STUDIES ON THE IMMUNE RESPONSE TO *AMBLYOMMA VARIEGATUM* IN CATTLE AND THE EFFECTS OF HAEMOPARASITISM ON THE ACQUISITION OF TICK RESISTANCE

A THESIS

SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE OF THE FACULTY OF AGRICULTURE, UNIVERSITY OF GHANA, LEGON IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(VETERINARY ENTOMOLOGY)

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DECLARATION

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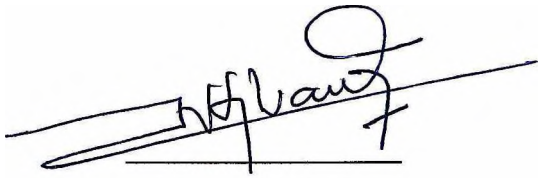

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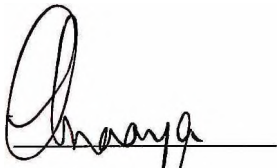

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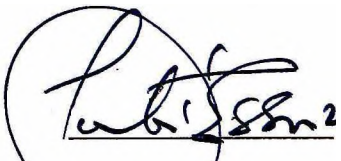

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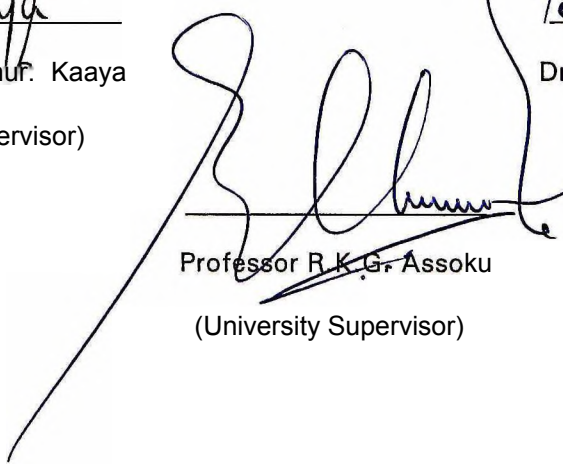

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ABSTRACT

Acaricides in tick control, had been thought to be in Africa the panacea for over a century. Environmental and economic constraints, development of acaricide-resistant strains due to acaricide misuse, have brought about the need for an alternative tick control strategy. The use of tick resistant cattle has then been advocated and echoed in the USA and Australia where it got its full expansion. In Africa, few works have been undertaken on tick resistance despite the fact that many tick species are found on the continent. Studies on acquisition of immunity to tick and especially to *A. variegatum* in Africa are very scarce so several questions need to be answered before the use of such a method.

Therefore, an attempt was made to shed some lights on the tick-cattle interactions and on some of the factors which could influence the acquisition of resistance. We have chosen the Boran cattle-A *variegatum* model. That model was compared to those of *A. variegatum*-Ayrshire cattle and *A. variegatum-crossbred* Friesian x Boran. The resistance was artificially induced by applying simultaneously at one month interval, 100 nymphs on one ear and 20 males and 20 females on the other ear. At the beginning of the experiment the average age of the 20 animals was one year.

For the first time, the status of acquired resistance was assessed with a multivariate analysis method. The principal component analysis (PCA) and fastclus procedure (SAS/STAT) were performed on the variables with high loads. It was noticed after the first infestation that, all the biological

parameters favoured tick survival. The percentage engorged (PENGD), the percentage dead (PDEAD), the feeding period (FPR), the engorgement weight (ENGWT), the percentage that moulted (PMLTD) and the percentage that engorged above critical weight (PEACWT), with loads > 0.45 proved to be the indicators for the resistance to nymphs and adults. Three groups (high, moderate, low) of resistance have been defined using the fastclus procedure. Each group has been subdivided into three lots with two individuals each. The resistance dynamic was assessed using the ANOVA procedure. It was observed with the Boran cattle that resistance to the nymphs was already induced from the third infestation. Resistance to adults did not show a defined trend. A decline in the immunity level to nymphal and adult stage was observed from the 4^{th} and 5^{th} infestation.

In order to assess the effects of trypanosomosis or babesiosis on the acquired immunity, the tick-immune animals from the above lots were infected separately with either 1 ml of 10^7 blood stream *T. congolense* or with 150 ml of infective blood from an immunosuppressed donor calf bearing 10% *B. bigemina* parasites. The same number of ticks was applied, in the same manner at the height of parasitaemia. There was a significant decrease in the PENGD, ENGWT, PMLTD, PEACWT, and a significant increase in the PDEAD and the FPR which indicated an increase in the resistance status. Acquired resistance to *A. variegatum* could also be demonstrated in the pure-bred *Bos taurus* Ayrshire as well as in the crossbred *Bos indicus* (Boran) x *Bos taurus* (Friesian) cattle, after four to five successive infestations though some individual variations could also be noticed.

Comparison of the resistance acquired by the Boran bleed to the one

acquired by crossbred Friesian x Boran and Ayrshire revealed that, the crossbred acquired significantly higher immunity than the other two breeds after the first two infestations. Although relatively lower, the resistance acquired by the Ayrshire was not significantly different from that of the Boran.

The profile of gel filtration chromatography of the tick-immune serum obtained from the Boran cattle showed 3 peaks corresponding to IgM, IgGs, and albumins. The IgGs run through affinity chromatography using protein A-Sepharose 4B^{R}, showed two peaks corresponding to the IgG1 and IgG2 fractions. The silver-stained profile revealed the heavy and light chains respectively around 54kDs and 29kDs while the IgM fraction showed three different bands of 77kDs, 69kDs and 29kDs.

SDS-PAGE gels of saliva and salivary gland homogenates, sequentially prepared from the ticks, showed that different molecules were injected into the host during attachment and feeding. Several molecules were recognized by the whole tick-immune serum as well as by the IgG1 and IgG2 obtained after fractionation and purification through gel filtration chromatography and gel affinity chromatography and the immunoblotting procedure.

Quantification of the antibodies implicated in the acquired immunity by indirect and direct ELISA showed that IgG1 was present and correlated with some of the parameters used to assess the acquisition of resistance. A higher host antibody concentration was observed after the sixth infestation when ticks were fed on the haemoprotozoan-infected animals. The IgM concentration remained unchanged during the infestations denoting its passive role in the mechanism of acquired immunity.

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CHAPTER ONE

INTRODUCTION

Ticks have been known and recognized as living things since biblical times (Shaw, R.D.; Thorburn, J.A. and Wallace, H.G., 1976). They are widely distributed throughout the world and particularly in the tropical and subtropical countries. It has been estimated that 80% of the world's 1,226 million cattle are infested with ticks (FAO, 1984). The ixodid ticks or hard ticks with 9 major genera *{Amblyomma, Boophilus, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Margaropus, Nosomma* and *Rhipicephalus)* supersede all other tick genera in economic importance. The majority of hard ticks are of veterinary importance. Among the soft ticks, one of the most veterinary important is *Argas persicus* parasite of man, bats and birds. Their importance as vectors of livestock debilitating-disease organisms was realized when it became necessary to increase the world livestock products to satisfy the needs of increasing human population living in the industrial areas. Cattle had to be moved into tick-infested areas where many died of tick burden and tick-borne diseases. Besides their importance as vectors of diseases, ticks cause other direct damages to livestock. Morel (1981) documented that a single *Amblyomma variegatum* tick can cause a loss of 2 ml of blood and a loss of productivity due to the diversion of nutrients and energy ingurgitated by the hosts. Sutherst et al. (1983), also documented that the daily loss by a cow due to an engorged *Boophilus microplus* female can reach 1,52g of weight. Due

to this double importance a lot of effort has been put into their control. Several methods have been used to fight the tick attacks.

Chemical control has been used since the turn of this century by cattle farmers in tick endemic areas as major means of control. These chemicals are often used in dipping tanks, spray machines (races or arches) and by hand spraying without any rigorous control measures. To this onslaught, the ticks have responded by developing resistance to different groups of chemicals (cross resistance phenomenon) (FAO, 1982; Matthewson, 1984). The cost of acaricide and labour, the increased cost involved in the development, testing and marketing of a new product, the rapidity with which resistance has developed against new acaricides in recent years and finally the chemical hazards in the environment, all create serious difficulties in the long term usage of chemicals to kill or control ticks (Latif, 1986). To avoid the tick resistance problem to whatever acaricide has been formulated alternative methods have been developed: pasture spelling in Australia, habitat modification, genetic control, use of chemical baited pheromones, anti-tick grasses and legumes such as *Stytosanthes sp.* and parasitoids *(Ixodephagus hookeri)* have been attempted. Among many of the control measures developed all over the world, the use of tick-resistant cattle has been given a considerable attention in Australia. A selection programme designed to develop a breed resistant to cattle tick *Boophilus microplus* was initiated in the sixties and seventies. Highly-resistant bulls were selected for production. In Africa, reports indicate that the

local breeds *(Bos indicus)* acquire a more effective natural resistance to tick infestation than the exotic ones *(Bos taurus)* (Latif, 1984a; 1984b; Norval, 1986).

An important question which is yet to be answered is whether, on a long term basis, natural resistance can control ticks absolutely. The question has arisen because it is felt that ticks should have been eliminated from Africa if the phenomenon of natural resistance was absolute. There has been no investigation whatsoever to answer this important question. ICIPE's Livestock Tick Research Programme at Rusinga Island in Kenya (Latif, unpublished data) indicates that the eventual outcome of natural resistance on a long term basis is the stabilization of tick population on the animal and in the field. This in itself suggests a non-absolutism of natural resistance in controlling ticks and a need to study those factors which hinder natural resistance from achieving absolute control of ticks.

This information is important in Africa where indigenous cattle keepers had relied mostly on natural resistance to ticks for several decades, in Africa where multiple parasitic infections in livestock are the normal situation, it is necessary to ascertain whether the stress exerted by infections is a factor to the sustainability of natural resistance against ticks. Indeed, in many tropical countries, animals including domestic livestock are under constant challenge by pathogens, some of which are tick-borne, and by ixodid ticks themselves.

1.1 Justification of study objectives

Remarkable advances have been made within the last decade in research on the feeding habits and pattern of tick infestations as well as their diurnal and seasonal activities. However, little is known about tick embryogenesis, development and survivability under natural conditions, tick population modelling, pheromone and biochemical studies, field sampling techniques and the phenomenon of natural host resistance to tick infestation (Dipeolu, 1989). The justification of these following studies lies in the above statement.

1.2 Study objectives

1.2.1 Aim

The aim of this investigation was to elucidate the effects of infections with parasites such as *Trypanosoma* and *Babesia* on the sustainability of natural resistance against tick infestation. Knowing the effects of *Trypanosoma congolense,* and *Babesia bigemina* infections a correct timing of their treatment may notably influence the acquisition and the sustainability of natural resistance against ticks. A comprehensive knowledge of this phenomenon may throw some light on the factors which should be considered in cattle vaccination against ticks.

1.2.2 Objectives

To achieve our goals we were to pursue three objectives:

(a) To monitor the resistance in different breeds of cattle against *Amblyomma variegatum* after repeated infestations using the biological parameters of Dipeolu (1990 and Dipeolu et al. (1992). (See details of parameters in methodology)

(b) To establish the correlation between the resistance status and the immunoglobulin at the isotype level.

