating to the nearby draining lymph nodes where they act as antigen-presenting cells for specifically reactive host lymphocytes, which results in production of circulating tick reactive antibodies (Wikel and Bergman, 1997). Studies using *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Ixodes hexagonus* have shown the presence of a relatively high concentration of IgG in the saliva of females that had fed for six days on guinea pigs (Wang and Nuttall, 1995). The IgG produced have a capability of passing from the gut lumen, through the gut wall into the haemocoel, while retaining its antibody activity. Further studies have shown that these immunoglobulins in haemolymph can target antigens of internal organs and interfere with the normal feeding or other physiological functions of the tick (Sauer *et al.*, 1994).

Based on the capability of IgG on targeting internal parts of feeding ticks, development of anti-tick vaccines is an area of research with considerable potential to control ticks in a cost-effective way. The most successful attempts of vaccine designs have been performed with antigens of *Boophilus microplus*. Three main antigens have been isolated from *B. microplus*. The Bm 86 gut antigen, which is present throughout all the stages, has been shown to be effective for the control of *B. microplus* and *B. annulatus* infestations (Lee and Opdebeeck, 1991). Partial protection has also been reported for *B. decoloratus*, *R. appendiculatus*, *R. sanguineus* and *H. a. anatolicum* (Ghosh and Khan, 1998; 2000). To date, this antigen has been produced by recombinant technology and acts by stimulating production of specific antibodies in vaccinated cattle, which damages the gut of engorging ticks, resulting in fertility reduction of up to 70% (Khalaf-Allah, 1999; Sharma *et al.*, 2001). The Bm 91 gut and salivary antigen have shown to elicit partial protection against *B. microplus* with some added

effect when used in combination with Bm 86 (Willadsen *et al.*, 1996). Bm 95 is also another gut antigen, which was isolated from argentine strain of *B. microplus* (strain A) and protects against a wider range of *B. microplus* strains when compared with the first antigen, Bm86 (Garcia *et al.*, 2000).

In addition to antibodies, T cells have immunoregulatory and effector functions in immunity to ticks. T helper 1 (Th1) cells mediate DTH reactions, which are effectors of the cutaneous basophil hypersensitivity component of acquired response (Mbow *et al.*, 1994). Studies in mice that have been repeatedly infested with *Ixodes ricinus* nymphs have revealed predominance of CD4+ T cells and infiltrating mononuclear cells that are positive for the inflammatory cytokines IL-1 β and TNF- α (Willadsen and Jongejan, 1999). Studies in BALB/C mice that have been infested by *Ixodes ricinus* nymphs have also resulted in the activation of infiltrating T-cells and cutaneous delayed type hypersensitivity (DTH) reaction (Mbow *et al.*, 1994). Inoculation of tick salivary gland extracts into cattle resulted in DTH reaction (Smith *et al.*, 1989). *In vitro* studies on peripheral blood lymphocytes from *Amblyomma americanum* infested *Bos indicus* calves have exhibited proliferation in the presence of salivary gland antigens from ticks of the genus *Amblyomma* (George *et al.*, 1985).

Analyses of cytokines that are expressed during tick-host interactions have been studied in various ways in mice models. Lymphocytes collected from *I. ricinus* infested BALB/C mice have resulted in high levels of IL-4 and low levels of IFN- γ , which increased after successive infestation when cultured *in vitro* with the T-cell mitogen concavalin A (Con A). In another attempt, IL-2, IL-4 and IFN- γ mRNA expression in skin at feeding sites and in draining lymph nodes of BALB/c mice during three successive infestations with *ixodus ricinus* nymphs was determined by *in situ* hybridization. Skin biopsies that are obtained near the mouthparts at 72 hour of primary infestation contained mononuclear cells in the dermis that

were positive for IFN- γ , a few were positive for IL-4 and none positive for IL-2. Cells positive for IL-2 and IFN- γ mRNA were found in the paracortical region of lymph nodes draining the attachment sites and IL-4 positive cells were seldom encountered. During the second infestation, more than half of the cells infiltrating the bite site were positive for IFN- γ mRNA, while 20-50% of cells were positive for IL-2 and/or IL-4 mRNA. The results of the third infestation were similar to those of the second exposure except the decreasing level of IL-4 positive cells. During second and third infestations, paracortical vessels contained numerous lymphocytes positive for IL-2 and IFN- γ mRNA. Very few IL-4 positive cells were detected, suggesting polarization towards a Th1 cell response (Wikel and Bergman, 1997). In addition, lymphocytes from lymph nodes draining sites of tick feeding elaborate significant levels of IL-2, TNF- α and GM-CSF when cultured with Con A and it has been observed that upon repeated infestation, IL-2 production increased (Mbow *et al.*, 1994).

In Both humoral and cell mediated immune response patterns to tick salivary antigen, sensitization with one species of tick leads to the production of immune effector elements that are reactive to other species and, even to other genera (George *et al.*, 1985, McTier *et al.*, 1981). Such attribute is very essential to the immunological control of multi-tick species.

Ticks possess host immunomodulatory strategies directed at both innate and specific acquired immune responses to tick infestation (Preston and Jongejan, 1999). They induce immunosuppression to complement components, NK cells, macrophages, T- and B-lymphocytes not only to survive but also to facilitate long term feeding from immunocompetent hosts. Immunosuppression of hosts during Ixodid tick infestation has been shown to occur both in laboratory animals and in cattle. Studies on the immunosuppressive roles played by saliva of *Ixodes scapularis* have shown that it inhibits complement components C3b and C5b from binding to sites where activation can occur. It also prevents

generation of the anaphylatoxin C3a, and contains a 49 Kda inhibitor of C3 hydrolysis. Moreover, a carboxypeptidase-N-like enzyme in the saliva of the same tick is found to inhibit complement generated anaphylatoxin (Wikel and Bergman, 1997). Such inhibition of complement activation and production of anaphylatoxins blocks the generation of chemoattractants and pro-inflammatory cytokines involved in inflammation and immunity. The cause of immunosuppression has been determined to be mainly due to prostaglandins, a high concentration of which is contained in the saliva of ticks (Bowman *et al.*, 1996).

Tick saliva contains a cocktail of pharmacologically active compounds such as analgesics, anticoagulants and antiplatelet aggregatory compounds that are able to facilitate feeding on host blood. Among the components of the saliva of ticks so far identified, prostaglandin E2 (PGE2) has been well established as one of the immunosuppressants (Goodwin and Ceuppens, 1983). It is found that prostaglandins act by inhibiting T-cell function, by suppressing IFN- γ and IL-2 production. They also inhibit the bioactivity of IL-2 on IL-2 dependent cells by reducing the expression of receptors in these cells (Bowman *et al.*, 1996). As a result, tick burden on the host becomes high and although not well studied, some findings also show that tick immunosuppression of the host enhance the establishment of tick-transmitted pathogens in the host. In laboratory animals and cattle that are resistant to tick infestations, tick-induced immunosuppression has not been observed and thus speculated that resistant hosts might have a mechanism of escaping the immunomodulatory act of feeding ticks that made such hosts capable of maintaining low burden of tick infestation (Wikel and Bergman, 1997). Knowledge of the mechanism is very essential for it helps in designing successful immunological strategies to control ticks in susceptible hosts.

Host resistance to ticks is the ability of a host, once primed, to mount an immune response to components of the saliva of feeding ticks, debilitating or killing them. The methodology for

studying the levels of host resistance to Ixodid ticks involves the assessment of several feeding and fertility parameters like reduced number of feeding ticks, engorgement weight, length of feeding period, egg production, percentage of egg hatching, and increased mortality of egg and larvae (Wikel, 1996; Utech *et al.*, 1978). As a result, tick population will be reduced significantly and enzootic stability to tick-borne diseases established (Willadsen and Jongejan, 1999).

Certain laboratory animals and cattle acquire resistance to repeated tick infestations. Studies show that certain strains of guinea pigs have developed resistance to various *Ixodid* species and such resistance has been shown to be an immunological phenomenon (Allen and Wikel, 1978). Both innate and acquired responses play significant roles in tick resistance. In resistant guinea pigs infested with *D. andersoni*, salivary gland antigen, complement components especially C3 and IgG were deposited at the dermal-epidermal junction of attachment sites and even at some distance from the attachment sites (Wikel and Whelen, 1986). Further studies have revealed the importance of complement and complement derived components in the expression of resistance to ticks. In guinea pigs that are resistant to *D. andersoni* adults, administration of cobra venom factor has resulted in blockage of rejection of the ticks after challenge infestation, which is expressed by increment of the numbers of attached larvae, increment in weights of engorgement of the attached larvae and reduction of the basophil packed lesion at the tick attachment site (Wikel and Allen, 1978).

Indirect studies on guinea pigs have revealed the presence of humoral component in the expression of tick resistance. When cyclophosphamide, an immunosuppressant which acts against the B-cell mediated arm of the immune response was administered to tick-resistant hosts prior to a second larval infestation, significant blockage of the expression of tick

resistance has been observed suggesting the role of the humoral arm in tick resistance (Wikel and Allen, 1978).

Regarding cell-mediated immune response to tick infestation, limited studies had been carried out in resistant and susceptible strains of laboratory animals (Wikel and Whelen, 1986). In an attempt to determine the role of cell mediated immune response to host resistance, viable lymph node cells from guinea pigs that are found to be resistant to *Dermacentor andersoni* have been passively transferred to guinea pigs of strain S-13 and as a result, the recipient guinea pigs have been shown to allow significantly fewer larvae to engorge than the controls that are previously unexposed to ticks (Wikel and Allen, 1978).

In cattle, several studies in one-host tick species have shown that resistance is proportionally related to the amount of Zebu (*Bos indicus*) genes in a breed (de Castro and Newson, 1993). First attempts to evaluate natural resistance of cattle against *B. microplus* were made using *Bos indicus* cattle to promote resistance against *B. microplus*. Later, studies showed that cattle resistance to ticks was due to the mounting of a protective immune response against ticks by bovine hosts, and this response was heritable in the cattle progeny (Wikel and Whelen, 1986). In general, pure and crossbred Zebu cattle had been worldwide acknowledged as more resistant to *B. microplus* and other ticks than European cattle (de Castro and Newson, 1993). Variation in resistance to ticks exists not only between breeds but also within breeds of cattle. This variation in tick resistance is stable and heritable. So it can be manipulated by selection and breeding for the control of ticks as it has been used by the Australian researchers for the control of *B. microplus* (Utech *et al.*, 1978, Wharton *et al.*, 1970, Latif, 1984).

The application of the resistance parameters together with selection of resistant cattle has led in Australia, Brazil and some regions of Africa, to develop tick-resistant cattle breeds after crossbreeding *B. taurus* and *B. indicus*. Evidence for a genetic trait for resistance to one host ticks mainly *B. microplus* in *Bos indicus* breeds is strong (Mattoli *et al.*, 2000). Studies conducted in *B. microplus* resistant cattle breeds have indicated that selection for resistance is not more difficult than selecting for milk yield or bodyweight (Wharton *et al.*, 1970). In Africa, studies performed on cattle resistant to *R. appendiculatus* have demonstrated that when these cattle are allowed to graze in *R. appendiculatus* infested paddocks, and no other host animals are available for the ticks to feed on, the tick population falls to very low levels, even near to disappearance (ICIPE, 1981).

Experimental studies investigating the mechanisms of resistance indicate that higher levels of host innate responses are observed in resistant than susceptible hosts, suggesting that innate resistance reflects differences in bovine immune response genes (Wikel and Whelen, 1986). Resistance appears to be stimulated in cattle in response to antigens inoculated in the saliva of the feeding tick. When ticks feed on a resistant animal, they excrete antigens in the saliva. These antigens stimulate an immediate type hypersensitivity reaction, and marked swelling occurs at the site of attachment of the tick within 20 minutes of attachment. This reaction interferes with the ability of the tick to feed properly. Higher concentrations of histamine, which mediates local skin reactivity and stimulates host grooming, have been found in tick resistant cattle than in susceptible cattle (de Castro and Newson, 1993). Another important finding is that tick- resistant cattle infected with ECF, greatly affect the infection rates in larvae or nymphae which feed on them, resulting in reduced transmission of the disease. Qualitative and quantitative characteristics of mediators released by mast cells vary among animal species (Gordon *et al.*, 1990). A comparative field study on tick susceptibility between N'Dama and Gobra Zebu have shown that a higher levels of circulating eosinophils

in N'Dama than the Gobra Zebu cattle which correlates with the lower numbers of ticks infesting N'Dama than Zebu (Claxton and Leperre, 1991).

Another approach that produces resistance in cattle depends on the observation that bovine gamma globulins ingested by ticks pass unchanged from the tick blood meal into the haemolymph (Wang and Nuttall, 1995). When target antigens from the ticks are inoculated into rabbits or cattle, antibodies are produced against the target antigens and when ticks are fed on these animals, a high tick mortality and reduced number of engorged female ticks is resulted. The few engorged ticks either do not lay eggs or produce eggs that have markedly reduced viability (Allen, 1994).

Dissemination of resistant cattle breeds (*Bos indicus* and their crosses), which have the inherent immunological capacity to severely limit tick numbers parasitizing them, has become an important tick control method. To date, Australia has been the most successful country in raising genetically manipulated tick resistant cattle with significant productivity and marked adaptability to the tropical environment (de Castro and Newson, 1993; Wharton and Norris, 1980). Different breeds have been developed by crossing Zebu and highly productive European breeds by selecting resistant genes for ticks from Zebu. In Africa, indigenous breeds like N'Dama have been shown to resist infestation with several tick species and have been promoted for integrated control of ticks and tick-borne diseases.

In Ethiopia, host resistance to ticks and tick-borne diseases has not yet been practised as tick control method. Nevertheless, its role in decreasing and maintaining the number of infesting ticks and the incidence of tick-borne diseases on indigenous cattle should not be underestimated. Information on the level of resistance of Ethiopian cattle to ticks is very important to facilitate the development of control strategies by using host resistance as a

complementary approach to chemical control. So far, studies have been undertaken to ascertain the role that host resistance could play in tick management in Ethiopia with special reference to comparing indigenous breeds and their crosses. Upon studying resistance of pure Boran and their crosses to tick infestation, it has been found that the pure breeds are more resistant than crossbreds (Sisay and Feseha, 1989). In another study involving indigenous breeds such as Boran and Horro (*Bos indicus*) and their crosses with Simmental, Friesian and Jersey (*Bos taurus*), it was observed that pure Horro and Boran breeds had lowest and intermediate tick burdens, respectively. The crosses of Horro with Simmental and Jersey showed relatively high resistance than all the other crosses and all crosses with Boran showed low resistance (Ali and de Castro, 1993). In a study done between Arsi, Boran and Boran Friesian cross breeds showed that Arsi breed had higher resistance than Boran and its cross (Solomon, 1993). However, no study has been conducted to assess the difference between host resistance status of indigenous cattle breeds alone and the possible factors of resistance.

Among the indigenous cattle breeds, Abigar, Gurage and Horro are used for meat, milk and work while Sheko is used for meat and work by the local people (DAGRIS 2002). The present study was conducted on these breeds to make a comparative evaluation in terms of host resistance to tick infestation. It was hypothesized that there are differences between the four breeds with regard to tick burden. Such evaluation is practically useful for it generates information that could be used in selection of breeds and developing further techniques to improve the resistance characteristics that may be employed in various areas where tick infestation is a major problem.

Objectives of the study

The general objective of this study is to generate information on the conditions of the four indigenous cattle breeds with respect to tick infestation. It will also address the following specific objectives:

- i. Assess and compare breed differences in tick burden,
- ii. Study the relationship between the tick burden and specific IgG levels in each breed
- iii. Provide baseline data on:
 - Tick species and their monthly dynamics in Ghibe valley (Tolle/ Gullele) and
 - Seroprevalence of tick-borne diseases/ pathogens in the study area

2. Materials and Methods

2.1. The Study Area

The study was conducted at the Ghibe valley, Gullele/Tolley station which is located between latitude 8°15'N and longitude 37°30'E and 230 Km south west of Addis Ababa (Fig. 1). The size of the area is about 2000 hectares, with altitude ranging between 1300 and 1400 meters above sea level. The slope of the river valley is gentle and the terrain undulating. The area is part of the Omo river system tsetse belt (Leak *et al.*, 1989), with medium to high tsetse density and trypanosomiasis risk (Leak and Mulatu, 1993; Leak *et al.*, 1993; Rowlands *et al.*, 1993).

The climate consists of a short rainy season in March and April and a main rainy season from late May to October. According to previous studies, the average annual rainfall ranges from 500 to 900 mm and average annual maximum and minimum temperatures range from 30°C to 44°C and from 10°C to 15°C, respectively (Tibbo, 1993). The monthly rainfall and minimum temperature of the area recorded during the study period was collected from the National Meteorology Agency weather station around the valley (Fig. 2).

Vegetation consists mainly of bush savanna with gallery forest along the drainage lines. There is plenty of growth during the wet season but during the dry season vegetation becomes sparse. The major flora include; Acacia, broad-leaved deciduous plants and the dominant grass, *Hyparrhenia spp.* The local farmers are engaged in cultivating maize, sorghum, teff, chickpeas and other cash crops.

The following wild animals were observed in the study area during the study period: Kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus scriptus*), warthog (*Phacochoerus*

aethiopicus), colobus (*Colobus gureza*), monkey (*Cercopithecus aethiops*), porcupine (*Hystrix africaeaustralis*), hippopotamus (*Hippopotamus amphibius*), and small number of crocodiles (*Crocodyles niloticus*).

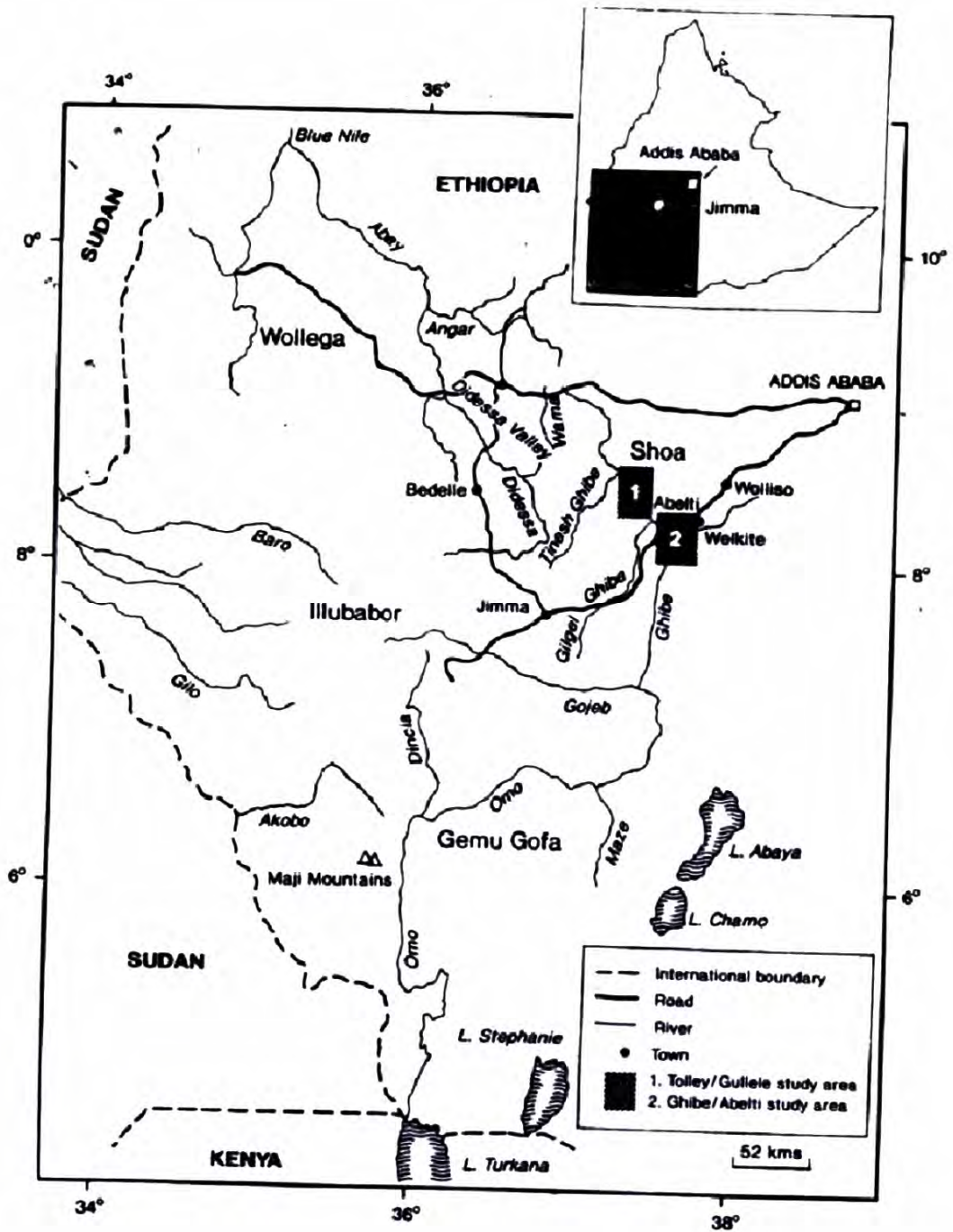


Figure 1 Map of Ghibe Valley including the study area (adapted from Leak and Mulatu, 1993)

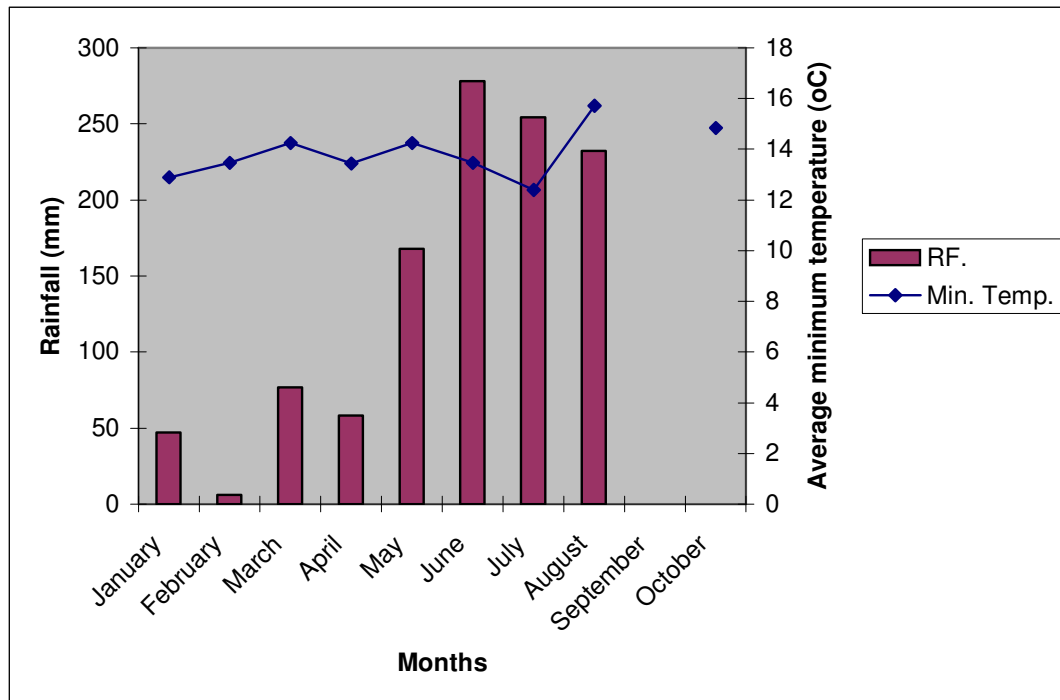


Figure 2 Monthly rainfall and average minimum temperature of the study area from Jan-Oct 2002

2.2. Study Animals

A total of 228 cattle, consisting of four indigenous breeds were brought to the study area on 2000 from four different locations of Ethiopia namely – Abigar, from Gambella; Gurage, from Gurage zone; Horro, from Wollega and Sheko, from Sheka zone, for the purpose of trypanotolerance study. They graze in a mixed herd controlled by three herdsman and are naturally exposed to tick infestation. At night all cattle were housed in their respective pens. The River Ghibe is the main source of water for the animals. Disease management was effected mainly by regular vaccinations against infectious diseases e.g. anthrax, black leg, pasteurellosis, foot-and-mouth-disease and through chemotherapy of cases of trypanosomiasis and other infections as required. A total of 60 randomly selected heifers (15 heifers from each breed), which were about 18 months old were used in the study. All are ear-tagged for permanent identification.

2.3. Collection of Feeding Ticks from Cattle

From January to October 2002, regular half body tick collections were carried out from the sample heifers for seven days at monthly intervals on alternative body sides. After fully restraining, visible ticks of all stages were removed by hand and using special forceps, holding the basis capitulum and screwing so as not to lose the mouthparts of the ticks. The collection was done on eight anatomical sites; head, ear, dewlap, back (side), ventre (abdomen), hoof, tail and ano-vulval areas according to the procedure of Kaiser (1987) and placed in eight separate universal bottles containing 70% ethyl alcohol that had been pre-labelled. Identification of ticks was subsequently done using light stereoscopic microscope employing standard keys of Hoogstraal (1956), Matthyse and Colobo (1987) and Morel (1980). Identified ticks were then counted and recorded by species, sex, instars, semi- or full engorgement. The count of ticks from half body zone of each animal was doubled to give the total number of ticks per animal, assuming equal number of infesting ticks on both sides of an animal. Further confirmatory identification was needed for some specimens and this was done by using the expertise and facilities available at the National Animal Health Research Centre (NAHRC) laboratory at Sebata.

2.4. Packed Cell Volume (PCV) Determination

Parallel with tick collection procedures, PCV of all study animals was determined. Blood was drawn from the marginal ear vein using capillary tubes filled to 3/4th of its length. One end of these tubes was sealed with cristaseal and then placed into a micro-haematocrit centrifuge for five minutes at 1200 rpm. The PCV was determined from the reading expressed as percentage of packed red cells in the total volume of blood components.

2.5. Serum Collection

Blood samples were collected by jugular venopuncture into vacutainer tubes without anticoagulants. The samples were then transported field to NAHRC laboratory (Sebata) at ambient temperature and allowed to stand overnight to clot and centrifuged for 10 minutes at 20,000 rpm. Sera from each sample were separated and kept in sterile, labelled epindorff tubes at 4⁰C overnight and then stored at -20⁰C until use. The collection was conducted in April, June, August and October. Sera for negative control were collected from unexposed calves found at ILRI Debre Zeit station.

2.6. Serological Analysis of Tick-Borne Diseases

Antibodies against the tick-borne pathogens *Anaplasma marginale*, *Babesia bigemina* and *Theileria mutans* were detected by Indirect Enzyme-Linked Immunosorbent Assay (iELISA). Pre-coated plates and reagents were supplied by ILRI ELISA Diagnostic Unit (Nairobi). The methods employed were according to the protocol of the test kits. In brief, the microtiter plates pre-coated with immobilized DNA based antigens of the above mentioned tick-borne pathogens were reconstituted and blocked by blocking buffer (300 µl/well) and incubated for 20 minutes at 37⁰C in a shaker incubator (Insel shaker incubator, UK). The plates were washed with wash buffer (PBS and 0.5% Tween 20). The wells were then filled with wash buffer and the process repeated three times. Weak positive (C+) control was prepared by mixing proportions of strong positive (C++) and negative (C-) control sera according to the instructions of the protocol (for *T. mutans*, 3 µl of C++ with 60 µl of C-, for *A. marginale* and *B. bigemina*, 5 µl of C++ with 55 µl of C-). Next, 150 µl of test and control sera (1:40 dilutions in 1% skimmed milk for *A. marginale*, 1:100 dilutions in 1% skimmed milk for *B. bigemina* and *T. mutans*) were added immediately in duplicate wells and incubated for 40

minutes at 37⁰C. For each control serum (background, strong positive, weak positive and negative controls) four replicates were used. The plates were washed four times as described above and immediately refilled with wash buffer with which wells were left to soak for 10 min. After soaking, the wash buffer in the wells were flicked and blotted as before. 150µl of purified rabbit anti-bovine immunoglobulinG (IgG) conjugated to Horse Radish Peroxidase enzyme solution (Sigma, USA) was added (1:40,000 dilution in 1% skimmed milk for *T.mutans* and 1:50,000 dilution in 1% skimmed milk for *A. marginale* and *B. bigemina*). After adding the conjugate the plates were incubated for 40 minutes at 37⁰C, and then washed four times as above. The HRP activities were detected by adding 100 µl/well of equal volumes of hydrogen-peroxide (H₂O₂) substrate and tetramethylbenzidine (TMB) (Kirkgard-Perry Laboratories, USA) chromogen and incubating the plates at 37⁰C for 15 minutes. The reaction was stopped by adding 100 µl /well of 1M orthophosphoric acid (H₃PO₄). The enzyme activities were measured spectrophotometrically at 450nm using Titerteck Multiscan Plus ELISA reader (Labsystems Multiscan Ex., Finland) connected to a personal computer and the readings were transferred to the PC in a program called PROCOMM. The absorbance values were expressed as percent positivity (pp) relative to the median absorbance values of the weak positive control (C+) by using the formula: -

$$pp = \text{OD of test sample} / \text{median OD of C+} * 100,$$

Where C+ represents weak positive control serum.