(c) To monitor the resistance dynamics in the *T. congolense* and *B.* b/gem/na-infected resistant cattle.

CHAPTER TWO

LITERATURE REVIEW

2.1 The ticks

2.1.1 Classification of ticks

Ticks are eight-legged arthropods belonging to the phylum Arthropoda and to the class Arachnida which consists of twelve orders. Among these twelve orders, the order Acarina comprises ticks and mites. They are obligate parasites and mostly live on both invertebrate and vertebrate animals. Cheng (1973) grouped the arthropod ticks under the suborder ixodoidea and subdivided them into three families: Argasidae or soft-bodied ticks, Ixodidae or hard-bodied ticks and Nuttalliellidae with one free living species.

Morel (1981) distinguished 3 superfamilies i.e Ixodoidea with the Amblyommidae and Ixodidae families, Argasoidea with one family (argasidae), and the superfamily of Nuttalliellidea with also one family, the Nuttalliellidae. FAO (1984), grouped the ticks in the suborder Metastigmata and the superfamily Ixodoidea which it subdivided into three different families: Argasidae, Ixodidae and Nuttalliellidae. Regardless of the author, ticks can broadly be divided into two groups: ixodid ticks with more than 650 species and argasid ticks with more than 170 species (FAO,1984), 1982). Members of the ixodid tick group have a dorsal scutum which covers the entire dorsal surface

as far as the males are concerned and partly in female species. Argasid ticks lack scutum but possess leathery integument. The majority of ticks of veterinary importance belong to the ixodid ticks and include *Haemaphysalis sp., Rhipicephalus sp., Dermacentor sp., Hyalomma sp., Boophilus sp, Ixodes sp.* and *Amblyomma sp.* (Hoogstraal, 1956).

2.1.2 Life cycle of ticks

Tick life cycle is characterized by three or more stages: eggs, larvae, nymphs and adults in case of hard-bodied ticks. With soft-bodied ticks one or more stages of preadults come in between nymph and adult stages. To keep to our subject, attention will only be focussed on ixodid ticks. Single large egg masses are laid in natural shelter (under stones, decaying matter) by engorged and detached females ixodid ticks. Depending upon the species the number of eggs can reach 1000 to 15000 (Morel, 1981). The egg laying operation is followed by the death of the female. Eggs hatch into six legged larvae which either actively look for a host or await in a resting place for a passing host. If the host is found, the larvae feed on it and drop off and then moult into eight-legged nymphs on the ground. The nymph undertakes the same activities as described above for the larva and moults into an eight-legged adult. According to their life cycle, ticks can be divided into three categories.

2.1.2.1 One host-tick life cycle

A remarkable example of this group is *Boophilus* species. Newly moulted nymph and adult remain on the host throughout the feeding and moulting phases of their life cycle. This is a successful adaptation which enables the members of this group to exist in harsh environments.

2.1.2.2 Two host-tick life cycle

This group comprises a few *Hyalomma* species eg. *H. detritum detritum* species and some *Rhipicephalus* eg. *R. bursa* species. The fed larva moults on the host to nymph which quickly recommences feeding. The engorged nymph detaches, drops off and moults to adult stage which then seeks for a final host.

2.1.2.3 Three host-tick life cycle

About 600 of the approximately 650 ixodid species have a three-host life cycle (FAO, 1984). The most common genera of this group in sub Saharan Africa are: *Rhipicephalus, Amblyomma* (*Amblyomma hebraeum* and *Amblyomma variegatum*), *Hyalomma (Hyalomma analolicum anatolicum).* Their life cycle comprises 3 stages: larva, nymph and adult. The mean life span of *Amblyomma variegatum* larvae and adults in laboratory conditions according to Voutoulou (1987) are 202 and 749 days respectively. *A.*

variegatum female feeds on naive susceptible animal for 12 to 15 days. The mean length of oviposition is 28 days (Dipeolu and Ogunji, 1980). The egg laying operation is preceded by a preoviposition period which, according to Voutoulou (1987) at 27° C, 84% R.H. is 11.35 ±1.517 days. After 50 to 53 days eggs eclode into larvae which seek for hosts and feed for 5 to 6 days, then drop off the hosts after repletion. The moulting period of larvae to nymphs is 15 to 20 days. The newly moulted nymph feeds for 6 to 8 days, drops off and moults into adult 12 to 15 days later. The whole cycle of *A. variegatum* from eggs to adults is 3 months.[1]

2.2 Economic importance

2.2.1 Direct damages

2.2.1.1 In animal productivity

According to Sutherst (1987) the breakdown of the economic losses caused by the cattle tick can be as follows:

increased labour costs	=	36%
loss of beef	=	20%
loss of dairy production	=	16%
loss by death	=	11 %
hide damage	=	5%
increased draught loss	=	5%

[1]Data on *A. variegatum* life cycle not referenced were obtained from the ICIPE Tick Rearing Unit.

2.2.1.2 Illness due to tick feeding per se

Paralysis from tick feeding on animals were documented by Gothe and Hoogstraal (1979). Cattle paralysis due to the feeding of *Dermacentor andersoni,* sheep and rabbit paralysis due to *R. appendiculatus R. evertsi evertsi* and *Ixodes rubicundus* infestation were recorded respectively by (Morel, 1981; Latif, 1986; Njau, 1985).

An exceptional mortality rate of 75% due to sweating sickness in calves and sheep resulting from *H. truncatum* infestations was reported by Morel (1981) in South Africa. Amblyomma feeding sites are prone to myiasis, *Dermatophilus* infections and toxicosis (Morrow et al.. 1989).

2.2.2 Indirect damages

This aspect has overshadowed all the above pathological changes brought about by ticks in their hosts. Ticks are carriers and transmitters of disease-causing organisms. Three of them are of major importance in Africa: *Theileria sp, Babesia sp* and *Cowdria sp.* In East Africa theileriosis or East Coast Fever (ECF) is considered the most significant and economically important among the tick-borne diseases. *Theileria parva parva* and *T. p. lawrencei,* causative agents of ECF, transmitted either by *R. appendiculatus*

and *R. evertsi evertsi* can kill about 40% of calves born in enzootic areas (Anonymous, 1974).

Bovine babesiosis or red water caused by intra-erythrocytic parasites of genus *Babesia* transmitted by *Boophilus sp* is one of the most important cattle diseases in the tropical and subtropical areas of the world. In Mexico, Hernandez-Ortiz et aL (1989) stated that babesiosis was responsible for loss of more than 100 million US dollars per year whereas de Vos and Every (1981) estimated the number of animals killed per year in South Africa as 8000. In other parts of Africa losses due to babesiosis have not yet been quantified in terms of income for livestock keepers but it can be estimated at 20% (Morel, 1981).

The third major tick transmitted disease in the tropics is rickettsiosis or heart water which infects cattle and sheep. The aetiological agent is *Cowdria ruminatium* transmitted by *Amblyomma variegatum, A. lepidum,* and *A. astrian* (FAO, 1984).

2.3 Tick Control

Control of ticks is of great importance to the entire world and especially for Africa where thousands of European breeds have been introduced in order to boost milk and beef production. Several methods have been used to control cattle ticks.

2.3.1 Chemical control

Tick control has relied mainly on acaricides. Several different chemical groups such as plant-extracts (Rotenone), synthetic molecules (Organochlorines, organophosphates and pyrethroids). Mixtures of some of these chemical groups ie. arsenic with rotenone and organophosphate; organophosphate and organochlorines; amidines and pyrethroids have also been used. Don Fronk (1985), according to the mode of action classified insecticides into four groups:

-physical poisons eg. oil, killing through physical actions (exclusion of pests from atmospheric oxygen);

-protoplasmic poisons eg. arsenic killing by precipitating the insect proteins;

-metabolic inhibitors eg. rotenone which interfere with the normal insect metabolism;

-nerve poisons: this group comprises most of the modern insecticides. They either kill by inhibiting enzymes such as cholinesterase (Organophosphates) or by affecting the permeability of the nerve membranes (Organochlorines and Pyrethroids). The acaricide misuse (lack of correct concentration, application, interval of usage and thoroughness of treatment) has favoured the rapid development of resistance. The resisted chemicals may no longer be used for a period (Matthewson, 1984). Return to their use at a later stage is not possible as resistance is again quickly expressed with control failure. Resistance originates in an area as a result of the expression of the requisite genes. Success in chemical control relies on the ingenuity of the synthetic

chemist to release on the market from time to time effective compounds to counter resistance. However, the development of a new insecticide estimated by Campell and Shugart (1985) is 25 to 30 million dollars. F.A.O. (1982) reported that chemical resistance was developped by *A. hebraeum* to arsenic in 1900 in (South Africa and Zimbabwe) and later to D.D.T in 1946. Resistance was later developped by the same tick against toxaphene-BHC-Dieldrin group in 1947, organophosphates in 1955 and amidine in 1971. Acaricide resistance in other parts of Africa needs to be studied and updated. Acaricides are poisonous to mammalians and should be handled with great care because of the acute oral toxicity, acute dermal toxicity, inhalation toxicity and subacute or chronic feeding toxicity. Toxicity varies according to the acaricide residue, the formulation and other abiotic factors. Residues on crops and in meat favour chronic poisoning as well as improper use of acaricides may upset the insect balance metabolism without killing the insects. Some chemicals are very destructive to predators and parasites of insect pests without being particularly effective against the pest. Acaricide misuse may adversely kill the pollinating insects resulting in poor fruit or seed set. Certain chemicals (chlorinated-hydrocarbons) exhibit a phenomenon known as biological magnification: a proportion of the chemical is stored in the fat of the animal that consumes the insecticide-contained organism. Top-placed animals in the food chain accumulate sufficient amount of the chemical as to interfere with their normal body functions. Insecticides may have deleterious effects on plants or accumulates in the soil to such an extent that it becomes impossible to grow plants. In order to reduce the environmental side effects and

counteract the resistance phenomenon, new approaches to tick control have been developed.

2.3.2 Alternative methods of tick control

2.3.2.1 Pasture spelling.

The whole idea is to prevent or sufficiently delay host finding by the ticks so that they die through dehydration. It was first used in Australia in 6. *microplus* control. Pastures were kept unstocked until the majority of *B. microplus* tick larvae had died (Wharton et al., 1969).

2.3.2.2 Pheromones

The use of pheromones baited with acaricides has been experimented with the genus *Amblyomma* but the species specificity and its short residual effect, have limited its application (Rechav and Whitehead, 1981).

2.3.2.3 Anti-tick vegetation

This control measure has been used in Australia. Some highly productive, nutritious varieties of the tropical pasture legumes *Stylosanthes sp.* and some grasses *{Andropogon sp.)* are covered with glandular trichomes or hairs which secrete viscous fluid. These sticky secretions immobilize larvae of *B. microplus* and *A. variegatum* (Sutherst and Wilson, 1986; Sutherst et al..

1982 and Zimmermann et aL, 1984). By preventing tick larvae to ascend stems, grasses, the plants trap the ticks and kill them. The ability to trap is related primarily to the density and the length of bristles on the stems rather than the degree of stickiness (Sutherst et al„ 1988).

2.3.2.4 Other control methods

The use of parasitic wasps *(Ixodephagus hookeri)* of ticks has been tried in the USA without conclusive results (Matthewson, 1984) but Mwangi et al. (1991), documented that /. *hookeri* could successfully be used in an integrated tick management programme as a biological agent of the programme.

2.3.2.5 Tick resistant animals

The utilization of host resistance to tick infestation as an alternative measure for tick control deserves careful consideration. It was observed early this century that different breeds of cattle tend to carry different numbers of ticks, *B. microplus* after one or more infestations (Johnston and Bancroft, 1918). Following reports on acquired immunity to ticks by Trager (1939), the first laboratory investigations were made by Kelly (1943) and Bonsma (1944). The development of procedures to immunomanipulate the potential host and render them resistant to tick infestation can only be achieved after a thorough understanding of the immunological aspects of the tick-host association. This was reviewed by Wikel and Allen (1982).

Resistance of cattle to tick infestation has been reported to have two components i.e innate and acquired resistance, with animals of innate resistance producing offsprings of similar resistance (Brown, 1985). Indeed, Kelly (1943), Riek (1962) and Francis and Little (1964), reported that *Bos indicus* cattle are innately more resistant to tick infestation than *Bos taurus,* with a wide range of resistance occurring in all herds but expressed more strongly in zebu cattle than their crosses (Riek, 1962). However Wagland (1975) strongly disagreed with the concept of innate resistance. However the use of resistant hosts by Australian workers as a result of tick infestation, received particular attention (Utech et aJ., 1978a; 1978b; Wikel and Allen, 1982).

In Africa, Latif (1984a; 1984b) found that crossbred cattle (*Bos taurus* x *Bos indicus)* carried four and half times more ticks than *Bos indicus* Kenana and Butana cows in the Sudan. He recorded that the weight at which fully-engorged female ticks feeding on Kenana cows detached was significantly lower than the weight of those fed on the crossbreds. The number of eggs laid by ticks fed on Kenana was also significantly lower. These findings were corroborated by de Castro (1985) with the East African *Bos indicus* Boran breed and by Norval (1986) in Zimbabwe with indigenous and exotic cattle tick against *R. appendiculatus* ticks. Following cumulative tick counts, Mattioli et aL (1993), Mattioli et al. (1995) in the Gambia, showed that in field conditions *A. variegatum* and *Hyalomma sp* tick burdens on *Bos taurus* N'dama cattle were fewer that those feeding on local Zebu and Zebu x N'dama crossbreds.

George et a|. (1985) observed that in pure-and crossbreds' resistance against A *americanum* after one infestation resulted in reduced significant weight. Jongejan et aL (1989), reported an acquired immunity to A *variegatum* in cattle, in which engorgement weights of female ticks at the 4th successive infestation were even greater than those obtained during the first feeding. Latif et a{. (1988) found that host resistance in rabbits against *A. variegatum* was manifested by strong premature female feeding inhibition, female mortality or pathologic reproduction.

The expression of resistance to ticks according to Rechav and Dauth (1987) varies greatly depending on the type of host and species of ticks. According to most of the reports, the resistance to the ticks *B, microplus, A. hebraeum, A. variegatum* and *H. a. anatolicum*, does not follow a straight and a definite pattern. Dipeolu et al. (1992) found that in Africa the degree of acquired resistance by Friesian cattle to *R. appendiculatus* larvae, nymphs and adults under varying conditions, rises after a number of infestations, stabilizes and then decreases. It later rises after challenge.

Adamson et al. (1991), after three repeated infestations in goats with *A. hebraeum* observed an acquired resistance associated with immediate hypersensitivity type I. Acquired resistance in sheep has been demonstrated against *A. americanum* by Barriga et al. (1991).

Concerning resistance to ticks by laboratory animals, Rubaire-Akiki and Mutinga (1980) found that rabbits infested with *Ft. appendiculatus* developed resistance. They stated this might be due to the host immunological reactions of immediate hypersensitivity types I and III in conjunction with the host non-specific and probably innate physiopathological reactions. Infestations with nymphs of *A. variegatum* and *A. hebraeum* on laboratory animals conferred resistance after a second infestation (Heller-Haupt et al., 1981). Dipeolu and Harunah in 1984 found that rabbits exposed to larvae, nymphs and adults of *A. variegatum* acquired resistance to the infestation with adults of this tick. The degree of acquired resistance was lowest in the rabbits exposed once previously to larvae and highest in those exposed twice previously to adults. However, Norval (1978) failed to demonstrate acquired resistance of rabbits to larvae and nymphs of *A. hebraeum,* after repeated infestations, although he found some wide individual differences in yield and great variations in mean engorgement weights. He attributed these to individual differences in grooming behaviour, seasonal influences and the long mouthparts.

Allen (1973), Wikel and Allen (1976) reported that a single infestation of *Dermacentor andersoni* on guinea pigs was sufficient to produce almost complete immunity with a reduction in the percentage of larvae engorging to less than 20%.

2.4 Host parasitism and tick resistance

Host resistance to tick infestation has been extensively studied. Many studies have shown that cattle under natural infestations develop immunity to tick feeding. Kaiser et al. (1982) in Uganda found that within a herd, 9-30% of cattle carry 50% of the total tick population infesting the herd. Latif et al. (1991) rating the survival of *R. appendiculatus* in Western Kenya, found that only 10% of the overall tick burden still fed on the highly resistant animals. Similar findings were observed in Cameroon by Starchuski, (1993) with *A. variegatum.* The above field observations could imply that host resistance has not been able to control ticks absolutely. Among many factors which could interfere with the acquisition of resistance, environment, season, physiology of the host and the nutritional status, the host health seems to be of a major importance. The host health status could be affected by different microbes eg. bacteria, protozoa or viruses. The effects of the interplay between pathogenic organisms such as trypanosomes and babesias and the slow feeding ixodid ticks eg *A. variegatum* will be the matter of concern.

2.4.1 Babesiosis and tick resistance

Babesiosis or red water or tick fever is a tick-borne protozoan disease. The causative agents belong to the genus *Babesia.* Several species infect cattle and human beings (McCosker, 1981). They are transmitted mostly by hard ticks of the genus *Boophilus.* The majority of the approximately 1.2×10^9

cattle of the world are exposed to one or more *Babesia* species (Young and Morzaria, 1986). *B. bigemina* and *B, bovis* occur in warm humid tropical areas (Young and Morzaria, 1986). *B. bovis* infections always cause an acute shock syndrome with high levels of mortality (de Vos et al.. 1987) whereas *B. bigemina* which is enzootic to Africa usually cause high parasitaemia resulting in haemolytic anaemia and haemoglobinuria. In most cases animals that survive the acute phase of the disease carry a chronic and usually subpatent infection for a considerable period of time (Carson and Phillips, 1981). The carriers develop strong immunity to the disease through circulating antibodies, cytophilic antibodies or opsonins and helper T-cells. Carson and Phillips (1981), ascribed three roles to the spleen in the development of cattle immunity to *B. bigemina-.* pitting of parasites from the infected red blood cells, phagocytosis of the parasitized cells and as an anti -*Babesia* antibody secretory organ.

Studies involving susceptibility to babesiosis dates back as far as 1897 when Pound, cited by Francis (1966) in Queensland (Australia), noticed that the first animals to succumb to tick fever were bulls, especially the old ones, followed by cows, then bullocks and sprayed cows. The last ones to die were calves. Host resistance to *Babesia* infection involves both non specific (host-parasite specificity, age, genetic and concurrent infections with other parasites) and specific immune mechanisms (Aragon, 1976). These studies were confirmed by de Vos et al. (1987).

Concerning the resistance of different breeds, Anonymous (1903) again cited by Francis (1966), observed that zebu blood can secure immunity to Texas fever (Kelly, 1943; Francis and Little, 1964). Morel (1981) confirming these observations, stated that zebu breeds were ten times more resistant and have such a high degree of resistance that it practically amounts to an immunity. The reactions of zebus to *B. bigemina* is so slight that the temperature charts show only very slight rises. According to Mahoney (1972) the apparent resistance is a consequence of a prior stimulation of the reticuloendothelial system by an heterologous antigen or alteration of the erythrocytes which block the entry to the *Babesia* parasites. However, Callow and Stewart (1978) have shown that infection of cattle with *Babesia bovis* caused immunosuppression against its natural tick vector, *B. microplus,* irrespective of the infection period. That observation revealed that the acquisition of resistance to *B. microplus* was hindered by *B. bovis* infection increasing thereby the tick survival. How do pure zebu cattle infected with *B. bigemina* to which they are said to be resistant, react against ticks especially A *variegatum* a three-host tick? Does *Babesia* infection alter the host-zebu's resistance?

2.4.2 Trypanosomosis and tick resistance

Trypanosomoses are in general insect-transmitted protozoan diseases. Tsetse flies the main vectors, infest approximately 10 millions km^2 of Africa. This represents 37% of the continent and affects 37 countries and

approximately 150 millions of cattle in these affected countries are exposed to the infections (Itard, 1981).

In cattle, three species of trypanosomes ('*Trypanosoma congolense, T. brucei,* and *T. vivax)* cause the disease as single species or mixed infection. They are characterized by cyclical transmission development in the flies whereby an infective fly remains infective for a long period. Cattle infections caused by *T. congolense and T. vivax* are by far the most serious, both in frequency and economic importance. Trypanosomosis caused by *T. brucei,* is of secondary importance as this trypanosome is only slightly pathogenic for cattle (Itard, 1981). Trypanosomosis due to *T. evansi* in cattle is extremely rare in Africa. The outcome of trypanosome infection is variable (Naylor, 1971). Many animals die after an acute or chronic illness, or if not fatal, trypanosomosis may cause poor growth, weight loss, low milk yield, reproductive failure and a reduced capacity for work. However, some breeds of cattle and many species of wild life possess the ability to survive and be productive in tsetse-infested areas without the aid of trypanocidal drugs, while other animals rapidly succumb to the disease (Murray et al.. 1982). The interest that African trypanosomosis has stimulated in immunologists is great because of the inevitability of the host death, due to mal-function of T-helper cells (Roelents and Pinder, 1984) and the induced immunosuppression mechanisms by the parasites (Holmes et al., 1974; Rurangirwa et al.. 1978; 1979; Masake et al.. 1981).

Literature on the resistance of trypanosome-infected animals against ticks is scarce. Heller-Haupt etal. (1983) reported that rabbits infected with *T. congolense* possessed lower resistance against the tick *R. appendiculatus.* According to these authors the degree of blocking of the immune response appeared to depend on the stage of the ticks involved in the primary infestation. They speculated that the improvement of the tick feeding could possibly be attributed to the marked reduction in complement, mainly C_3 and C_5 elements. What would be the effects of trypanosome infected cattle on the biology of the tick *A. variegatum* which, unlike *R. appendiculatus* has long mouthparts and feed longer than the later one?