Cut-off values were calculated by computing the mean pp of the negative controls and multiplying by two (Wright *et al.*, 1993).

2.7. Measurement of Serum Antibody Levels Against *Amblyomma variegatum*

2.7.1. Antigen Preparation

Antigen from *A. variegatum* larvae was prepared according to the method described by Fivaz *et al.* (1991). Approximately 1000 unfed laboratory bred larvae which were kept at a temperature and of 28⁰C relative humidity and 80% were brought from NAHRC laboratory and washed by centrifugation in 100% ethyl alcohol followed by a wash in phosphate-buffered saline (PBS), PH 7.2. The larvae were then macerated in a tissue grinder to which was added 3ml of PBS. The suspension was centrifuged at 10,000g for 1 hr at 4⁰C. The supernatant was then drawn out and divided into 0.5ml aliquots. The protein content of the larval antigen was determined according to the method of Lowry (Copeland, 1994) using Bovine Serum Albumin (BSA) as a standard. Briefly, 0.3 mg/ml of BSA was pipetted into 10 test tubes with the following volumes: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0 ml. In three other test tubes, 0.1 ml of the test samples was added. The total volume of each test tube was brought to 1.0 ml by adding distilled water. 1.0 ml of Lowry reagent was added to each tube and incubated for 15 minutes after vortexing thoroughly. 3 ml of 1:10 diluted Folin-Phenol reagent was then added to each tube and immediately mixed thoroughly. After incubating for 45 minutes, the absorbance of each solution was determined by a spectrophotometer (Beckman DU-40, USA) at 540nm. The absorbance was then plotted as a function of BSA concentration and the best-fit straight line was calculated. Using the average absorbance for the three samples, the concentration was read from the plot. The aliquots of the samples were stored at -20⁰C until used in the assay.

2.7.2. Determination of IgG Levels Against *Amblyomma variegatum*

Optimal dilutions for antigen, serum and conjugate were initially determined by checkerboard titration (Voller, *et al.*, 1976). The optimal dilution was found to be 1:40 in PBS (PH 7.2 ± 0.2) for the antigen, 1:40 in 3% skimmed milk for the test and control sera and 1:20,000 in PBS-T for the conjugate. 96-well flat-bottomed polystyrene microtitre plates (Nunc Immulon, Denmark) were coated overnight at 4⁰C with 100 µl/well of 340µg/ml crude larval homogenate diluted appropriately. The plates were emptied by flicking the contents into a sink followed by washing manually with PBS-T once. Blocking was performed by adding 300 µl/well of 0.5% BSA in PBS and incubating the plates for 30 minutes at 37⁰C. The plates were washed three times with PBS-T, flicked and blotted on paper towel. 100 µl of diluted test sera were added in duplicate wells and the control sera (strong positive, weak positive, negative and conjugate controls), in four replicate and then incubated for 40 min at 37⁰C. The plates were washed four times as described above and immediately refilled with wash buffer and left for 10 minutes to be soaked at room temperature. After soaking, the wash buffer in the wells were flicked and blotted as before. Appropriately diluted Horseradish peroxidase (HRP)-conjugated rabbit Anti-bovine IgG (FAO/IAEA, Austria), in PBS-T was then added to all wells (100 µl/well) and incubated for 40 min at 37⁰C. Following incubation, plates were washed four times and soaked as described above. The HRP activities were detected by adding 150 µl/well of equal volumes of substrate (H₂O₂) and chromogen (TMB) and incubating the wells at 37⁰C for 15 minutes. The reaction was stopped by adding 100 µl /well of 1M H₃PO₄. The enzyme activities were measured spectrophotometrically at 450nm in ELISA reader connected to a personal computer and the readings were transferred to the PC in a program called PROCOMM. The absorbance values were used as a measure of IgG levels directly.

2.8. Data Management and Analysis

The total, mean per animal and percentage abundance of each species of male, female, fully engorged females and immature ticks removed from each breed on each month were presented. Abundance of the major tick species over the study period was presented as the total count of ticks of a given species within a breed in a specific month and the presence /absence of significant difference in burden of these tick species between the breeds was analysed by employing GLM (General Linear Model) univariate analysis at $P < 0.05$ level of significance in SPSS (version 11.0) statistical program. Analysis for variation in tick burden within each breed was performed by GLM repeated measures analysis.

On log transformed values, comparison of breeds with respect to the overall tick burden, adult ticks and fully fed female ticks was performed by GLM univariate model at $P < 0.05$ and $P < 0.01$ levels of significance and ranking of the breeds according to significant differences in total tick count was done using Student-Neuman- Keuls test at $P < 0.001$.

Mean values of PCV measurement in each month were presented and GLM univariate analysis was employed to compare the mean PCV difference between breeds. Period seroprevalences of the major tick-borne diseases (pathogens) were determined and test for significant differences in mean seropositivity of tick-borne pathogens between breeds was analysed by One way Analysis of Variance (ANOVA).

The mean level of anti *A. variegatum* IgG in each breed of cattle was obtained from the absorbance values and comparison was done using One Way Analysis of Variance (ANOVA) followed by Student-Neuman-Keuls test on SPSS programme. The pattern of the relationship between IgG levels and the respective tick burden of each individual animal was determined by employing Pearson correlation analysis in 95% confidence interval.

3. Results

3.1. Abundance and Monthly Variation of Identified Tick Species

Ticks infesting cattle in the study area belong to the genera *Amblyomma*, *Boophilus*, *Rhipicephalus*, *Hyalomma* and *Haemaphysalis* in decreasing order of abundance (Table 2). The most common tick species were found to be *Amblyomma variegatum*, *Boophilus decoloratus*, *A. cohaerens*, *Rhipicephalus evertsi evertsi* and *R. praetextatus*. The relative abundance of each tick species is listed in table 3. Species of very little abundance include, *Haemaphysalis aciculifer*, *Hyalomma marginatum rufipes*, *Rhipicephalus bergeoni*, *R. lunulatus*, *R. muhsamae* and *R. pravus*, all of which comprise about 0.31 % of the total adult tick species. Because of their low abundance, these species were excluded from subsequent analyses. The general pattern of abundance of all the ticks over the study period shows that ticks were abundant from January to May, when temperature was higher and rainfall lower and abundance was low from June to October, when rainfall was lower (Fig. 3).

Table 2 Relative abundance of adult and immature ticks by genera on the experimental herd in Ghibe/ Tolley station between January and October 2002

Genus	Count (%)
<i>Amblyomma spp.</i>	53903 (63.33)
<i>Boophilus spp.</i>	23488 (27.61)
<i>Rhipicephalus spp.</i>	7650 (8.99)
<i>Hyalomma spp.</i>	25 (0.03)
<i>Haemaphysalis spp.</i>	10 (0.01)
Total ticks	85076 (100)

Table 3 Relative abundance of adult tick species identified on the four breeds in Ghibe / Tolley station between January and October 2002

Tick species	Abigar	Gurage	Horro	Sheko	Total Count (%)
<i>Amblyomma variegatum</i>	3690	3717	1814	4956	14163 (39.51)
<i>Boophilus decoloratus</i>	4974	2920	1632	4432	14016 (39.10)
<i>A. cohaerens</i>	1060	878	531	1344	3765 (10.50)
<i>Rhipicephalus e. evertsi</i>	675	268	576	903	2395 (6.68)
<i>R. praetextatus</i>	352	374	159	528	1388 (3.87)
<i>R. pravus</i>	18	7	6	2	33 (0.09)
<i>R. bergeoni</i>	4	13	4	5	26 (0.07)
<i>Hyalomma m. rufipes</i>	13	4	4	4	25 (0.07)
<i>R. lunulatus</i>	7	2	10	2	21 (0.06)
<i>Haemaphysalis aciculifer</i>	2	4	0	4	10 (0.03)
<i>R. muhsame</i>	3	0	0	0	3 (0.01)
Total	10798	8187	4736	12180	35845 (100.00)

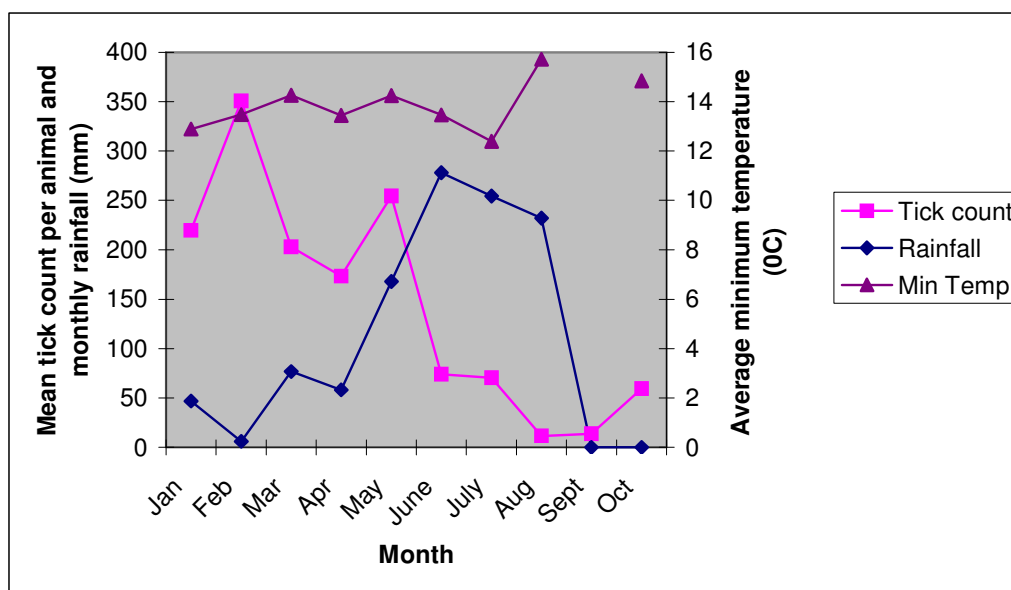


Figure 3 Pattern of abundance of tick species on the sample herd with respect to rainfall and minimum temperature in Ghibe/ Tolley station over the study period (Jan- Oct 2002)

Amblyomma variegatum was the most abundant species over the study period comprising about 39.5% of the total tick count. This tick species was present throughout the study period with peak infestation in May (Fig. 4). In this month, the burden was highest in Sheko breed with total count of 2205 and lowest in Horro with total count of 615 ticks. In Abigar and Gurage breeds, the total count was 1575 and 1335 respectively. The least square mean difference in infestation by this tick species was significant ($P < 0.05$) between Horro and the other three breeds (Abigar, Gurage and Sheko) while the difference between the latter three breeds was not significant (Table 4). Fully engorged *A. variegatum* female ticks were most abundant in May ($n=12$) and the highest proportion of them was from Sheko cattle but the mean difference was not significant ($P > 0.05$). The overall male to female ratio of this species was 3:1, which shows a higher proportion of males than the females (Table 5). The

body zone with highest frequency of attached *A. variegatum* was the ventral area followed by dewlap and hooves. The frequency of attachment in the ventral site is significantly higher than the other body zones ($P<0.05$) (Table 6). Body zones with least frequency of this tick species were ear, tail and head.

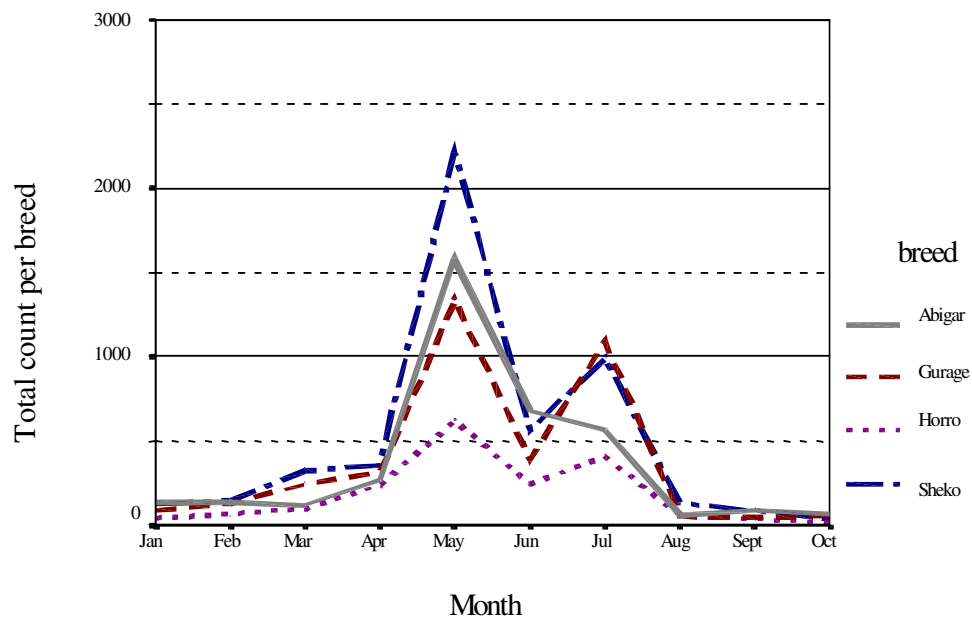


Figure 4 Monthly abundance of *A. variegatum* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

The second most important tick species in the study area was *Boophilus decoloratus*. It comprised about 39.1% of the total tick species. The activity of this species was high from January to June and peaked in February (Fig. 5). In this month, the average burden was 117 ticks per animal in Abigar, 88 in Gurage, 39 in Horro and 118 in Sheko. The mean difference of the burden in February was significant between Abigar and Gurage and between Abigar and Horro breeds ($P<0.05$) (Table 4). In March, infestation by *B. decoloratus* became highest only in Abigar breed with an average of 130 ticks per animal but the mean difference was not

significantly different from the rest of the breeds ($P > 0.05$). The number of fully engorged females in Sheko and Abigar was significantly greater than those of Gurage and Horro ($P < 0.05$). In March, highest number of fully engorged female ticks was collected from Abigar ($n=300$), but not significantly different than the others ($P > 0.05$). Unlike in the other species, the proportion of female *B. decoloratus* ticks was higher than that of the males in all breeds, being about 1:2 in Abigar, Gurage and Horro; while about 1:3 in Sheko (Table 5). The attachment site with highest frequency of this species was the back (side) of the animals ($P < 0.05$), followed by dewlap, ventre (abdomen) and ear (Table 6).