2.4.2.1 Effects of trypanosomosis on the lymphoid system

The mechanism of immunodepression has been extensively studied in murine animals and the mechanism involved may be fully operative only in the spleens of mice (Roelents and Pinder, 1984). Considerable evidence has been accumulated showing that trypanosome-infected mice have impaired antibody responses to a large variety of soluble and particulate antigens. Lymphocytes from such mice give lower response to B- and T-cell mitogens. Several groups of workers have studied the mechanism of immunosuppression in murine experimental trypanosomosis. According to some reports, immunodepression in *T.brucei-Iniected* mice is mediated primarily by a potent immunosuppressor T-cell which releases factors having an affinity for macrophages that become suppressors for both T-cell and B-cell responses (Roelents and Pinder, 1984).

The series of experiments performed by the Mill Hill group of Cambridge showed the involvement of T-suppressor cells and macrophages but the cell interactions were not clearly specified. Mansfield and Bagasra, (1978) concluded that the immunodepression did not affect the intrinsic B-cell function which remained intact throughout the infection and that B-cell failure preceded death and the observed unresponsiveness was associated with the mal-function of T-helper cells. However, Wellhausen and Mansfield (1979) came to the conclusion that the suppressor activity in African trypanosomosis in mice was attributable to a suppressor macrophage. Other investigators showed that suppressor cells are all a subpopulation of Thy I^+ cells and that macrophages might play a secondary role despite the fact that some experiments showed strong suppression in the presence of macrophages (Pearson et al., 1978; 1979). In agreement, Wellhausen and Mansfield (1979) could not detect in lymph nodes, thymus or bone marrow of mice infected with *T. congolense* any unresponsiveness or suppressive activities.

2.5 Nature of the immunological response of the host to tick infestation

2.5.1 Expression of immunity

Rabbits have been frequently used and were proved to mount resistance to ticks, *Ixodes ricinus* (Bowessidjaou et al.. 1977), *R. appendiculatus* (Rubaire-Akiki and Mutinga, 1980), *R. evertsi evertsi* (Njau and Nyindo, 1988), *R. sanguineus* and *A. maculatum* (Dipeolu, 1990) and *A. variegatum* (Dipeolu and Harunah, 1984).

The way immunity is expressed varies greatly according to the host. The effects range from simple rejection of the parasite, apparently with little or no damage to it, to interference with feeding, prolongation of feeding time, reduction in engorgement Weights, inhibition of egg laying, decreased viability of ova and death of the parasite on the host (Wikel, 1984).

Roberts (1968) stated that the main expression of resistance in cattle *Bos taurus* infested with *B. microplus* larvae is the rejection of larvae in the first 24 hours. The same results have been shown in zebu cattle (Roberts, 1968; Wagland, 1975). One major expression of immunity in host infested with ticks is the removal of the ticks through grooming as cattle exposed to *Ixodes holocyclus* or *Haemaphysalis longicornis* mount resistance expressed by the removal of the ticks (Sutherst et al., 1979; Wikel and Allen, 1976). A single infestation of *Dermacentor andersoni* on guinea pigs was sufficient to produce almost complete immunity as shown by the reduction in the percentage of larvae engorging to less than 20% and frequently almost to zero (Allen, 1973; Wikel and Allen, 1976). Schneider et al. (1971) showed that a number of amphibian species become immune to *A. testudinis* resulting in reduced engorgement weights, egg numbers and feeding rate. The list is not exhaustive and the expression of immunity described so far has covered so many species.

2.5.2 Quantification of hosts' resistance levels

According to Wikel (1984), resistance could be expressed in many ways: (a) reduced number of engorged ticks; (b) reduced blood meal intake; (c) reduced number and viability of ova; (d) prolonged period of engorgement; (e) death of the tick on the host. Bennett (1969) and Utech et a[. (1978b) determined resistance level against *B. microplus* as the percentage of larval ticks that failed to survive to maturity following infestations with 20,000 larvae. This method is not applicable to two-or-three-host ticks. The commonest method of determining the resistance status of cattle against African ixodid ticks among others, is by assessment of the number of ticks engorging or percent reduction in engorgement weight (Latif, 1984a; 1984b; Chiera et al., 1985; Rechav, 1987; Jongejan et al. 1989). Dipeolu (1990) quantified the resistance of rabbits to the tick *A. maculatum,* using the following biological indices to estimate three components of the manifestation of resistance to ticks: Decreased Tick Weight Index (DTI), Decreased Engorgement Period Index (DEI) and Reject Index (RI) to quantify the degree of resistance Tick Resistance Index (TRI).

$$\text{DTI} = \frac{\text{Average Weight of ticks on challenge.}}{\text{Average Weight of ticks on control}}$$

$$\text{DEI} = \frac{\text{Average feeding period of ticks on control.}}{\text{Average feeding period of ticks on challenge}}$$

$$RI = \frac{\text{Number of ticks engorged on challenge}}{\text{Number of ticks engorged on control}}$$

and TRI = DTI x DEI. In resistant animals, TRI and RI tend towards zero (o).

From the discriminant function procedure, Latif et al. (1991) derived a resistance index from cattle infested with *R. appendiculatus* and obtained three groups of resistance : high resistant group with index around 0.70, moderate resistant with index of 0.80 and susceptible with an index of 1.15. However a different approach to the assessment of acquired resistance could be sought for since individuals from the same breed could show differences in their physiological and behavioural pattern, which might reflect on their immunological reactions. So grouping animals with identical status could be useful.

2.5.3 Humoral responses

Antibody synthesis depends on several factors such as: (a) interaction and association between macrophages and its effects on cells (T-and -B cells); (b) age of the host; (c) quantity and type of antigen (T-or B-dependent); (d) route of administration; (e) health status of the host; (f) immunopotentiation by drugs and adjuvants and (g) the host genotype. The first laboratory studies on humoral factors to tick infestation dated as far back as 1939 when Trager after a transfused serum from a sensitized host to *Dermacentor variabilis* produced

partial immunity in the recipient. Brossard and Girardin (1979) having injected serum from immune rabbits into susceptible recipients at a rate of about 0.25 ml/10Og body weight, 4 hours before an initial infestation with ticks, found the females feeding on treated rabbits weighing significantly less than those on comparable control hosts. Bowessidjaou et aL (1977) noted that antibody to *Ixodes ricinus* salivary glands, measured by indirect immunofluorescence, appeared towards the end of a first infestation and reached high titres on a second infestation but did not increase thereafter although the host became progressively more immune while Njau and Nyindo (1987a) demonstrated antibody synthesis in rabbits as early as 4 days after *R. appendiculatus* feeding. The persistence of the antibody would depend on the trapping of antigen in extracellular spaces by dendritic cells and the stable retention of antigens by macrophages (Agyei, 1992; Allen et al.. 1979). Although a serum transfer with *Dermacentor andersoni* infestations is not followed by acquired immunity in the recipient (Wikel and Allen, 1976), there is no doubt that antibody is involved in this mechanism. Immunoglobulin G, homocytotropic and precipitating antibodies have been detected by Brossard and Girardin (1979), Njau and Nyindo, (1987b). The antibody production does not all the time correlate with the degree of immunity because Willadsen et al. (1978) found that high levels of precipitating antibodies to *B. microplus* feeding correlated with low levels of acquired resistance. Rechav (1987) found a positive correlation between the tick burden on the cattle and the serum gamma globulin levels. According to Butler (1983) and Musoke et al. (1986), bovine immunoglobulins are composed of Immunoglobulin IgA, IgG1, IgG2, IgE and IgM. The existence of an IgG3 has been reported by Butler etal. (1987). The

difference between IgG1 and IgG2 reside in the capability of IgG2 to mediate adherence and phagocytosis by neutrophils and fresh monocytes (McGuire et al, (1979). According to Brown et al. (1982) immunity of guinea pigs to *B. microplus* could be associated with IgG 1 synthesis.

2.5.4 Cellular responses/Cutaneous responses

The relation between circulating antibody and or skin reaction and the onset of resistance was established in the nineteen seventies. Wikel (1979) stated that cutaneous responses of animals to tick infestations lead to deleterious effects being exerted on the attached tick and its subsequent rejection. Among the five types of hypersensitivity defined by Roitt (1991) hypersensitivity I which is antibody-mediated and hypersensitivity IV, which is cell-mediated, have been ascribed roles in the expression of resistance by hosts to tick infestation.

2.5.4.1 Immediate hypersensitivity reactions or anaphylaxis

Resistant cattle exposed to *B. microplus* larvae were intensely irritated. Papular reactions were seen around nymph and adult feeding sites and there was a transient increase in blood histamine levels (Riek, 1956; 1962). Intra-dermal injection of egg, larval and nymphal antigens gave immediate edematous dermal reactions (Riek, 1962; Binta and Cunnigham, 1984). Three allergens have been purified from ticks which can trigger edematous reactions after 20 min and sensitivity to these allergens was

correlated with the level of immunity (Willadsen et al.. 1978). The underlying mechanism is the release of the vasoactive substances and the binding of the allergens to the IgE Fc receptors borne by the mast cells.

Histological analysis of the tick feeding sites in immune guinea pigs demonstrates a dominant basophil response (30 to 80%) of the infiltrate with a marked eosinophil presence as early as 24 hours after attachment (Brown and Askenase, 1981; Brown et al., 1983; 1984; Brown, 1985). Langerhans cells present in the skin are thought to act as antigen-presenting cells in the induction of contact hypersensitivity reactions and to bind tick antigen in the skin of guinea pigs infested with ticks (Brown, 1985). The major cell populations involved in the anaphylactic reactions are the basophils concerning *Bos taurus* (Brown et al., 1984) and the mast cells with *Bos indicus* (Riek, 1962). The basophil recruitment at the feeding site could be favoured by the vasopermeability initiated by the mast cell degranulations. Koudstaal et al. (1978) suggested that immediate hypersensitivity was partly responsible for the resistance of cattle to the tick *B. microplus.* Through grooming, fifty percent of the infesting larvae could be eliminated. Larvae made shorter, repeated attachments on hosts of high resistance. Injection of histamine beneath *B. microplus* larvae induced detachment of the feeding tick (Kemp, 1978; Kemp and Bourne, 1980). However, Tatcheli and Bennett (1969) found that histamine could be advantageous to the newly attached larvae, hence a secondary role could be attributed to that amine in the mediation of immunological responses. Chinery and Ayitey-Smith (1977) and Willadsen et al. (1979) suggested that ticks secrete a histamine-blocking factor which they

possibly use to regulate histamine levels at the feeding site thus avoiding damages.

Immediate hypersensitivity reactions to other tick species eg. *D. andersoni, I. holocyclus, H. leporipalustris* have also been reported by Allen (1973), Allen and Humphreys (1979) and Boese (1974) respectively.

2.5.4.2 Cell-mediated immune response cutaneous basophil hypersensitivity or delayed-type hypersensitivity immunity

Delayed type hypersensitivity (DTH), are protracted T-cell mediated immune reactions encountered in hosts repeatedly attacked by arthropods (Mitchell, 1980). Such cell-mediated cutaneous reactions have been shown to exert lethal effects and rejection of ticks by affected hosts (Wikel, 1979; Brown et al.. 1984). This phenomenon has been demonstrated in hosts resistant to *B. microplus* (Roberts, 1968), *D.andersoni* (Wikel and Osburn, 1982), D. *variabilis* (Allen, 1973), *I. holocyclus* (Bagnall, 1975) and *R.appendiculatus* (Binta and Cunnigham, 1984; Smith et al.. 1989). Passive transfer experiments using lymph and peritoneal exudate cells have shown that cell-mediated reactions are involved and confer greater protection (39-90%) compared to serum transfer (Brown, 1985). The cutaneous basophil hypersensitivity is the result of an interaction between the tick saliva, the primed macrophages and the T-cells. The primed macrophages stimulate the sensitized T-cells which release soluble factors that mediate hypersensitivity by attracting and activating macrophages. T-helper cells secrete lymphokines which help in the destruction

of the foreign body. The fact that tick could be rejected in the presence of a histamine antagonist reveals the plausible role of a basophil derived factor involved in tick resistance (Brown, 1985). Willadsen (1980) reviewing DTH attributed the delayed lymphocyte blastogenesis to tick induced immunosuppression. This may indicate that some ticks avoid possible harmful effects of DTH inflammatory reactions contrary to the notion that death of ticks is attributed to the predominance of inflammatory cells in the feeding lesion (Nelson et al.. 1977). Host DTH responses to tick infestations are characterized by various functional cell types. Lymphocytes and basophils have been shown to dominate the reactions depending on the animal species (Allen, 1983; Wikel et al.. 1978; Brown and Askenase, 1981; Brown et al., 1984). Eosinophils, neutrophils, mononuclear cells eg. macrophages and Langerhans cells have also been reported by Allen (1979), Brown (1985) and Latif et al. (1991). Wikel et al. (1978) found that an intra-dermal injection of 50 microgrammes *(jjg*) of salivary gland antigenic material from partially fed D. *andersoni* into tick-resistant guinea pigs resulted in a significant delayed hypersensitivity reaction with a transient increase in skin thickness reaching a maximum at 48 hrs. An induced lymphocyte blastogenesis was observed, which was significant at the end of the tick engorgement period. The main cells found in early lesions are the neutrophils which may be found either occluding or cuffing the venules and may be accompanied by varying numbers of lymphocytes and eosinophils. As feeding progresses, neutrophils invade the tissue beneath the hypostome and lymphocytic cuffing may appear around deeper vessels of the corium. Lysis of collagen fibers beneath the hypostome follows neutrophils invasion with resultant cavity formation. However, Eveleigh

et al. (1974) found only lymphocytes and eosinophils in their preparations. The mechanism of the skin reaction to tick bite is obviously a complex phenomenon involving irritation and pharmacologic effects of oral secretions (saliva, material regurgitated from the gut of the parasite) and of substances produced by the hosts' cells. Morehouse (1968), later confirmed by Tatchell and Morehouse in 1970 found that during tick-host interactions, there was specific vascular damage resulting from the tick saliva while tissue damage (cavity formation) was caused by the host response.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Studies on the acquisition of resistance

3.1.1 Hosts

3.1.1.1 The Boran cattle

Twenty Boran steers aged 12 months (Plate 1), were acquired from Mutara (Agricultural Development Corporation, ADC, Kenya) Ranch at the beginning of the experiment. Mutara ranch is located on the Equator at 2000 metres (m) above sea level in Laikipia (005'N, 360 42'E) and covers about 2600 hectares (ha) of good grazing land for cattle with a mean annual rainfall of 600 millimetres (mm).

According to studies carried out by de Castro et al. (1991) the predominant ticks in the ranch were *Rhipicephalus evertsi, Hyalomma truncatum, Rhipicephalus pulchellus, Rhipicephalus appendiculatus and Rhipicephalus hurti.* The cattle were tick free when collected as a result of intensive acaricide use. Before the experiments, the naivety of the animals was checked by running the pre-infestation sera on agar double diffusion technique (Ouchterlony, 1958) and Western blot (Burnette, 1981). Pre-*Babesia* or trypanosome infections were respectively checked according to Barry et al. (1982) and Murray et al. (1977).

Plate 1. **Boran *(Bos indicus)* cattle**

These cattle were treated by immunization against Rinderpest, Anthrax, Brucellosis, Foot and Mouth Disease and Lumpy Skin Disease before use. The animals were kept in the Large Animal Accommodation Unit at The International Centre of Insect Physiology and Ecology (ICIPE), where they were fed with hay, concentrates and water ad libitum for three months before the commencement of experimental treatments. Each of the Boran steers was ascribed an identification number on an eartag and on the skin.

3.1.1.2 The Ayrshire breed cattle

Five purebred Ayrshire steers aged between 10 to 12 months (Plate 2), were bought from the ADC Ranch, Zea in Kitale. They had been bred in tick free paddocks. ADC Zea Complex is located in the Kenya Highlands within the western plateau in Trans-Nzoia District at an altitude of 2220 m above sea level. The farm is tick free and no outbreak of tick-borne diseases have been noticed for many years. The cows and bulls are registered with the Kenya stud book. The cattle were immunized against the major cattle infections (Rinderpest, Anthrax, Brucellosis, Foot and Mouth Disease and Lumpy Skin Disease).

3.1.1.3 The crosses between Boran and Friesian cattle

Boran x Ayrshire crosses were not available for purchase so five crossbreds (Boran bulls and Friesian dams), aged 10 to 12 months were bought from the same farm (Plate 3). They were immunized as above, against the major cattle infections.

Ayrshire *{Bos taurus)* cattle

<u>Plate</u> 3. Crossbred Boran *(Bos indicus)* x Friesian *(Bos taurus)* cow is shown by an arrow.

3.1.2 The parasites

Three parasites were used:

(a) Ticks *Amblyomma variegatum* Fabricius 1794. Unfed and fed specimen are displayed in plates 4 and 5.

(b) Haemoparasites, *Babesia bigemina* Smith and Kilbourne 1892, and *Trypanosoma* (Nannomonas) *congolense* Broden 1904

3.1.2.1 Ticks

The *A. variegatum* ticks used were obtained from a disease-free laboratory colony of ICIPE Tick Mass Rearing Unit. They had been maintained on naive rabbits and for good reproductive activities at 28°C, 85 or 97% RH according to Irvin and Brocklesby (1970). The nymphs and adults were between 4 to 6 weeks old at the time of the infestations.

Plate 4 Unfed tick, A *variegatum* adults: male (right) and female (left)

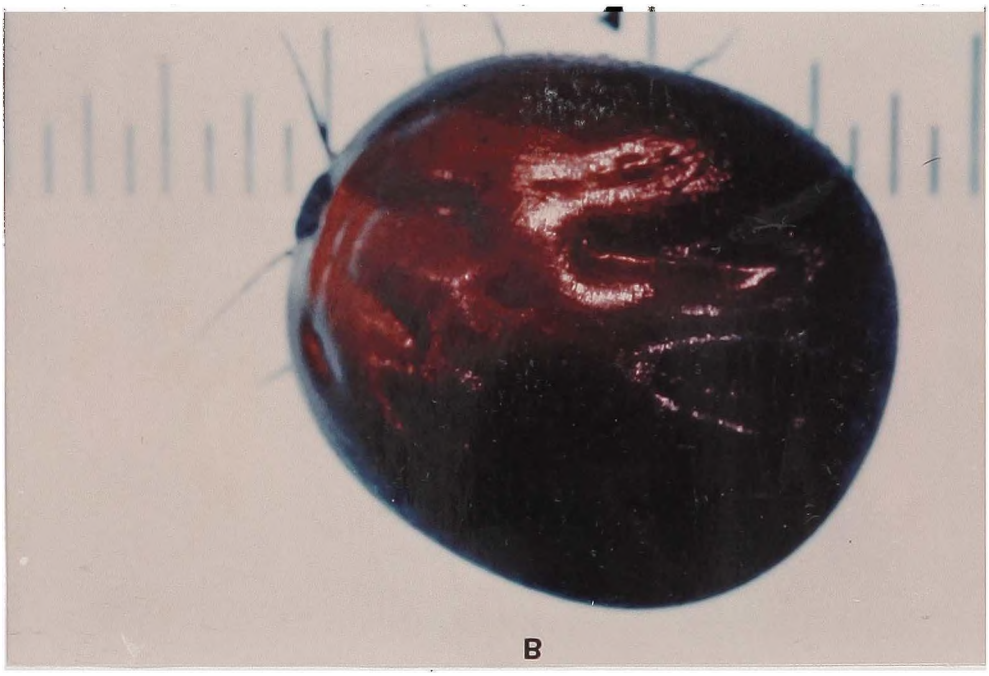

Plate 5 **Fed tick, *A. variegatum* female.**

3.1.2.2 Tick challenges and data collection

To simulate field conditions the experimental animals were infested concomitantly with both nymphs on the right ear and adult tick on the left ear but for clarity and convenience experiments will be related separately.

Infestations were performed using, each time, laboratory reared new batches of nymphs and adult ticks 30 days after all the ticks from the previous challenge had dropped. The 20 Boran steers and the five crossbreds were five times infested with *A. variegatum* nymphs and adults while the Ayrshire were challenged four times since we noticed later that immunity to ticks could be induced after four repeated infestations.

The infestation procedure was as followed. One hundred 4 to 6 weeks old nymphs were applied in a bag fixed to the right ear of all Boran cattle as well as on those of the five purebreds and crossbreds according to Bailey (1960). The ticks were contained in cloth sleeves glued with chloroprene adhesive, Stick Tite(R) (Car and General Kenya Ltd), on a band of stocking nets which were also glued to the base of the ears. The glued bags on the cattle were left for 24 hours before ticks were applied. The outer end of the bag was opened daily to inspect the ticks and closed afterwards with adhesive tape (Leucoplast^{R}, Beierssdorf Germany). When opened the earbag was shaken into a white enamel tray to collect all the detached or engorged nymphs. All harvested nymphs from a particular animal, on a given day, were individually weighed with a sensitive chemical balance Mettler 100^{R} and then put together in a 7.5 x 2.5 cm flat bottomed Samco^{R} glass tube. The feeding

period was written on each tube. The harvested nymphs out of the initial number was counted and handled as described by Irvin and Brocklesby (1970). The tube was plugged with cotton wool and then incubated at 28°C in an aluminum can containing 40% potassium chloride (May and Baker Ltd.) solution which gives approximately 85% RH. The tubes were examined daily to record the moulting period. The percent moult of the harvested nymphs, the percent which died before or after feeding on a particular animal, were recorded at the end of each infestation.