Table 4 Mean values of *A. variegatum*, *B. decoloratus* and fully engorged female ticks of *B. decoloratus* in each breed of cattle during the study period (Jan-Oct 2002)

Ticks	Abigar	Gurage	Horro	Sheko
<i>A. variegatum</i>	0.86 (0.51) ^b	0.84 (0.52) ^b	0.69 (0.45) ^a	0.88 (0.54) ^b
<i>B. decoloratus</i>	0.67 (0.40) ^b	0.64 (0.38) ^a	0.59 (0.37) ^a	0.78 (0.43) ^b
Fully engorged <i>B. decoloratus</i> females	0.62 (0.43) ^b	0.41 (0.23) ^a	0.49 (0.19) ^a	0.63 (0.33) ^b

^{a, b} Within variable row, means with different superscripts are different at $P < 0.05$.

Figure 6 (a,b) shows the abundance of larval and nymph populations of *B. decoloratus*. The activity of both stages was high from January to May and low from June to October. Dewlap was the body zone with highest frequency of immature *B. decoloratus* (Table 6).

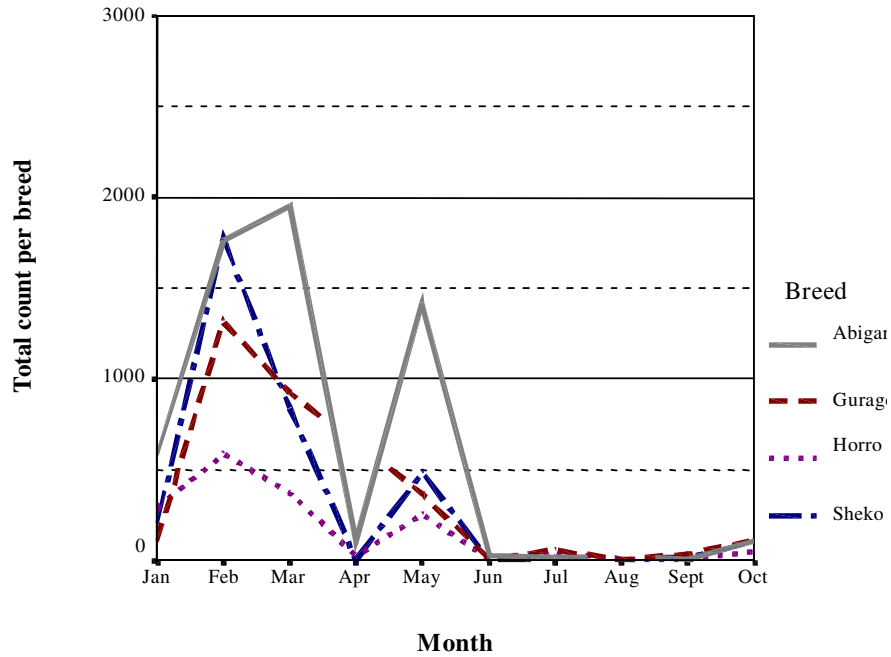
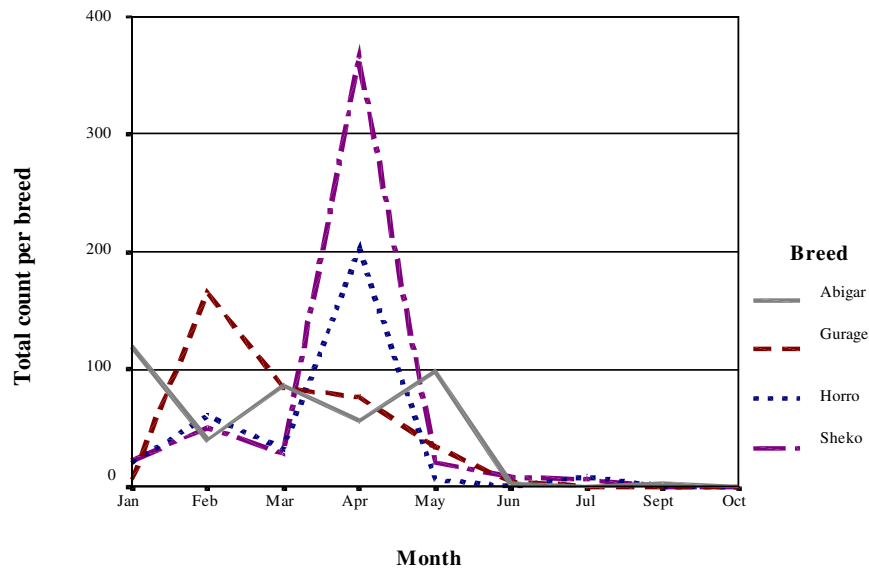


Figure 5 abundance and monthly variation of Adult *B. decoloratus* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

a. Larvae



b. Nymphs

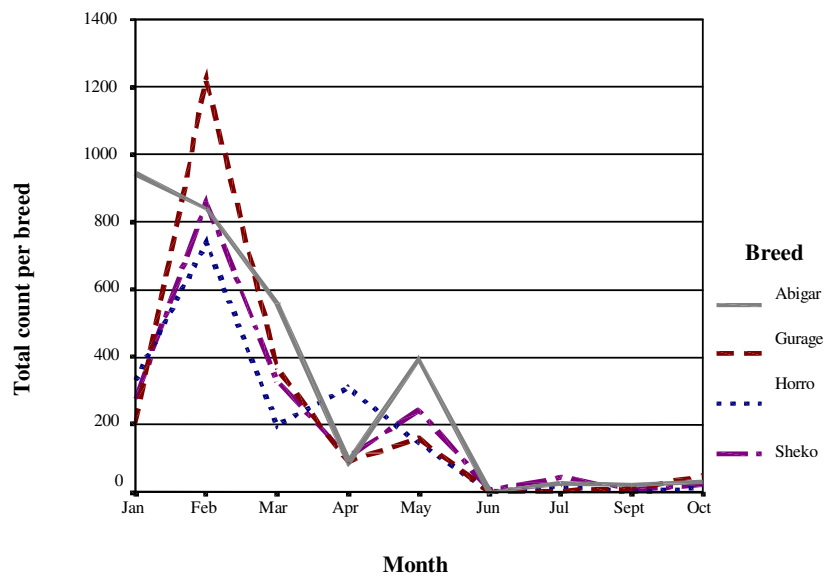


Figure 6 Abundance and monthly variation of a) larvae and b) nymphs of *B. decoloratus* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

The third abundant species, *Amblyomma cohaerens* was present in almost all months of the study period, with peak activity in January. May and October were also found to be months of higher activities (Fig. 7). The number of fully engorged females of *A. cohaerens* was low in all months of the study period. The proportion of male ticks was higher than that of the females in the four breeds with ratio of about 2:1 in Abigar and Gurage breeds, and about 3:1 in Horro and Sheko breeds (Table 5). Abdomen was the body zone with high frequency of attached ticks, but not significantly higher than the other body zones (Table 6).

Larvae and nymphs of *Amblyomma spp.* were the most abundant immature tick species in the study animals. The months with peak activities of larval forms of *Amblyomma spp.* were April and May, when the temperature was higher. The larval population decreased in the months when rainfall was higher and temperature lower (June – August), and in September, the population started to rise up (Fig. 8a). Regarding the nymphal forms, peak activity was observed in January and the population decreased from April to October in all breeds (Fig 8b).

R.e.evertsi was present in all months of the study period with highest frequency in January, when temperature was high (Fig. 9). The number of fully engorged females was lower than those of *A. variegatum* and *B. decoloratus* throughout the months. The proportion of males and females was similar in all breeds (Table. 5). The most preferred anatomical site of this tick species was the anovulva (Table 6). Very few ticks of this species were collected from the hooves and ventral sites, and hardly any ticks from dewlap areas.

Infestation by *R. praetextatus* was highest in October, when there was less rainfall and high temperature (Fig. 10). The number of fully engorged females was low in the four breeds of cattle with no significant difference between them. The male to female ratio was almost

equivalent (1:1) except in Sheko, which was about 2:1 (Table 5). Ear was the most preferred site for attachment, followed by the dewlap. The other body zones were with significantly less number of attached *R. praetextatus* ticks (Table 6).

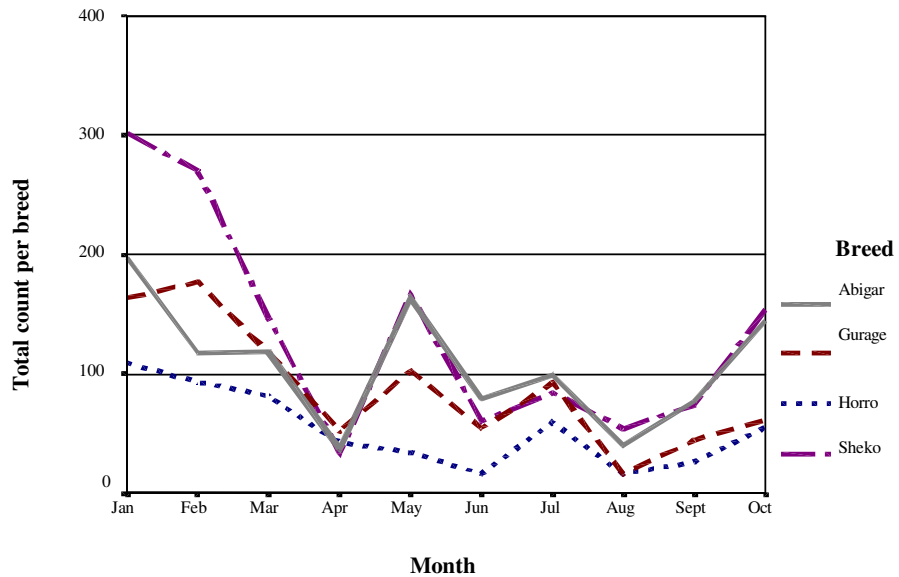
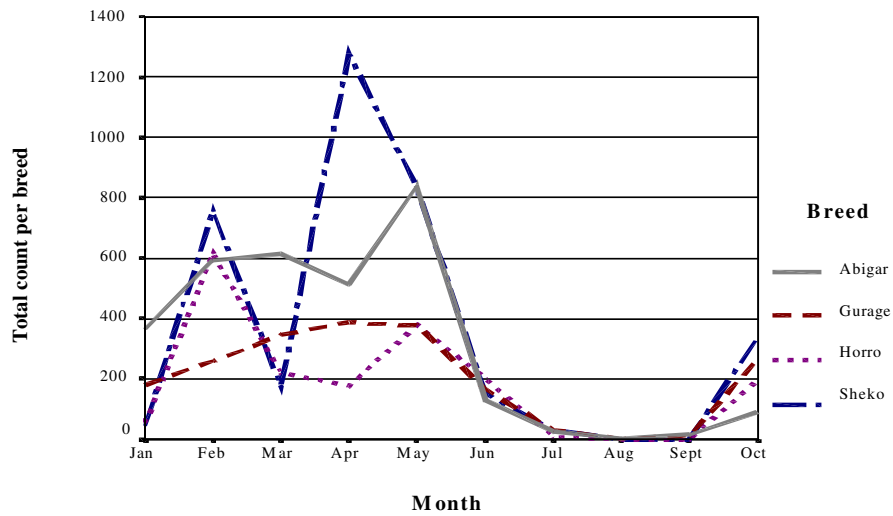


Figure 7 Abundance and monthly variation of *A. cohaerens* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

a. Larvae



b. Nymphs

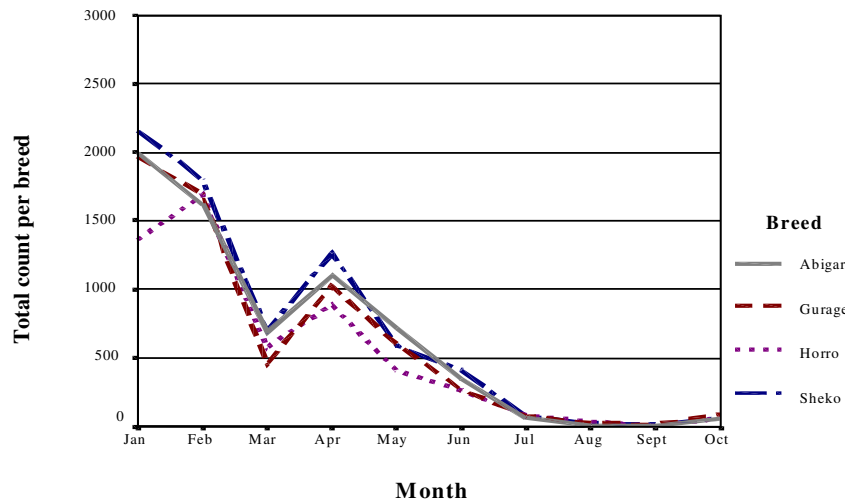


Figure 8 Abundance and monthly variation of a) larvae and b) nymphs of *Amblyomma spp.* in the four breeds of cattle in Ghibe/ Tolley station over the study period (Jan- Oct 2002)