Simultaneously, 20 unfed male ticks were applied in the same manner as the nymphs, on the left ear of each animal. Twenty unfed female ticks obtained from the tick colony were applied 5 to 6 days later when all males had attached. In fact after feeding for approximately 5 days, male *A. variegatum* emitted a pheromone which attracts unfed male and female A *variegatum* and stimulates attachment and feeding on the host (Norval and Rechav, 1979). Harvested replete and detached females, were individually weighed and stored at 28°C, 97% RH maintained by a saturated potassium sulphate solution (AR Park Scientific Ltd). The following biological parameters were recorded: the preoviposition period (number of days taken by the female tick to start laying eggs), the feeding time (time taken by the ticks to feed to repletion), the weight of the egg mass, the engorgement weight, the number of ticks which oviposited and the number of ticks which engorged above the critical weight. The critical weight documented by Dipeolu and Harunah (1984), with /A. *variegatum* is 2g. According to Dipeolu et al. (1992) it is the weight of an engorged female tick above which the feeding status does not influence anymore the mass of eggs

laid. In order to record the percentage of eggs which hatched, the engorged females were randomly divided into two equal groups. One group of the engorged females was allowed to oviposit. The oviposited eggs of each tick were weighed to get the weight of the egg mass. The eggs laid by the other group were allowed to hatch into larvae and the percentage of total number of eggs which hatched was estimated as follows: the degree of hatching was initially assessed by visual inspection by comparing proportion of empty egg shells or hatched larvae with that of unhatched eggs for each egg mass. The tubes containing larvae were frozen at -20°C for 1 hr. From each tube approximately 10% of the total sample were counted on a white background. The number of larvae counted expressed as a fraction of the number of eggs estimated for the egg mass was the egg hatch which when multiplied by 100 gave a percent hatch rate for each egg mass.

3.2 The immune response in cattle repeatedly infested with the ticks

3.2.1 Preparation of antibodies

3.2.1.1 Sera collection

Twenty five ml of blood were collected from the jugular vein of each animal in a clean universal bottle before each infestation. The blood sample was kept at room temperature for two hours, then kept at 4°C overnight. Sera obtained after centrifugation at 1000g for 15 min was stored at -20°C. Subsequent bleeding were done just after each infestation when all ticks had dropped off their hosts.

3.2.1.2 Preparation of immunoglobulins

Two ml of whole serum were withdrawn from each of the above sera. They were pooled according to breed and the infestation level. The pooled sera from the experimental animals was diluted by half with distilled water and precipitated with an equal volume of saturated ammonium sulfate pH 7. After centrifugation at 1000g at 4°C for 15 min, the precipitate was dissolved in an equal volume of 0.15M phosphate-buffered saline (PBS) pH 7.2. The solution was precipitated twice with a saturated ammonium sulfate solution. The last precipitate was brought to the initial volume of serum with distilled water. The solution was dialysed for one hour against distilled water and later against 0.15M PBS pH 7.2 for 24 hrs with regular changes of the dialyzing buffer. The immunoglobulin solution was centrifuged again at 10OOg at 4°C for 15 min to get rid of any remaining precipitate. The presence of any eventual sulfate anions was checked with 0.1 M solution of barium chloride (Analar, BDH, UK). The immunoglobulins were aliquoted and stored at -20°C until required.

3.2.1.3 Preparation of immunoglobulin subclasses

3.2.1.3.1 Separation of IgGs by gel filtration method

The IgGs were separated from IgM and albumins using the gel filtration method (Hudson and Hay, 1989) at 4°C. Bio-Gel A-1.5m^{R} (Bio-Rad Laboratories USA), was washed with several changes of 0.15M of PBS pH 7.2 to remove the fine particles and degassed. The gel was packed in a 2x170 (Econo- column" Bio-Rad Laboratories, Richmond, USA). It was equilibrated

and run with 0.1 M acetate buffer pH 5 at 4°C according to the method of Hudson and Hay (1989). The column was connected to a UV monitor (Uvicord^{R}, Pharmacia), a fraction collector (FRAC-100^{r}, Pharmacia, Uppsala Sweden) and calibrated with the Bio-Rad calibration kit. One milligramme (mg) of Dextran 2000^{R}, (Pharmacia), was added to the column to observe the migration of the dextran molecule. Two hundred and fifty microlitres (/ul) of IgGs in 750 *fi*\ of 0.1 M acetate buffer pH 5 was loaded each time onto the column. Elution was monitored at 280 nm. The different peaks corresponding to a given number of tubes were pooled, concentrated in polyethylene glycol 6000, (PEG, Serva^{R}). The concentrates were dialysed against 0.15M PBS at 4°C overnight. The purity of the different peaks was assessed by SDS-PAGE.

3.2.1.3.2 Fractionation of IgGs into different subclasses

Three grammes (gm) of Protein A-Sepharose 4B^{R} (Pharmacia) was swollen and washed according to the manufacturer's instructions. One ml at each time, of the IgG solution at a concentration of 9.5 mg per ml, was loaded onto a K 9/15^{R} column (Pharmacia), packed with Protein A-Sepharose 4B^{R}. After the collection of the unbound, the bound fraction was eluted with 0.1M glycine-HCL, pH 2.8 in 1M Tris HCL pH 8.5. The purity of the eluants was assessed by SDS-PAGE.

3.2.2 Preparation of antigens

3.2.2.1 Preparation of salivary gland antigens (SGA)

Salivary glands of 600 *A. variegatum* adult males and females at a ratio of 1:1 were partially fed for each period on tick naive 10 to 12 month old Boran steers for three, six, nine and twelve days in glued earbags as above. They were dissected into cold 0.1 M phosphate-buffered saline with ethylenediamine tetra-acetic acid (PBSE) and 0.001 M Phenylmethylsulfonylfluoride (PMSF) respectively. The removed glands were thrown into liquid nitrogen and later stored at -70°C until required.

For the antigen preparation, the glands were washed two times in PBS (1000g at 4°C for 15 min). They were then ground in Pyrex^{R} glass tissue grinder N° 7227, USA in the appropriate volume of (1:2v/v) of PBS pH 7 with 1 mM of PMSF and 0.2% Triton X 100^{R}. The samples were frozen and thawed three times in liquid nitrogen and water respectively in order to break the salivary gland membranes through thermic shocks. The homogenized glands were ultra sonicated in a ultrasonic transducer Soniprep^{R} 150 MSE Ltd (Crawley, Sussex) at an amplitude of 14 microns per second for five cycles. The homogenate was centrifuged at 20,000g per minute at 4°C for 30 min. The supernatant was collected and the pellet resuspended in the extraction buffer and the procedure repeated as above. The two supernatants were pooled, concentrated, and stored at -70°C until required.

3.2.2.2 Preparation of saliva antigens (SA)

Replete females of A *variegatum* ticks collected on Day 12, were water-cleaned. The legs were removed and the ticks immobilized onto a glass slide covered with a double-sided adhesive cellophane tape. Microcapillary tubes held with plastocine were slipped over the chelicerae of the ticks. The ticks were injected under the cuticle, in the shoulder region with 10 microlitres of 10 percent w/v pilocarpine hydrochloride solution. The ticks were then put in humid environment at 37°C. Saliva collected in the tubes after one to two hrs were pooled and stored at -70°C. The dispositions are shown in plate 6.

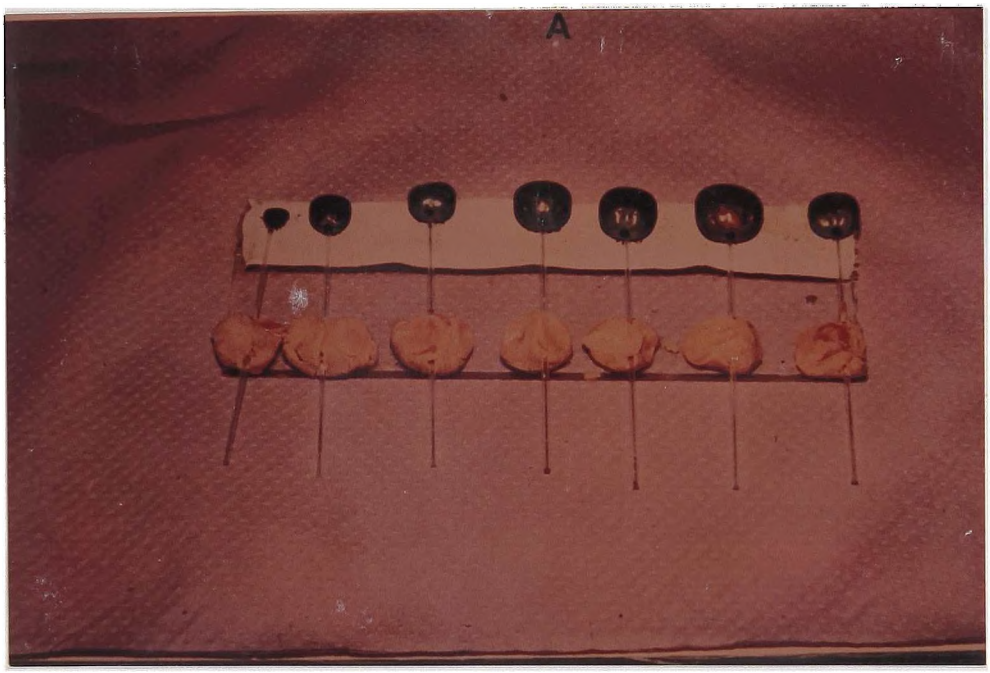

Plate 6. **Collection of saliva from fed female *A. variegatum* ticks**

3.2.2.3 Preparation of nymphal antigens (NA)

Six hundred nymphs partially fed for 4 days on a tick-naive Boran steers in glued earbags as earlier indicated were homogenized in 0.1 M PBSE pH 7 containing 0.2% Triton X 100 and 1mM PMSF with a mixer (Sorvall^{R} Omni Mixer 17106 Dupont Instruments). Five cycles of homogenization were performed on ice. Each lasted 30 seconds followed by 30 seconds of rest. The final mixture was thereafter ultrasonicated and centrifuged as for salivary gland homogenates for one hr. The supernatant was aliquoted and stored at -70°C until required.

3.2.3 Protein content determination

The protein content of the different homogenates and all the fractions obtained from the chromatography, was estimated according to Smith et al.. (1985) using the Pierce bicischoninic acid (BCA). The standard used was the Pierce bovine serum albumin. The samples were read on a spectrophotometer Beckman DU-50^{R}, USA at 562 nm.

3.2.4 Analysis of the different antigens by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Seven jug of each of the above SGA, NA were subjected to gel electrophoresis according to a modified method of Laemmli (1970), On 180 x

160 x 1.5 mm discontinuous slabs. The gels consisted of 3% stacking gel and 5 to 20% of gradient separating gel containing (30:1), acrylamide:bis acrylamide. Gradient was cast using a gradient former, Hoefer SG50^{R} (Hoefer Scientific Instruments, San Fransisco, USA). Samples were dissolved in 124 mM Tris-HCL buffer (pH 6.8) containing 0.4% SDS, 1.25% glycerol, 1% mercaptoethanol and 0.1% bromophenol blue. The samples were heated for 5 min in a boiling water bath. Electrophoresis was carried out at constant current 25 mA, through the stacking gel and at 35 mA in the separation gel. The running buffer was Tris-glycine with 49.5 mM Tris and 411 mM glycine. Gels were stained with silver nitrate according to a modified technique used by Morrissey (1981). Molecular weights were estimated from a standard curve obtained from known molecular weights and their corresponding relative mobilities (Rf).

3.2.5 Identification of the different antigens

3.2.5.1 The double immunodiffusion test

An antigen antibody precipitation test was performed according to a slightly modified method of Ouchterlony (1958). The modification was in the use of 2% agarose (Electran^{R}, BDH, UK), in barbitone buffer. The antigens SGA, SA and NA, were loaded in the centre and the pooled serum of the tick-

infested animals after the different infestations in the peripheral wells. Different dilutions of antibodies (1:10, 1:50, 1:100) and of antigens (1, 1:2, 1:5, 1:10) were also used. Diffusion was allowed to proceed at room temperature for 48 hrs in a humid chamber.