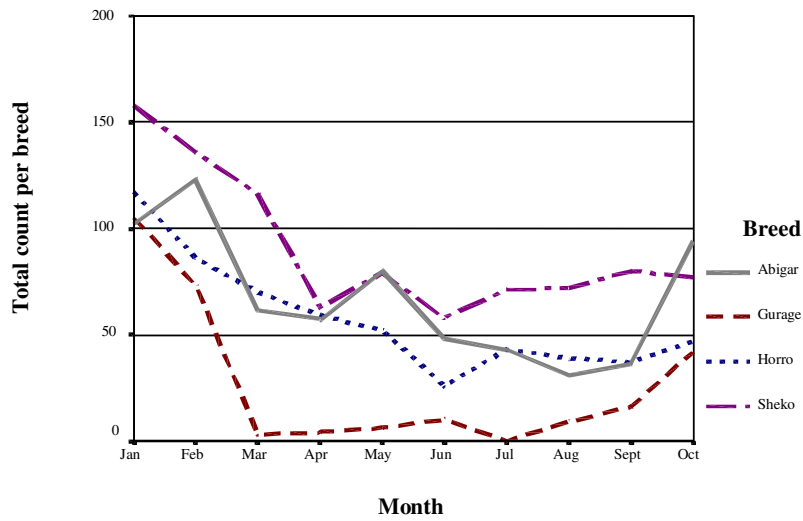


Figure 9 Abundance and monthly variation of *R. e. evertsi* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

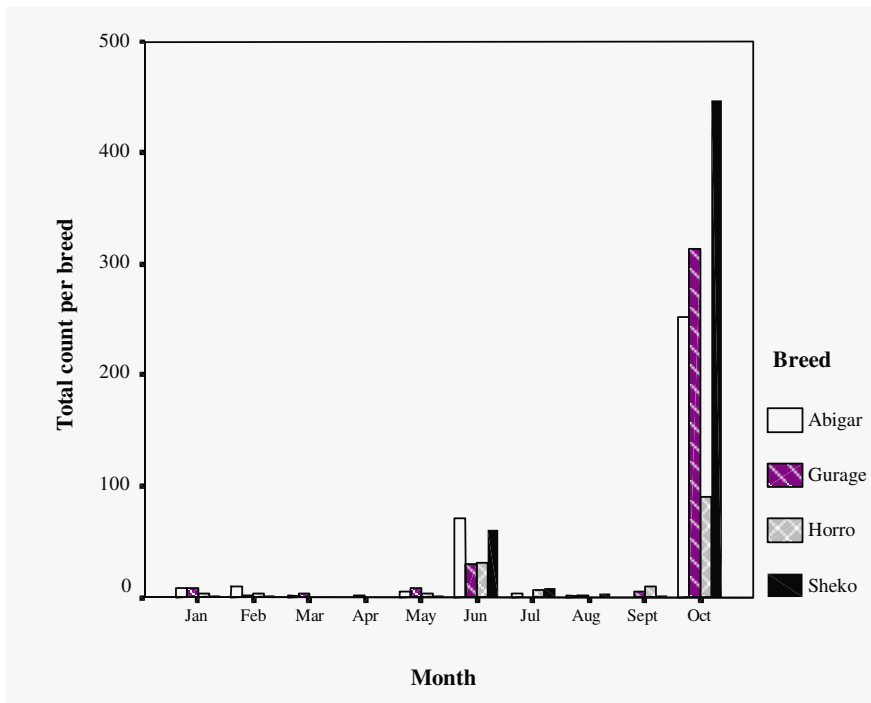
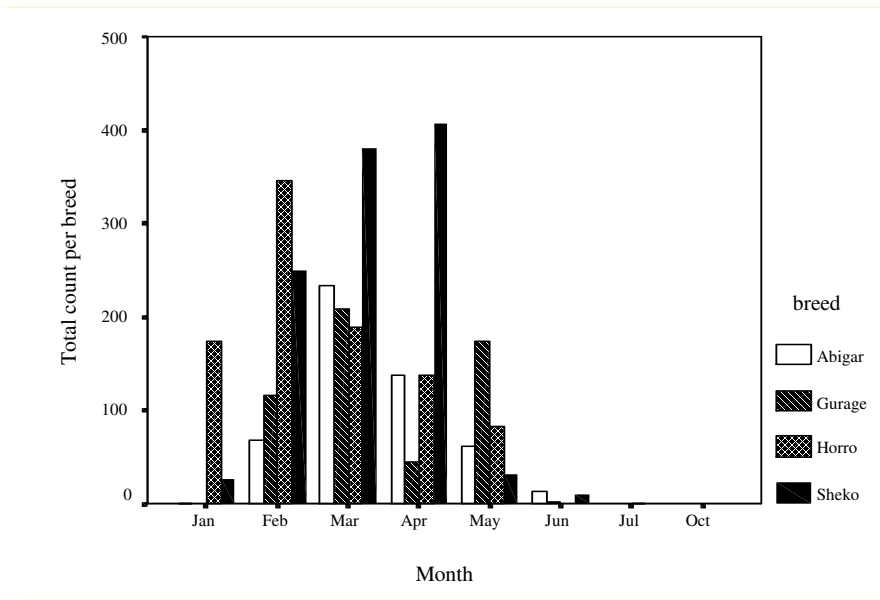


Figure 10 Abundance and monthly variation of *R. praetextatus* in the four breeds of cattle in Ghibe/ Tolley station between January and October 2002

Larval forms of the genus *Rhipicephalus* were abundant in February, March and April with peak infestation in April (Fig. 11). Although Sheko and Horro breeds had higher number of larval ticks, the mean difference was not significant ($P > 0.05$). The level of infestation of the nymphal forms was observed to exhibit variation between breeds in the months from January to May but the mean difference was not significant ($P > 0.05$). Both the larval and nymphal forms were most frequently found attached deep in the ear canal than the other body sites (Table 6).

a. Larvae



b. Nymphs

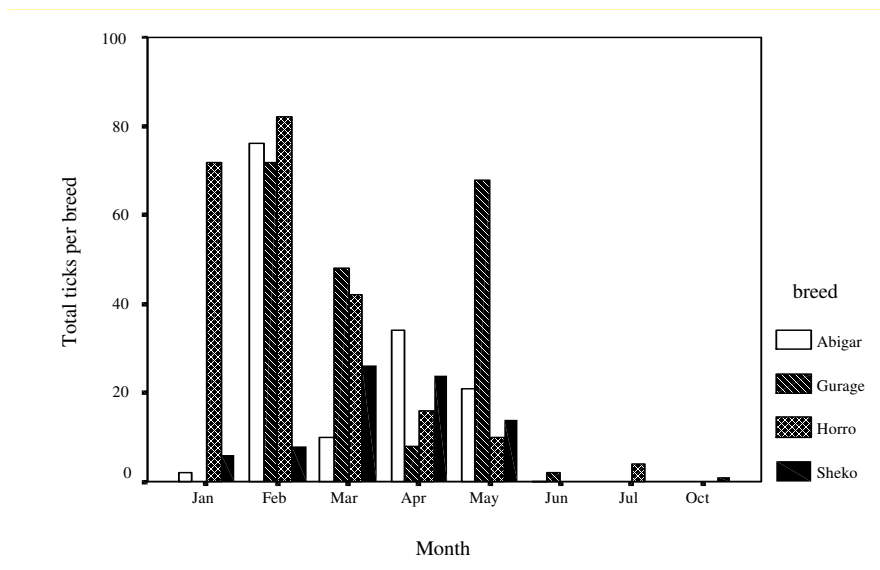


Figure 11 Abundance and monthly variation of a) larvae and b) nymphs of *Rhipicephalus spp.* in the four breeds of cattle in Ghibe/ Tolley station over the study period (Jan-Oct 2002)

Table 5 Overall male to female ratio of ticks in the four breeds of cattle in Ghibe/ Tolley station over the study period (Jan- Oct 2002)

Tick species	Abigar			Gurage			Horro			Sheko			Overall
	Male	Female	M: F Ratio	Male	Female	M: F Ratio	Male	Female	M: F Ratio	Male	Female	M: F Ratio	M: F Ratio
<i>A. variegatum</i>	2864	826	3.47: 1	2793	924	3.02: 1	1263	373	3.39: 1	3860	1096	3.52: 1	3.35: 1
<i>A. cohaerens</i>	751	309	2.43: 1	622	256	2.43:1	348	122	2.85: 1	1012	332	3.05: 1	2.68: 1
<i>B. decoloratus</i>	2127	3847	1: 1.81	1034	1886	1: 1.82	420	970	1: 2.31	912	2520	1: 2.76	1: 2.05
<i>H. aciculifer</i>	0	2	0	2	2	1: 1	0	0	-	2	2	1: 1	1: 1.5
<i>H. m. rufipes</i>	10	3	3.3: 1	4	0	0	3	1	3: 1	4	0	0	5.25: 1
<i>R. e.evertsi</i>	451	224	2.01: 1	162	106	1.53: 1	361	181	1.99: 1	592	311	1.9: 1	1.91: 1
<i>R. bergeoni</i>	2	2	1:1	8	5	1.6: 1	2	2	1: 1	3	2	1.5: 1	1.36: 1
<i>R. lunulatus</i>	4	3	1.3: 1	0	2	0	2	8	1: 4	0	2	0	1: 2.5
<i>R. muhsame</i>	2	1	2: 1	0	0	-	0	0	-	0	0	-	2: 1
<i>R. pravus</i>	10	8	1.25: 1	2	5	1: 2.5	4	2	2: 1	2	0	0	1.2: 1
<i>R. praetextatus</i>	185	167	1.11:1	209	165	1.27: 1	67	84	1:1.25	324	204	1.59: 1	1.27:1

Table 6 Mean counts (on transformed values) of the major tick species at different attachment sites of the sample animals in Ghibe/ Tolley station during the study period (Jan-Oct 2002)

Tick species	Log 10 transformed mean (S.D) of tick counts at the respective body sites							
	Head	Ear	Dewlap	Ventre	Back	Hoof	Tail	Ano-vulva
<i>A.variegatum</i>	0.44 ^{b,c} (0.30)	-	0.74 ^c (0.50)	1.01 ^d (0.51)	0.57 ^{b,c} (0.35)	0.65 ^{b,c} (0.37)	0.11 ^a (0.16)	0.37 ^b (0.38)
<i>B.decoloratus</i>	0.60 ^b (0.34)	0.79 ^{b,c} (0.51)	0.92 ^c (0.52)	0.85 ^c (0.52)	0.97 ^c (0.56)	0.62 ^b (0.39)	0.41 ^a (0.39)	0.29 ^a (0.29)
<i>A. cohaerens</i>	0.30 ^{a,b} (0.00)	0.30 ^{a,b} (0.00)	0.62 ^{b,c} (0.33)	0.83 ^c (0.39)	0.40 ^{a,b} (0.21)	0.44 ^{b,c} (0.21)	0.10 ^a (0.17)	0.23 ^{a,b} (0.29)
<i>R.e.evertsi</i>	0.50 ^{a,b} (0.35)	0.30 ^a (0.00)	-	0.25 ^a (0.12)	0.30 ^a (0.00)	0.36 ^a (0.18)	0.12 ^a (0.16)	0.63 ^b (0.32)
<i>R. praetextatus</i>	0.38 ^{a,b} (0.14)	0.95 ^c (0.51)	0.83 ^{b,c} (0.56)	0.40 ^{a,b} (0.17)	0.30 ^{a,b} (0.00)	0.38 ^{a,b} (0.21)	0.31 ^{a,b} (0.31)	0.20 ^a (0.26)
<i>Amblyomma spp.</i>	0.80 ^c (0.45)	0.79 ^c (0.46)	0.90 ^c (0.44)	1.16 ^d (0.52)	0.88 ^c (0.46)	1.14 ^d (0.49)	0.28 ^b (0.34)	0.14 ^a (0.19)
<i>Boophilus spp.</i>	0.61 ^{c,d} (0.36)	0.83 ^{d,e} (0.51)	0.98 ^e (0.55)	0.69 ^{c,d} (0.45)	0.85 ^{d,e} (0.51)	0.54 ^{b,c} (0.31)	0.33 ^b (0.38)	0.11 ^a (0.24)

^{a, b, c, d, e} Within variable row, means with different superscripts are significantly different at P< 0.05.

Variation of individuals within each breed with respect to the overall tick burden was tested.

Variation was observed within the Abigar, Horro and Sheko breeds but not in the Gurage breed (Table. 7).

Table 7 Mean tick counts of individual animals of the four breeds of cattle in Ghibe/ Tolley station over the study period (Jan- Oct 2002)

Abigar		Gurage		Horro		Sheko	
Animal ID	Mean count (S.D)	Animal ID	Mean count (S.D)	Animal ID	Mean count (S.D)	Animal ID	Mean count (S.D)
0028	1.51 (0.91) ^a	0128	1.45 (0.74) ^a	0114	1.42 (0.62) ^a	0037	1.63 (0.64) ^a
0020	1.65 (0.60) ^a	0126	1.48 (0.80) ^a	0093	1.44 (0.72) ^a	0046	1.76 (0.49) ^a
0015	1.72 (0.78) ^a	0125	1.53 (0.89) ^a	0119	1.44 (0.78) ^a	0061	1.89 (0.74) ^a
0027	1.79 (0.76) ^a	0122	1.59 (0.73) ^a	0085	1.57 (0.82) ^a	0062	1.90 (0.78) ^a
0019	1.85 (0.66) ^a	0133	1.79 (0.82) ^a	0076	1.62 (0.58) ^a	0035	1.92 (0.56) ^{a,b}
0022	1.94 (0.59) ^a	0120	1.82 (0.66) ^a	0089	1.63 (0.72) ^{a,b}	0049	2.00 (0.56) ^{a,b}
0002	1.94 (0.70) ^a	0132	1.84 (0.79) ^a	0079	1.64 (0.62) ^{a,b}	0039	2.00 (0.67) ^{a,b}
0018	1.98 (0.70) ^a	0135	1.96 (0.91) ^a	0084	1.69 (0.86) ^{a,b}	0060	2.02 (0.71) ^{a,b}
0003	2.02 (0.80) ^a	0127	1.97 (0.85) ^a	0102	1.77 (0.61) ^{a,b}	0066	2.03 (0.80) ^{a,b}
0001	2.10 (0.69) ^a	0123	2.04 (0.77) ^a	0081	1.86 (0.54) ^{a,b}	0042	2.06 (0.54) ^{a,b}
0007	2.14 (0.75) ^a	0130	2.05 (0.54) ^a	0118	1.98 (1.03) ^{a,b}	0040	2.16 (0.62) ^{a,b}
0023	2.16 (0.66) ^a	0137	2.05 (0.61) ^a	0105	2.01 (0.69) ^{a,b}	0065	2.18 (0.61) ^{a,b}
0013	2.18 (0.55) ^a	0139	2.12 (0.85) ^a	0095	2.09 (0.66) ^{a,b}	0050	2.26 (0.67) ^{a,b}
0014	2.28 (0.65) ^a	0138	2.15 (0.61) ^a	0092	2.10 (0.60) ^{a,b}	0058	2.35 (0.51) ^{a,b}
0030	2.39 (0.79) ^b	0124	2.28 (0.72) ^a	0101	2.15 (0.53) ^b	0070	2.47 (0.66) ^b

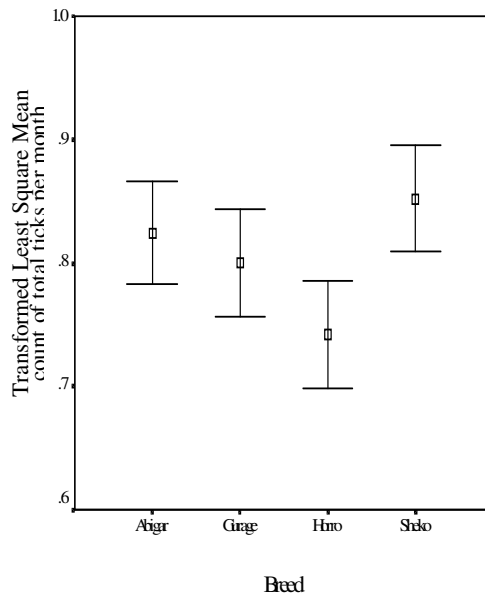
^{a, b} Within variable column, means with different superscripts are different at P<0.05

ID – Tag number

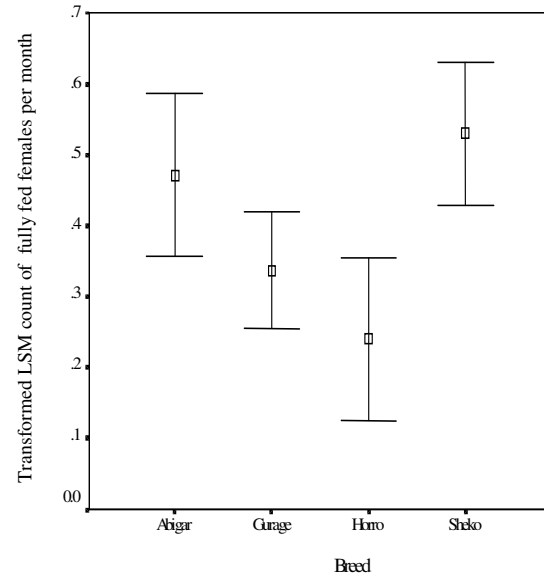
3.2. Comparison of Tick Burden in the Four Breeds

The relative resistance of the four breeds to tick infestation was measured by using three assessment parameters: the total count of fully engorged females, the total count of adult ticks and the total count of all attached ticks. Significantly lowest burden ($P < 0.001$) of total ticks was found in Horro. The breeds with highest total tick burden were Sheko and Abigar but the mean difference between the two breeds was not significant ($P > 0.001$). In Gurage, intermediate total tick burden was found ($P < 0.001$) (Fig 12 a). Significantly highest burden of fully engorged female ticks was found in Sheko and lowest burden was found in Horro. The rest two breeds had intermediate burden of fully engorged ticks ($P < 0.05$) (Fig 12 b). Significant difference in adult tick count was found between Horro, Sheko and Abigar and Gurage breeds ($P < 0.01$) (Fig 12 c).

a.



b.



c.

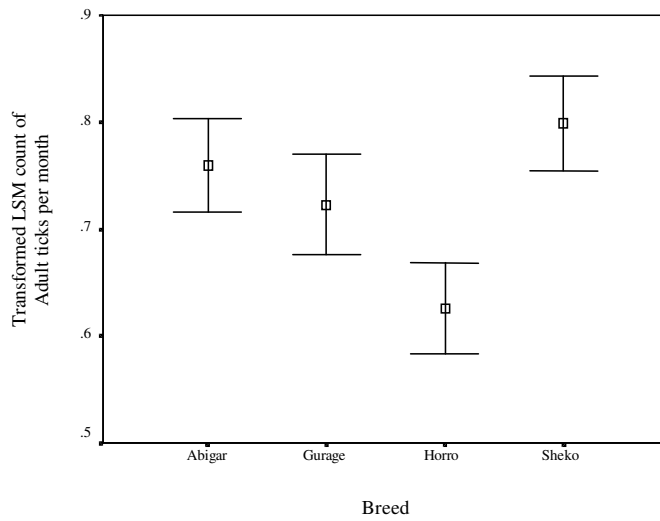


Figure 12 Mean tick counts in each breed of cattle over the study period (Jan- Oct 2002) in Ghibe/ Tolley station (a) Overall tick population; (b) Fully engorged females and (c) Adult ticks only

3.3. Comparison of Breeds with Respect to Specific IgG against *Amblyomma variegatum*

The mean values of IgG level measurements in the four breeds of cattle showed the level of serum IgG that reacts against the crude homogenates of *A. variegatum* to be highest in Horro breed (Fig. 13) ($P < 0.05$).

The level of anti- *A. variegatum* serum IgG and the respective burden of *A. variegatum* were assessed in each breed. Weak negative correlation ($r = - 0.2$) was observed between IgG and *A. variegatum* infestation in Horro breed, but not statistically significant ($P > 0.05$). Positive correlation was observed in Abigar ($r = 0.3$, $P > 0.05$), Gurage ($r = 0.4$, $P < 0.05$) and Sheko ($r = 0.5$, $P < 0.05$) breeds. In April, when the population of *A. variegatum* was high on the animals, animals of the Horro breed were observed to maintain low infestation with *A. variegatum* while having high concentration of IgG (Fig. 14). In contrast, in the Sheko breed, individuals harbouring high *A. variegatum* infestation had lower concentration of IgG (Fig. 14).

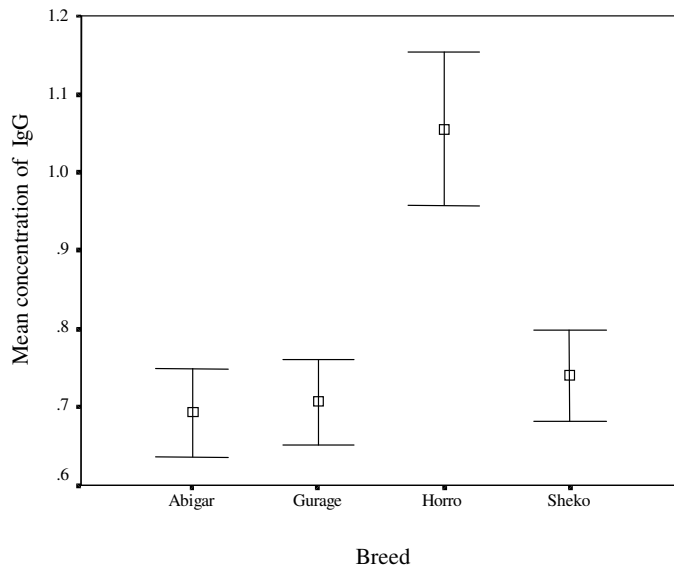


Figure 13 Concentration of IgG antibodies in the four breeds of cattle against *A.variegatum* crude larval homogenates

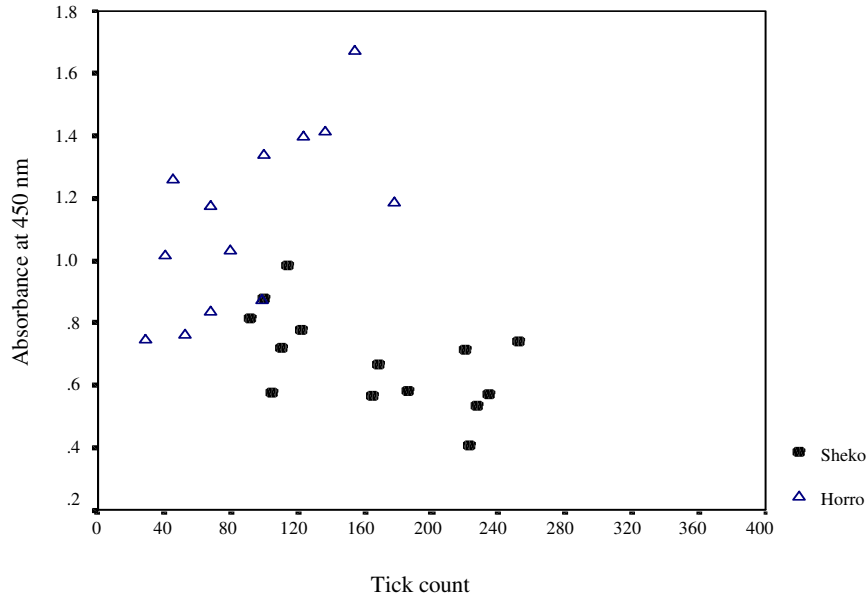


Figure 14 Pattern of relationship between the burden of *A. variegatum* tick species and specific IgG level in breeds with lowest (Horro) and highest susceptible (Sheko) breeds in Ghibe/ Tolley station on April 2002

3.4. Measurement of Packed Cell Volume (PCV)

The general trend of the PCV values showed negative correlation with tick burden in all the breeds over the study period ($r = -0.8$ in Abigar, Gurage and Sheko; $r = -0.95$ in Horro, $P < 0.05$), indicating the effect of tick burden on the volume of red blood cells in the animals. From January through May, PCV values were lower and from June to September, PCV values were higher in all the cattle breeds (Fig. 15). In January, June and October, significantly highest PCV values were recorded in the Sheko breed ($P < 0.05$).

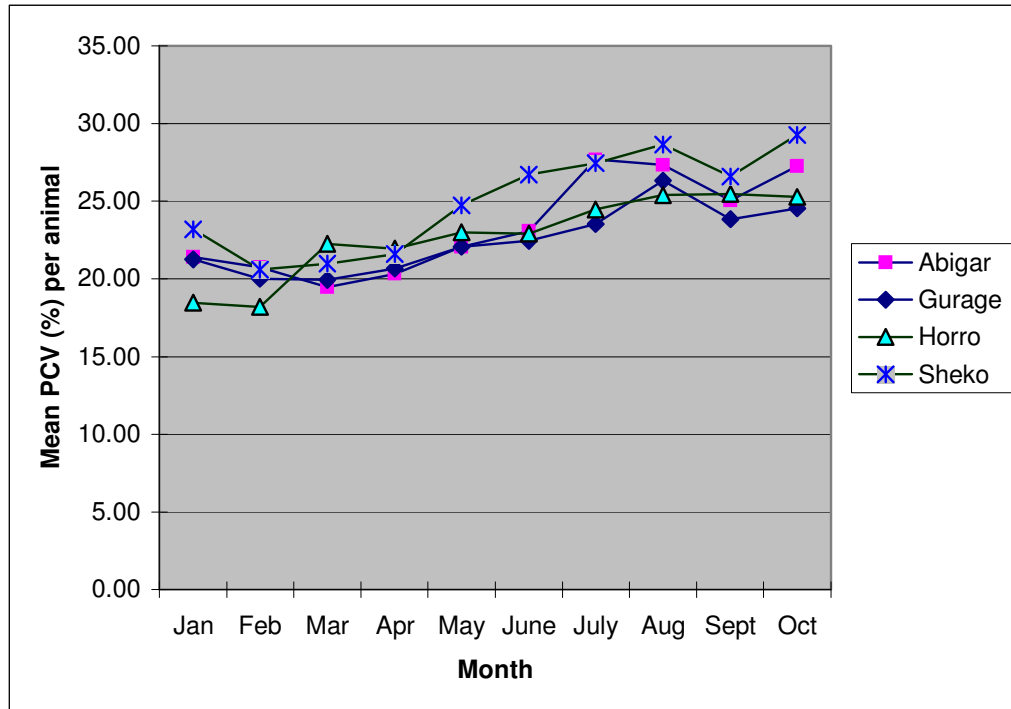


Figure 15 Mean monthly PCV values of each breed over the study period

3.5. Seroprevalence of Tick-Borne Pathogens

Table 8 shows the results of serological examinations for the three tick-borne diseases/ pathogens (*T. mutans*, *B. bigemina* and *A. marginale*). High seroprevalence rates were obtained in the animals for all of the tick-borne pathogens. Significantly higher seropositivity for *A. marginale* was detected in Horro breed ($P < 0.05$). Regarding *B. bigemina* and *T. mutans*, the mean seropositivity between the breeds was not significantly different from each other ($P > 0.05$).

Table 8 Seroprevalence of tick-borne pathogens based on Indirect ELISA: (a) *Anaplasma marginale*, (b) *Babesia bigemina* and (c) *Theileria mutans* in the four breeds of cattle

(a) *A. marginale*

	April	June	August	October	
Breed	N (%)	N (%)	N (%)	N (%)	Overall period prevalence
Abigar	15 (100)	11 (73.3)	11 (73.3)	9 (60)	77 ^{a,b}
Gurage	15 (100)	15 (100)	8 (53.3)	7 (46.7)	75 ^b
Horro	15 (100)	15 (100)	14 (93.3)	12 (80)	93 ^a
Sheko	14 (93.3)	15 (100)	13 (86.7)	13 (86.7)	92 ^{a,b}
Overall	59 (98.3)	56 (93.3)	46 (76.7)	41 (68.3)	84.2

(b) *B. bigemina*

	April	June	August	October	
Breed	N (%)	N (%)	N (%)	N (%)	Overall period prevalence
Abigar	14 (93.3)	8 (53.3)	12 (80)	14 (93.3)	80 ^a
Gurage	13 (86.7)	12 (80.0)	13 (86.7)	15 (100)	88 ^a
Horro	15 (100)	12 (80.0)	14 (93.3)	15 (100)	93 ^a
Sheko	15 (100)	11 (73.3)	13 (86.7)	13 (86.7)	87 ^a
Overall	57 (95)	43 (71.7)	52 (86.7)	57 (95)	87.1

(c) *T. mutans*

	April	June	August	October	
Breed	N (%)	N (%)	N (%)	N (%)	Overall period prevalence
Abigar	13 (86.7)	5 (33.3)	8 (53.3)	4 (26.7)	50 ^a
Gurage	9 (60)	8 (53.3)	7 (46.7)	8 (53.3)	53 ^a
Horro	13 (86.7)	8 (53.3)	8 (53.3)	7 (46.7)	60 ^a
Sheko	11 (73.3)	7 (46.7)	8 (53.3)	6 (40)	53 ^a
Overall	46 (76.7)	28 (46.7)	31 (51.7)	25 (41.7)	54.2

^{a, b} Within variable column, means with different superscripts are different at P < 0.05

N = Number of animals serologically positives

4. DISCUSSION

On the whole a total of 85,076 ticks of all stages, which belong to 11 species and subspecies were collected from the four breeds of cattle. The tick species identified in the present study were also found in previous surveys that were conducted in western and central parts of Ethiopia (de Castro, 1994; Mekonnen *et al.*, 2001,).