3.2.5.2 The immunoblotting technique (Western Blot)

The procedure was according to that of Towbin et al. (1979) and Burnette (1981). Saliva, extracts of glands obtained from partially fed A *variegatum* males and females, for 9 days and whole nymph extract as described above were resolved on 5-20% gradient SDS-PAGE. The analysis was done under the same conditions as described above. After completion of the electrophoresis, the gels were removed and pre-equilibrated for 15 min in the transfer buffer composed of Tris (25 mM), glycine (192 mM) and 20% methanol. A transfer sandwich was prepared according to the following arrangement:

i scotch brite pad,

ii 3 layers of blotting paper,

iii gels

iv nitrocellulose paper Schleicher and Schuell (Germany) of 0.45 micron porosity,

v 3 layers of blotting paper,

vi scotch brite pad.

The two grilles were gripped firmly together and put in the transfer cell (Trans-Blot^{R}, Bio-Rad UK). The cell was filled with adequate volume of buffer. The running was done at 30 volts for 16 hrs at 4°C (Jongejan et al.. 1989). All components in contact with the slab gels were pre-wetted with the electrode buffer. Thereafter, the electro-transferred proteins were revealed with a rouge ponceau solution (50% rouge ponceau in 3% trichloroacetic acid). The blots were cut into strips and incubated for 2 and a half hours in quenching buffer consisting of 5% fat-free milk in tris buffered saline (TBS) pH 7.5 (20 mM Tris and 100 mM NaCI). The strips were washed with the same blocking solution. They were then incubated at room temperature overnight with the bovine antisera at the dilution of 1:100 while the fractions were in a solution of 1 to 25 under constant shaking. The dilution was done with 1% fat-free milk in TBS. Free antibodies were removed with 5 washes in 5% fat-free milk in TBS at 15 min intervals before incubation with the second antibodies (whole anti-bovine IgG, lgG1 heavy chain specific, lgG2 heavy chain specific and IgM *p* chain specific all conjugated to horse radish peroxidase). These antibodies had been raised in sheep and were acquired from Bethyl Laboratories, USA. The working dilution was 1:1000. The unbound were removed by washing as described above and the strips incubated in 10 mM Tris-HCL, pH 6.8 for 15 min. Before visualization with 0.03% of 4 chloro naphtol in ice cold methanol, 60 /jl of cold 30% hydrogen peroxide were finally added (Pierce, 1992). The reactions were stopped by rinsing the strips with distilled water after 10 min. They were later dried between blotting paper and photographed.

3.2.6 Quantification of antibody subclasses in tick-infested cattie

3.2.6.1 Quantification of IgG1 and IgG2

In order to assess the humoral responses to *A. variegatum* SGA, lgG1, lgG2 and IgM were sequentially quantified after each infestation using the enzyme linked immunosorbent assay (ELISA) according to Kemeny (1991). The antigen used throughout the experiment was the Day 9 SGA. Optimum dilution of antigen and antibody were predetermined by checkboard titrations. One hundred jul of SGA diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6) were added to each well of a flat bottom microtitre plate Dynatech Immulonl The dilution was such that each well received 0.70 jug of proteins. The plates were put in a humid environment for one hr at 37°C and then overnight at 4°C. The antigen coated plates were washed the following day with 0.15M PBS pH 7.2 containing 0.05% of poiyoxyethylenesorbitane monolaureate (Tween 20) and dried on tissue (Quick Dry^{R}, Chandaria Industries, Kenya). Two hundred *p*\ of a 3% gelatine in 0.15M PBS with 0.05% Tween 20^{R} as a blocking agent were added into each well and incubated at 37°C. After one hr, the plates were washed again and 100 jul of a serially-diluted antisera from 1:100 to 1:12800 in 1% gelatin-Tween 20^{R} solution were added again for one hr. The plates were thereafter washed and dried. One hundred *fj*\ of appropriately diluted horse radish peroxidase conjugated sheep antibovine lgG1 were pipetted into each well and the other plate had sheep antibovine lgG2 in each well. Both plates were incubated for one hr. The plates were washed as above

and 100 /ul of the substrate, orthophenylenediamine hydrochloride (OPD, Sigma Chemicals USA), in citrate buffer pH 5.0 and 22 /i\ of 30% H_20_2 w/v were added. The plates were kept in the dark for 30 min. The reaction was stopped by adding 50 *[u* of 2M sulfuric acid. A Titertek Multiskan^R Elisa plate reader (Flow Laboratories, UK), was used to read the optical density of the reaction at 450 nm. Four repetitions of each treatment were carried out.

S.2.6.2 Quantification of IgM

IgM was quantified using a double sandwich class-capture assay (Kemeny, 1991). One hundred *y* of an affinity purified antibovine IgM *JJ* chain specific raised in sheep, diluted 1:5000 in carbonate-bicarbonate solution pH 9.6, were used to coat the plates. Washing and incubation were done as described above. After washing, the different reactants were put as follows:

100 /JI of serially diluted from 1 to 1:6800 of bovine anti-tick antiserum,

100 jul of SGA diluted as above,

100 /il of the bovine anti-tick antiserum,

100 /il of horse radish peroxidase conjugated antibovine IgM *fj* chain specific raised in sheep diluted 8000 times. The substrate was OPD in citrate buffer pH 5.

As above four repetitions were carried out for each treatment.

3.3 Studies on the effects of the haemoparasitism on the acquired resistance

3.3.1 The parasites

3.3.1.1 The trypanosomes

The trypanosome used was a *Trypanosoma congolense* IL 2642 obtained from the International Livestock Research Institute (ILRI), Kenya. This parasite was first isolated from a naturally infected cow on the 26 February, 1962 in Uganda under the name EATRO 209. After successive passages through mice, rats and tsetse flies with cloning in between, it has been kept in liquid nitrogen since then as stabilates under the strain name of IL 2642. The strain has been proved to be infective for tsetse flies and cows. IL 2642 strain elicited chronic trypanosomosis (Morrison et al.. 1978; Nantulya et al.: Akol et al.. 1986).

3.3.1.1.1 Multiplication of the trypanosomes

The above parasite was inoculated into Swiss mice immunosuppressed with a solution of cyclophosphamide monohydrate (Sigma Laboratories) at 150 mg per kg. Prior to the infection two capillary tubes containing the stabilate were suspended in one ml of phosphate saline glucose (PSG), pH 8. Viability, motility and concentration were checked according to Mattioli (1988) and found to be 10^5 trypanosomes per ml. Each mouse was injected with 200 *p*\ of the

infective mixture. Parasitaemia was checked thrice a week. When the parasitaemia was high, between 10^5 and 10^6 according to Mattioli (1988) the animals were bled by heart puncture into a heparinized vial. Rats were inoculated with 200 *fj*\ of the infective blood stream parasites in 1 ml of PSG.

3.3.1.1.2 Induction of trypanosomosis

Before haemoparasite infection, the 16 tick-immune Boran steers were grouped into 2 lots composed of six tick-immune Boran animals (2 from the highly-resistant group, 2 from the moderately-resistant group and 2 from the lowly-resistant group). The remaining four were free of haemoparasites. The first lot of 6 was inoculated intravenously with 1 ml of 10^6 bloodstream trypanosomes from peak parasitaemia of a donor rat according to Olubayo et al. (1990). Rectal temperature, clinical signs and parasitaemia were measured daily. Nymphal and adult tick stages were applied after 32 days and 38 days post-infection respectively as indicated in paragraph 3.1.2.2 when high parasitaemia estimated according to Mattioli (1988) at 10^s per ml was established. Nymphs and adult ticks were left to feed to repletion and detached. The latter results were compared to those observed before haemoparasite infections.

3.3.1.2 The *Babesia* sp.

3.3.1.2.1 Preparation of tick stabilate of *Babesia* sp.

Babesia bigemina strain 275 of Muguga Veterinary Laboratory (Kenya) was used. It was obtained from a natural calf infection from Kerua (Kiambu, Kenya). The calf was immunosuppressed with dexamethasone and 20,000 nymphs of *Boophiius decoloratus* were fed on for 13 days. The fed ticks were ground in 125 ml of minimum essential media (MEM) plus bovine plasma albumin (BPA) and centrifuged at 500g for 3 min. To the supernatant were added 120 ml of MEM and BPA containing 20% of dimethyl sulfoxide (DMSO). The solution was allowed to equilibrate in gas phase of liquid nitrogen for 24 hr and later transferred into liquid nitrogen. The tick stabilate was preserved in liquid nitrogen until required.

3.3.1.2.2 Multiplication of the *Babesia* sp.

A young splenectomized, dexamethasone-immunosuppressed calf was inoculated with 4 ml of tick stabilate into the jugular vein at Muguga Veterinary Laboratory, Kenya. The parasitaemia was checked daily by blood smear. The immunosuppressed calf was bled at 10% parasitaemia. One litre of infective blood was collected and centrifuged at 500g for 10 min. The plasma and the buffy coat were carefully removed with a pasteur pipette and an equal volume

of 0.01 M of cold sterile PBS pH 7.2 was added to the blood sample and centrifuged again at 500g for 10 min. The supernatant and the buffy coat were removed. The washing operation was repeated 2 times. The pellet was resuspended to 50% packed cell volume (PCV).

3.3.1.2.3 Induction of *Babesia* infection

A lot of six tick-immune Boran steers composed as above (case of trypanosomosis induction) was inoculated with 150 ml per animal of the Babes/a-infected blood through the jugular vein according to Hernandez-Ortiz et al. (1989). Presence of *Babesia* parasites was checked daily through blood smears. Rectal temperature, clinical signs and parasitaemia were also recorded. Nymphal and adult tick stages were applied 32 and 38 days after *Babesia* infections respectively.

3.4 Statistical analysis

A multivariate analysis technique, the Principal Component Analysis (PCA) was used to descriptively show the pattern of co-variation of the various biological parameters among the animals such that the different variables may be reduced to a smaller set of independent components which could be plotted to reveal groupings of the animals. Cluster analysis was therefore used to

classify the tick-infested animals into distinct groups of resistance status for each infestation and according to the stage of ticks used. Analysis of variance (ANOVA) was used for the determination of the differences between means. The T-test and Student's Newman-Keuls test at the significance level of 0.05 were used. For normalization, arcsin-transformation was performed on all the data. All analyses were carried out using the SAS/STAT (1987) package.

CHAPTER FOUR

RESULTS

4.1 Repeated infestations of Boran cattle with *A. variegatum*

4.1.1 Infestations with the nymphal stage

Out of the twenty Boran cattle, two died of trypanosomosis and two of meteorization. The different parameters recorded for quantifying the acquisition of immunity to the tick feeding are shown in Table 1. The corresponding abbreviations will be used throughout the text. The different means and standard deviations of the different parameters are presented in Table 2. At the start of the experiments all parameters were given equal weight in determining the resistance status. By the means of the Principal Component Analysis (PCA) procedure, seven linear combinations of the different parameters or Principal Components (Prin) or axis corresponding to the earlier stated parameters were derived. Table 3, showed that all the Prins were characterized by an eigenvalue which is the value of the variance explained by a principal component (Prin) out of the overall variance. Only the first four Prins with eigenvalues greater than 0.9 were considered. Here the first Prin accounts for 39.24% of the total variation while the second accounts 17.27%. The third and fourth accounted respectively for 14.2 and 13.11%.