In all the four breeds of cattle, *Amblyomma variegatum* and *B. decoloratus* were found to be the dominant tick species for the period studied. Previous studies in western Ethiopia also showed that *A. variegatum* and *B. decoloratus* were the most widespread tick species (de Castro, 1994). All the breeds showed similar monthly fluctuations in counts of both ticks except in the case of *B. decoloratus*, where highest frequency was seen in Abigar breed in March, while the abundance in the other breeds was low. Based on the observed monthly variation and the available meteorological data, the tick species seemed to depend on the ambient temperature of the study area. Because of limited baseline data on weather variables, it was not possible to adequately relate the monthly variation of the ticks with the relevant climatic conditions of the study area.

In this study the male to female sex ratio of each tick species was not equivalent, which is different from the natural condition where equivalent proportions of male and female ticks are expected. Since the present study dealt with only the on- host the population dynamics of ticks, it could not clearly show the abundances of the two-host ticks such as *R. e. evertsi*, *H. m. rufipes*, and three- host ticks such as *A. variegatum* and *A. cohaerens* which spend some time off their hosts.

Immature forms of *Amblyomma* and *Rhipicephalus spp.* were recovered and it was difficult to determine to which species they belong. Studies on tick biology and ecology indicated that immature forms *A. variegatum* and *A. cohaerens* can parasitize cattle, wild mammals, birds and rodents. Similar studies show that immature forms of *R. e. evertsi* are found deep in the ear (Hoogstraal, 1956). Therefore, larvae and nymphs of the genus *Rhipicephalus* which were collected from the inner part of the ear may be immature forms of *R. e. evertsi*.

Previous tick surveys have revealed that two species of the genus *Boophilus* were found in Ethiopia that is, *B. decoloratus*, which is distributed in almost all areas of Ethiopia (Morel, 1980) and *B. annulatus*, distributed in western Ethiopia near the Sudan border (Pegram *et al.*, 1981). Based on this and on the fact that no other species of *Boophilus* was identified during the study period, it is inferred that larvae and nymphs of *Boophilus spp* that were found in this study belong to *B. decoloratus*.

Specificity of ticks to different attachment sites of a host is a common behaviour in most species of ticks. The most abundant species in the study area, *A. variegatum*, was mostly found in abdominal sites. Due to this, the damage of udder and cases of severe myiasis, teat perforation and amputation, and cases of mastitis particularly due to bites by *A. variegatum* were recorded in the Sheko breed. Likewise, localized wound infections in the brisket areas were observed especially in the months of April and May in which *A. variegatum* was most abundant. Such wounds were observed to cause inability to move and temporary lameness of the forelimbs of the animals. *R. e. evertsi* was mostly found on delicate skins of the anus and vulva areas and severe infestation was observed to cause inflammation of these areas. *B. decoloratus* was mostly attached on the back and dewlap and were observed to cause inflammatory lesions on the attached sites. Moreover, the number of female *B. decoloratus* ticks was higher than the males, indicating the possible risk of maintaining a high tick

population on the animals.

The pattern of dispersal of ticks within their host population is useful for quantitative assessment of their impact on the host. Several studies reported that ticks tend to overdisperse within host populations, which means that some hosts have many ticks while others have only few ticks (Petney *et al.*, 1990). Such a skewed distribution does not allow for normal methods of statistical analysis due to the presence of high variation around the mean, which is usually reflected by higher magnitude of standard deviations than the means. In order to tackle the problem of overdispersion in such cases, it has been suggested that at least one of the following approaches must be used (Petney *et al.*, 1990):

- Carry out a preliminary survey to determine the presence of variability before starting the study;
- Ensure that the sample size is sufficiently large so that correct statistical tests could be performed or else, data should be replicated between years to determine seasonal variation;
- Use non-parametric statistical tests on direct count data or use parametric statistical tests on log or lan transformed data.

Since the present data exhibits overdispersion, transforming the data was found to be important necessary in order to have a clear picture of the statistical analyses that are performed for breed comparison.

Estimates of resistance can be obtained in the field by ranking cattle according to their relative tick burdens when grazing on infested pastures (Utech *et al.*, 1978; Spickett *et al.*, 1989). This approach was used in the present study. The other technique available is to experimentally infest animals with a known number of ticks and observe the numbers that successfully attach and engorge (Utech *et al.*, 1978). Both these methods have been widely

used in dealing with estimation of tick resistance and its heritability in several breeds of cattle. In the present study, based on the three parameters used for estimating resistance between breeds (i.e. Total tick count, fully engorged females and adult ticks), the Horro breed demonstrated a relatively high level of resistance against all the tick species with significantly lower mean tick counts than those of the other three breeds. In terms of fully engorged female ticks, Horro and Gurage breeds were found to have significantly lower count than Abigar and Sheko breeds. Previous studies made at Bako research centre on Horro, Boran and their crosses have also shown the Horro cattle to be more resistant than the others (Ali and de Castro, 1993).

Information on the resistance status of individuals within breed is important for it provides basis for selecting individuals with consistently low tick burden and for culling individuals with consistently high infesting ticks on them (Utech *et al.*, 1978). In this study, within breed variation was observed in Horro, Sheko and Abigar breeds with respect to the overall tick burden. In order to obtain improvement on the overall resistance of the herds, culling of the individuals that showed higher tick burden should be practised as part of the management programme in the study area.

Adaptation of a host to various ecological factors such as altitude, climate and vegetation have been shown to affect tick counts on the host (Ali and de Castro, 1993). The Abigar (Sanga subgroup), which were brought from Gambella and the adjacent eastern areas of South-western Ethiopia have shown high tick burden. According to tick surveys that were carried out between 1986-1989 in western Ethiopia (Illubabor, Keffa and Wollega), the geographical and ecological features of Gambella is different from Ghibe–Tolley area where the study animals have been kept (de Castro, 1994). Similarly, the Sheko cattle, which belong to humpless shorthorns (*Bos taurus*), were brought to the study area from warm and humid parts

of southwestern Ethiopia around the Bench-Maji zone. These areas have generally warm but wet climate and thus are different from the study area. The more adverse ecoclimatic conditions in Ghibe- Tolley might have predisposed Sheko and Abigar breeds to have high level of infestation.

The Horro cattle, which belong to Zenga (Zebu + Sanga) breed group, were brought from Anger Guten valley and Dimtu localities of Eastern Wollega. The ecoclimatic conditions of these areas are quite different from Ghibe/ Tolley area. Likewise the Gurage breed, which belongs to the Abyssinian Shorthorn Zebu subgroup, were brought from the Gurage and Hadiya areas, which are found in close proximity to the tsetse infested valleys of the tributaries of the Ghibe River. Despite this, the ecological features are not the same as that of the study area. Unlike the case of Abigar and Sheko breeds, effect of the ecological factors for Horro and Gurage breeds to harbour lower tick burden looks insignificant.

Seroprevalence results showed the presence of three tick-borne pathogens: *Theileria mutans*, *Babesia bigemina* and *Anaplasma marginale*. Retrospective data on of the clinical cases showed that there were cases with clinical signs such as weakness, loss of appetite and rough coat and their diagnosis showed the presence of *Theileria spp.* in blood smear but no other haemoparasites. Upon treatment of these cases with Oxytetracyclin recovery was observed. This indicates the presence of benign theileriosis in the animals. However, there is no evidence for the causative agent of such disease condition to be due to *T. mutans*. Further serological studies should be done with other *Theileria* species such as *T. orientalis*, which are present on areas adjacent to the study site. Furthermore, a strain of *T. mutans* (Aitong) is present in East Africa and is found to be pathogenic causing mild anaemia. The vector of this strain is *Amblyomma variegatum* (Kettle, 1990). Therefore, further serological screening tests should be performed against this specific strain in order to evaluate the pathogenicity of *T.*

mutans in the study animals.

The seroprevalence results of *A. marginale*, *T. mutans* and *B. bigemina*, as demonstrated by Norval *et al.* (1983), can be considered to be in the range of a situation approaching enzootic stability in the animal population. Moreover, the fact that *B. decoloratus* was the second most abundant tick species in the study area and that mortalities due to babesiosis were recorded in the animal population to which the study animals belonged with the typical post mortem findings of red water (haemoglobinuria), indicated the endemicity of *B. bigemina* in the study animals, as shown also in the present study.

The point seroprevalences for the tick- borne pathogens in each breed were variable in the four months. Although the reason for such variability is not immediately clear, the possible explanations could be either because of the presence of endemic stability in which the host immune system suppresses the parasite, or the cross- effect of trypanocidal drugs on the tick- borne pathogens.

During months of low tick burden (June - October), a marked rise in packed cell volume was observed indicating the effect of tick infestation in PCV level. However, significantly higher PCV values were found in Sheko cattle in January, June and October. Although the mechanism behind this phenomenon is unclear, assumptions can be made based on the previous findings that this breed is believed to have a characteristic for trypanotolerance at some level (DAGRIS 2002). This might be expressed by their ability to resist the development of severe anaemia (Trail *et al.*, 1993) while the other breeds were exposed to RBC destruction by trypanosomes together with the presence of tick-borne pathogens and blood intake by ticks. It can be speculated that during the specified months, the major determinant of anaemia in the area might be trypanosomiasis as the Sheko cattle showed

higher PCV values than the rest of the breeds. Another possible reason is that the PCV values could also be affected by other blood- sucking ectoparasites, haemoparasites as well as gastrointestinal parasites. Therefore, tick infestation cannot be considered as the sole factor affecting the PCV of the animals.

Testing the IgG response of the animals to the most important tick species, *A. variegatum* infestation, it was necessary to control for the presence/absence of cross-reactivity with transovarially transmitted tick-borne pathogens and antibodies that would react against the larval stages, to the adult ones. Studies on the lifecycle of tick-borne diseases and pathogens that are transmitted by *A. variegatum* (all species of *Theileria* and *Cowdria*) have indicated that transstadial (stage to stage) transmission is possible. Nevertheless, It has been shown that *Theileria spp.* cannot be transmitted transovarially (from the adult female to newly hatched larvae via the egg) (Walker, 1990). Earlier findings on the lifecycle of *Cowdria ruminantium* have also showed rare transovarian transmission in the vector *Amblyomma hebraeum*, which was not reported in *A. variegatum* (Camus *et al.*, 1996). Therefore, the crude antigens of *A. variegatum* prepared from the larvae can be considered free from antigens of tick-borne pathogens that may cause false-positive results as a result of cross-reactivity. The reliability of homogenates from unfed ticks as antigen was also reported by Smith *et al* (1989). Regarding specificity of the responses in different stages of ticks, Jongejan *et al* (1989) showed that in rabbits, successive infestation by nymphs of *R. appendiculatus* resulted in reduction in mean engorgement weights of adult *R. appendiculatus* ticks, suggesting that immunity to infestation induced by feeding ticks of one stage can confer resistance to other tick instars. Furthermore, Fivaz *et al* (1991) have demonstrated a significant reduction in engorgement weight of nymphal stages of *R. zambeziensis* by repeated infestation of rabbits with the adult ticks.

Animals develop resistance to ticks after natural infestation or after immunization with tick-derived antigens. Both antibody and cell-mediated immunity are involved but the degree of either immune response may depend on the host and the tick species (Smith, *et al.*, 1989). The present study revealed that the different breeds exhibit different magnitudes of antibody responses to tick infestations, which showed that the host immune response plays a partial protective role in acquisition of resistance. Infestation with *A. variegatum* in Horro cattle was significantly the lowest of all breeds, which showed that the pick-up rates of ticks in this breed were low. Although not significant, engorging female of *A. variegatum* ticks were highest in the Sheko cattle, which indicated that the degree of engorgement of ticks were larger than the other three breeds. Assessment of the magnitude of humoral immune responses also revealed that the level of antibody responses to *A. variegatum* was significantly the highest in Horro breed, but is weakly negatively correlated with the burden ($P>0.05$). The production of antibody in response to tick infestation has been reported in a number of tick- host associations. In early studies, Barriga (1991) observed that substances extracted from tick salivary glands stimulate antibody response in the host, suggesting the contribution of humoral immunity to tick resistance. Rechav (1991) also demonstrated the presence of negative correlation between mean weight of engorged female ticks and the level of serum gamma globulins in the host, suggesting the role of antibodies in bovine resistance to ticks. In contrast to our finding, studies on comparison of parasite burdens between N' Dama and Zebu cattle showed no significant difference in antibody levels to adult and nymphal *A. variegatum* antigens while positive correlation was observed between the level of eosinophils and resistance to tick infestation (Claxton and Leperre, 1991). Inverse relationship between resistance to *Ixodes ricinus* infestation and serum IgG levels in sheep was also observed, suggesting the shift of immune response to Th2 type for acquisition of resistance (Ogden *et al.*, 2002).

The results of this study suggest that among the four indigenous cattle breeds, the Horro breed shows resistance to tick infestations and the possible factors for the exhibited resistance include host immunological factors, specifically antibodies. Future studies on the determination of level of resistance of cattle to tick infestation should include other immunological parameters such as level of eosinophils and test for the presence of delayed-type hypersensitivity reactions on feeding sites of the ticks, since these factors have been considered to be related with host resistance to ticks. The effect of immunological factors on fertility of female ticks should also be assessed in terms of difference in engorgement weight, number of eggs produced and their hatchability. Moreover, long- term population dynamics study of the present tick species should be carried out in order to have a consistent rank of tick burden in the individual animals. It also helps to achieve in selection of individuals with factors for tick resistance.

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Appendix I Least square ANOVA of log transformed counts of *A.variegatum* in the four breeds of cattle

Multiple Comparisons

Dependent Variable: LGTOT

LSD

(I) breed code	(J) breed code	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
					Lower Bound	Upper Bound
Abigar	Gurage	.0195	.04660	.675	-.1008	.1398
	Horro	.1678*	.04943	.001	.0402	.2954
	Sheko	-.0204	.04576	.656	-.1385	.0978
Gurage	Abigar	-.0195	.04660	.675	-.1398	.1008
	Horro	.1483*	.04957	.003	.0204	.2763
	Sheko	-.0399	.04591	.385	-.1584	.0786
Horro	Abigar	-.1678*	.04943	.001	-.2954	-.0402
	Gurage	-.1483*	.04957	.003	-.2763	-.0204
	Sheko	-.1882*	.04878	.000	-.3141	-.0623
Sheko	Abigar	.0204	.04576	.656	-.0978	.1385
	Gurage	.0399	.04591	.385	-.0786	.1584
	Horro	.1882*	.04878	.000	.0623	.3141

Based on observed means.

*. The mean difference is significant at the .01 level.

**Appendix II Least Square ANOVA of Log Transformed counts of *B. decoloratus*
in the four breeds of cattle**

Multiple Comparisons

Dependent Variable: LGTOT

	(I) breed code	(J) breed code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Abigar	Gurage	.0962*	.04628	.038	.0054	.1871
		Horro	.1105*	.05078	.030	.0109	.2102
		Sheko	.0113	.04768	.813	-.0823	.1049
	Gurage	Abigar	-.0962*	.04628	.038	-.1871	-.0054
		Horro	.0143	.05321	.788	-.0901	.1188
		Sheko	-.0849	.05026	.092	-.1836	.0137
	Horro	Abigar	-.1105*	.05078	.030	-.2102	-.0109
		Gurage	-.0143	.05321	.788	-.1188	.0901
		Sheko	-.0992	.05443	.069	-.2061	.0076
	Sheko	Abigar	-.0113	.04768	.813	-.1049	.0823
		Gurage	.0849	.05026	.092	-.0137	.1836
		Horro	.0992	.05443	.069	-.0076	.2061

*. The mean difference is significant at the .05 level.

**Appendix III Least square ANOVA of log transformed engorged tick counts of
the major tick species collected during the study period (Jan-Oct
2002)**

Multiple Comparisons

Dependent Variable: LGFFFC

	(I) Tick code	(J) Tick code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	A. variegatum	A. cohaerens	.0653	.16443	.692	-.2595	.3901
		B. decoloratus	-.1301	.09684	.181	-.3214	.0612
		R.e.evertsi	.3269*	.10538	.002	.1187	.5350
		R. praetextatus	.1589	.13702	.248	-.1118	.4296
	A. cohaerens	A. variegatum	-.0653	.16443	.692	-.3901	.2595
		B. decoloratus	-.1954	.13970	.164	-.4714	.0806
		R.e.evertsi	.2616	.14575	.075	-.0263	.5495
		R. praetextatus	.0937	.17004	.583	-.2422	.4296
	B. decoloratus	A. variegatum	.1301	.09684	.181	-.0612	.3214
		A. cohaerens	.1954	.13970	.164	-.0806	.4714
		R.e.evertsi	.4570*	.05987	.000	.3387	.5753
		R. praetextatus	.2890*	.10609	.007	.0795	.4986
	R.e.evertsi	A. variegatum	-.3269*	.10538	.002	-.5350	-.1187
		A. cohaerens	-.2616	.14575	.075	-.5495	.0263
		B. decoloratus	-.4570*	.05987	.000	-.5753	-.3387
		R. praetextatus	-.1679	.11394	.143	-.3930	.0571
	R. praetextatus	A. variegatum	-.1589	.13702	.248	-.4296	.1118
		A. cohaerens	-.0937	.17004	.583	-.4296	.2422
		B. decoloratus	-.2890*	.10609	.007	-.4986	-.0795
		R.e.evertsi	.1679	.11394	.143	-.0571	.3930

*. The mean difference is significant at the .05 level.

Appendix IV Least Square ANOVA of *A. variegatum* counts on the respective predilection sites

Multiple Comparisons^a

Dependent Variable: LGTOT

	(I) Prdeiliction site code	(J) Prdeiliction site code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	HEAD	DEWLAP	-.2940*	.10634	.006	-.5027	-.0853
		VENTRE	-.5577*	.09462	.000	-.7434	-.3721
		BACK	-.1223	.10651	.251	-.3313	.0867
		HOOF	-.2016*	.09911	.042	-.3961	-.0071
		TAIL	.3345	.18765	.075	-.0338	.7028
		ANOVULVA	.0746	.11208	.506	-.1454	.2946
	DEWLAP	HEAD	.2940*	.10634	.006	.0853	.5027
		VENTRE	-.2637*	.05646	.000	-.3745	-.1529
		BACK	.1717*	.07470	.022	.0251	.3183
		HOOF	.0925	.06370	.147	-.0326	.2175
		TAIL	.6285*	.17160	.000	.2917	.9653
		ANOVULVA	.3686*	.08245	.000	.2068	.5304
	VENTRE	HEAD	.5577*	.09462	.000	.3721	.7434
		DEWLAP	.2637*	.05646	.000	.1529	.3745
		BACK	.4355*	.05678	.000	.3240	.5469
		HOOF	.3562*	.04125	.000	.2752	.4371
		TAIL	.8922*	.16460	.000	.5692	1.2153
		ANOVULVA	.6324*	.06665	.000	.5016	.7632
	BACK	HEAD	.1223	.10651	.251	-.0867	.3313
		DEWLAP	-.1717*	.07470	.022	-.3183	-.0251
		VENTRE	-.4355*	.05678	.000	-.5469	-.3240
		HOOF	-.0793	.06398	.216	-.2048	.0463
		TAIL	.4568*	.17171	.008	.1198	.7938
		ANOVULVA	.1969*	.08267	.017	.0347	.3592
	HOOF	HEAD	.2016*	.09911	.042	.0071	.3961
		DEWLAP	-.0925	.06370	.147	-.2175	.0326
		VENTRE	-.3562*	.04125	.000	-.4371	-.2752
		BACK	.0793	.06398	.216	-.0463	.2048
TAIL		.5361*	.16722	.001	.2079	.8642	
ANOVULVA		.2762*	.07288	.000	.1331	.4192	
TAIL	HEAD	-.3345	.18765	.075	-.7028	.0338	
	DEWLAP	-.6285*	.17160	.000	-.9653	-.2917	
	VENTRE	-.8922*	.16460	.000	-1.2153	-.5692	
	BACK	-.4568*	.17171	.008	-.7938	-.1198	
	HOOF	-.5361*	.16722	.001	-.8642	-.2079	
	ANOVULVA	-.2599	.17522	.138	-.6038	.0840	
ANOVULVA	HEAD	-.0746	.11208	.506	-.2946	.1454	
	DEWLAP	-.3686*	.08245	.000	-.5304	-.2068	
	VENTRE	-.6324*	.06665	.000	-.7632	-.5016	
	BACK	-.1969*	.08267	.017	-.3592	-.0347	
	HOOF	-.2762*	.07288	.000	-.4192	-.1331	
	TAIL	.2599	.17522	.138	-.0840	.6038	

*. The mean difference is significant at the .05 level.

a. Tick code = *A. variegatum*

Appendix V Least Square ANOVA of *B. decoloratus* counts on the respective predilection sites

Multiple Comparisons ^a

Dependent Variable: LGTOT

	(I) Prdeiliction site code	(J) Prdeiliction site code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	HEAD	EAR	-.1909*	.08280	.021	-.3534	-.0284
		DEWLAP	-.3247*	.07814	.000	-.4781	-.1714
		VENTRE	-.2543*	.07660	.001	-.4046	-.1040
		BACK	-.3722*	.07656	.000	-.5225	-.2220
		HOOF	-.0203	.09504	.831	-.2068	.1663
		TAIL	-.1883*	.09504	.048	.0018	.3748
		ANOVULVA	.3047*	.11262	.007	.0837	.5257
		ANOVLVA	.3047*	.11262	.007	.0837	.5257
	EAR	HEAD	.1909*	.08280	.021	.0284	.3534
		DEWLAP	-.1339*	.06001	.026	-.2516	-.0161
		VENTRE	-.0634	.05799	.274	-.1772	.0504
		BACK	-.1814*	.05794	.002	-.2951	-.0676
		HOOF	-.1706*	.08080	.035	.0120	.3292
		TAIL	.3792*	.08080	.000	.2206	.5378
		ANOVULVA	.4956*	.10089	.000	.2976	.6936
		ANOVLVA	.4956*	.10089	.000	.2976	.6936
	DEWLAP	HEAD	.3247*	.07814	.000	.1714	.4781
		EAR	.1339*	.06001	.026	.0161	.2516
		VENTRE	.0704	.05111	.169	-.0299	.1707
		BACK	-.0475	.05106	.352	-.1477	.0527
		HOOF	.3045*	.07601	.000	.1553	.4537
		TAIL	.5131*	.07601	.000	.3639	.6622
		ANOVULVA	.6294*	.09710	.000	.4389	.8200
		ANOVLVA	.6294*	.09710	.000	.4389	.8200
	VENTRE	HEAD	.2543*	.07660	.001	.1040	.4046
		EAR	.0634	.05799	.274	-.0504	.1772
		DEWLAP	-.0704	.05111	.169	-.1707	.0299
		BACK	-.1179*	.04866	.016	-.2134	-.0224
HOOF		.2340*	.07442	.002	.0880	.3801	
TAIL		.4426*	.07442	.000	.2966	.5887	
ANOVULVA		.5590*	.09586	.000	.3709	.7471	
ANOVLVA		.5590*	.09586	.000	.3709	.7471	
BACK	HEAD	.3722*	.07656	.000	.2220	.5225	
	EAR	.1814*	.05794	.002	.0676	.2951	
	DEWLAP	.0475	.05106	.352	-.0527	.1477	
	VENTRE	.1179*	.04866	.016	.0224	.2134	
	HOOF	.3520*	.07439	.000	.2060	.4980	
	TAIL	.5605*	.07439	.000	.4146	.7065	
	ANOVULVA	.6769*	.09583	.000	.4888	.8650	
	ANOVLVA	.6769*	.09583	.000	.4888	.8650	
HOOF	HEAD	.0203	.09504	.831	-.1663	.2068	
	EAR	-.1706*	.08080	.035	-.3292	-.0120	
	DEWLAP	-.3045*	.07601	.000	-.4537	-.1553	
	VENTRE	-.2340*	.07442	.002	-.3801	-.0880	
	BACK	-.3520*	.07439	.000	-.4980	-.2060	
	TAIL	.2086*	.09330	.026	.0255	.3917	
	ANOVULVA	.3250*	.11115	.004	.1068	.5431	
	ANOVLVA	.3250*	.11115	.004	.1068	.5431	
TAIL	HEAD	-.1883*	.09504	.048	-.3748	-.0018	
	EAR	-.3792*	.08080	.000	-.5378	-.2206	
	DEWLAP	-.5131*	.07601	.000	-.6622	-.3639	
	VENTRE	-.4426*	.07442	.000	-.5887	-.2966	
	BACK	-.5605*	.07439	.000	-.7065	-.4146	
	HOOF	-.2086*	.09330	.026	-.3917	-.0255	
	ANOVULVA	-.1164	.11115	.295	-.1018	.3345	
	ANOVLVA	-.1164	.11115	.295	-.1018	.3345	
ANOVULVA	HEAD	-.3047*	.11262	.007	-.5257	-.0837	
	EAR	-.4956*	.10089	.000	-.6936	-.2976	
	DEWLAP	-.6294*	.09710	.000	-.8200	-.4389	
	VENTRE	-.5590*	.09586	.000	-.7471	-.3709	
	BACK	-.6769*	.09583	.000	-.8650	-.4888	
	HOOF	-.3250*	.11115	.004	-.5431	-.1068	
	TAIL	-.1164	.11115	.295	-.1018	.3345	
	ANOVLVA	-.1164	.11115	.295	-.1018	.3345	

*. The mean difference is significant at the .05 level.

a. Tick code = B. decoloratus

Appendix VI Least square means of the burden of fully engorged females, adult and total tick counts in the four breeds (analysis done on log transformed values of the parameters)

		Horro	Gurage	Abigar	Sheko	Total
Fully fed females count	Mean*	0.24 ^a (0.26)	0.34 ^{ab} (0.25)	0.47 ^{bc} (0.42)	0.53 ^c (0.34)	0.43 (0.36)
Adult tick count	Mean**	0.63 ^a (0.42)	0.72 ^b (0.49)	0.76 ^{bc} (0.50)	0.80 ^c (0.49)	0.73 (0.48)
Total count	Mean***	0.74 ^a (0.48)	0.80 ^{ab} (0.51)	0.82 ^b (0.52)	0.85 ^b (0.52)	0.80 (0.51)

*P< 0.05

**P< 0.01

***P< 0.001

^{a, b, c} Within variable row, means with different superscripts are significantly different at P< 0.05

Appendix VII Least square ANOVA of IgG levels against *A. variegatum* ticks among the four breeds of cattle

Dependent Variable: OD values

LSD

(I) BREED	(J) BREED	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Abigar	Gurage	-.04629	.050931	.364	-.14663	.05404
	Horro	-.32463*	.050931	.000	-.42496	-.22429
	Sheko	-.01737	.050931	.733	-.11770	.08297
Gurage	Abigar	.04629	.050931	.364	-.05404	.14663
	Horro	-.27833*	.050931	.000	-.37867	-.17800
	Sheko	.02893	.050931	.571	-.07141	.12926
Horro	Abigar	.32463*	.050931	.000	.22429	.42496
	Gurage	.27833*	.050931	.000	.17800	.37867
	Sheko	.30726*	.050931	.000	.20692	.40759
Sheko	Abigar	.01737	.050931	.733	-.08297	.11770
	Gurage	-.02893	.050931	.571	-.12926	.07141
	Horro	-.30726*	.050931	.000	-.40759	-.20692

*. The mean difference is significant at the .05 level.

**Appendix VIII Eco-climatic characteristics of natural habitats of the four breeds
and that of the study area**

(Source: de Castro, 1994)

Locality	Altitude (m)	Annual rainfall	Vegetation	Remark
Gambella	400-600	1200-1400	Grasslands	Trypanosomiasis endemic
Ghibe	1200-1400	1000-1200	Coniferous forest	Trypanosomiasis endemic
Nekemte	2200-2400	1600-1800	Broadleaf forest	-
Sheko	1600-1800	2200-2400	Broadleaf forest	-
Tolley	1400-1600	1200-1400	Broadleaf forest	-