Table: 1 Abbreviations given to the biological parameters of *Amblyomma variegatum* used for the assessment of acquired resistance in Boran cattle

Parameters	Biological parameters
PENGD	Percentage of nymphs engorged out of 100
PMLTD	Percentage of nymphs moulted out of the harvested
PUMLTD	Percent of nymphs which did not moulted
PDEAD	Percentage of nymphs which died before and after feeding
ENGWT	Engorgement weight of harvested nymphs
FPR	Feeding period (Time taken to feed)
MPR	Moulting period (Time taken to moult into adults)

Table: 2 Means and variations of different biological parameters of tick *A. variegatum nymphs* after repeated infestations on Boran cattle

Parameters (Means±Sd)*	Infestations				
	1	II	III	IV	V
PENGD (%)	67.53±7.89	68.34±6.70	50.09±6.21	50.09±13.45	54.15± 14.24
PMLTD (%)	59,92+9.01	65.12±6.36	36.81 ±4.19	42.62±14.32	50.63±12.51
PUMLTD (%)	13.31 ±5.78	10.01 ±5.25	27.94±5.77	19.20±4.17	11.45±4.36
PDEAD (%)	25.70±9.36	22.56±4.89	52.80±4.95	47.38±14.32	38.75±12.16
ENGWT (g)	0.653±0.009	0.657±0.01	0.643±0.008	0.642±0.008	0.646±0.009
FPR (days)	7.78±1.27	9.36±1.84	16.74±3.99	17.10±4.97	14.11 ±4.18
MPR (days)	23.78±1.09	27.43±1.48	22.57±1.80	23.52±7.30	22.06±1.3

Table: 3 Eigenvalues of the selected axes by nymphal infestation

Infestation	Axes	Eigenvalue	% Variation accounted for	Cumulative percentage
1	1 II III IV	2.74698 1.20913 1.00036 0.91767	39.24 17.27 14.29 13.11	39.24 56.52 70.81 84.00
II	1 II III	3.11686 1.81952 1.01305	44.53 25.99 14.47	44.53 70.52 84.99
III	1 II III	2.54040 1.83842 1.33005	36.29 26.26 10.00	36.29 62.55 81.55
IV	1 II III	3.39456 1.03906 0.94378	48.49 14.84 13.48	48.49 63.33 76.82
V	1 II III	3.09553 1.59789 0.94818	44.22 22.83 13.54	44.22 67.04 80.59

The results of the second infestation showed that the variation explained by Prin 1, 2 and 3 were respectively 44.53, 25.99 and 14.47%. After the third infestation, the percentage explained by axis 1, decreased, though still higher, while that of axes 2 and 3, shot up denoting a more active participation of axes 2 and 3 in the acquisition of the resistance. The results of the 4^{th} and 5^{th} infestations, showed that axis 1 accounted for more than 40% in the acquisition of the resistance. Since the aim of our investigation, was to bring out the first important parameters which could be used in the quantification of the acquisition of the resistance in cattle and to simplify the analysis, only the first two Prins which from the second infestation, explained more than 60% of the total variation had been examined further.

In determining the major parameters which should be considered within the two axes, the high loads were computed and displayed in Table 4. It showed that PENGD and PMLTD, PDEAD which made up axis 1 are opposed in each infestation whereas PUMLTD, FPR or MPR dominated the second principal component in each of the infestations. Load on MPR, was higher than those on FPR and PUMLTD. A plot of Prin 1 dominated by PENGD, PMLTD and PDEAD versus Prin 2 consisted of FPR, MPR is shown in Fig.la. Fig. 1a which represents the plot of the different values of Prin 1 made up by PENGD, PDEAD and PMLTD against values of Prin 2 dominated by PUMLTD, FPR and MPR, showed that cattle M and N, as

Table: 4 Scores of the major parameters on the selected axes after nymphal successive infestations

Axis	Infestations -				
	1	II	III	IV	V
1	Pengd(0.483) Pmltd(0.554) Pdead(-.571)	Pengd(-.533) Pm!td(-.546) Pdead(0.538)	Pengd(.556) Pm ltd (.56) Pdead(-.558)	Pengd(.516) Pmltd(.529) Pdead(-.529)	Pengd(.553) Pmltd(.558) Pdead(-.557)
II	Pumltd(.787) Fpr(.469)	Pum!td(-.41) Fpr(0.648) Mpr(0.615)	Pumltd(.422) Fpr(-.572) Mpr(.576)	Pumltd(.44) Mpr(.838)	Fpr(.62) Mpr(-.59)
III	Engwt(.971)	Engwt(0.96)	Pumltd(.605) Engwt(-.489)	Pumltd(.789) Engwt(-.423)	Pumltd (0.92)
IV	Pumltd (-.409) Fpr(0.865)	"	"	”	

well as I and F fall in different groups. The distribution of the animals according to the level of resistance after the second infestation (Fig. 1 b), showed that U has a higher values of Prin 2 while Q and G, with lower Prin 2 and higher Prin 1 values, belong to different groups. A plot of values of Prin 1 and Prin 2 after the third infestation displayed in Fig. 1c showed that cattle C with lower values of Prin 1 and higher Prin 2, forms its own group which was different from the bulk of the other animals. P, I and A which had higher value for Prin 1 and lower values for Prin 2.

Results of infestation 4 in Fig. 1d, showed that animal B, with negative coordinates on Prin 1 and Prin 2, followed a trend different from the group composed of animals U, R, P, S, N, F, J, C and H; the latter different from the cluster of cattle Q, L, E, K, A and O. Fig. 1 e shows that B belongs to a different group from that of cattle O represented by the highest Prin 1 and the lowest Prin 2. Animals U, Q, G, H, S fall in a group different from that of L, A, C, K, I, P, N and R.

The cluster analysis produced three main clusters in each infestation. Group I was composed of animals which allowed fewer ticks to engorge and moult. The feeding process on those animals, took longer time. Group III was consisted of animals which allowed significantly ($P < 0.05$) more ticks to feed and moult than the group I. Ticks which were fed on those animals showed a lower death rate. Group II, was that of animals with

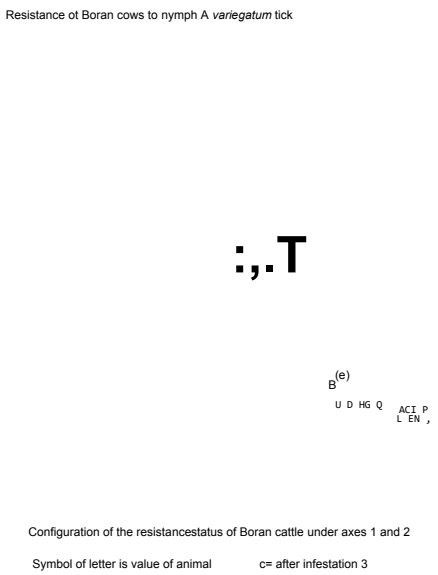

Fig. 1 Configuration of the resistance of Boran breed cattle repeatedly infested with *A. variegatum* nymphs under axes 1 and 2.

Table : 5 **Resistance level of animals by nymphal infestation number and group composition**

Groups (Clusters)	Infestations				
	1	II	III	IV	V
1 Highly-resistant animals	A, B, H, L, 0, Q, U	U	H, N, G, B, R, L, C	P, U, N	B, Q, U, D
II Moderately-resistant animals	N	A, B, N, L	P, J, A, 1	R, S, 1, C, H, B	L, A, C, N, P, E, G, R, H, S
III Least-resistant animals	C, D, G, P, R, S, E, 1	0, R, E, H, S, P, C, 1, D, G, Q	Q, E, S, M, 0, U	A, 0, Q, L, G, E, D	0

values of parameters falling between those of the two previous groups.

The different groupings after each infestation, are shown in Table 5. After infestation I, cattle, Q, O, H, L, B, U, and A were put in group I while N falls in group II, Group III was composed of E, R, S, D, P, C, G, and I. The clustering of the animals after second infestation, showed that animals U, belongs to group I and N, A, L, and M, to group II, Members of group III were O, R, E, H, S, P,C, I, D, G and Q.

The clustering results of the third challenge, showed that cattle N, G, B, R, L, D and C were in cluster I while P, I and A were in cluster II. Q, E, S, O and U were in the third cluster.

Examination of the results obtained after the fourth infestation showed that P, U and N were grouped in group I while R, S, I, C, H, and B were gathered in cluster II. Animals A, O, Q, L, G, E and D were allocated the third group. After the fifth infestation, B, Q, U, and D were put in group I while O was alone in group III. All the other animals were allocated to group II.

Examination of Tables 6, 7 and 8 with Figs. 2A and B revealed that after successive nymphal infestations, cattle of high , medium and low resistance allowed significantly ($P < 0.05$) fewer nymphs to engorge and moult after engorgement. There was a significant increase ($P < 0.05$) in the PDEAD and in the FPR. The engorgement weight was also reduced at the-same significance level.

Table: 6 Means and variations of the major biological parameters of *A. variegatum* nymphs after repeated insfestations on high tick-resistant Boran cattle

Biological parameters (Means+SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	87.50±2.10a	87.50±1.04a	54.75±2.25b	34.5+1,85c	49.75+11 .Obc
PMLTD (%)	89.09±2.98a	93.00±5.17a	62.35±3.14b	57.16±7.37b	92.32+2.63a
PUMLTD(%)	7.13±0.92a	6.70+5.17a	38.63±3.25b	43.01 ±7.73b	7.67±2.63a
PDEAD (%)	12.50±2.10a	12.5+1.04a	66.00±1.41b	80.50±2.33c	52.25±9.51d
FPR (days)	8.05±0.00a	9.80±0.47a	17.09±0.93bc	18.63±1,30b	14.56+1.08c
ENGWT (g)	0.051 ±0.001 a	0.059±0.001b	0.039±0.001c	0.0322±0.00d	0.043+0.000e
MPR (days)	23.45±0.19a	27.90±0.27b	22.57±0.21ac	23.58±0.24a	22.07+0.47C

*Data were analysed by analysis of variance and SNK test
Means in the same row not followed by the same letter are statistically different ($P < 0.05$)

Table: 7 Means and variations of the major biological parameters of *A. variegatum* nymphs after repeated infestations on moderate-tick resistant Boran cattle

Biological parameters (Means±SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	82.00±2.31a	81.33 ± 8.41a	62.67±6.36a	43.75±15.70a	52.50±6.70a
PMLTD (%) ■	95.87±1.90a	95.15 ± 3.73a	60.89±10.96a	62.64±12.11a	86.27±4.04a
PUMLTD (%)	4.56±2.30a	4.85 ± 3.73a	39.11±10.96b	34.53±10.33a	13.03±4.02a
PDEAD (%)	23.33±7.42a	18.67 ± 8.41a	62.67±6.36b	67.25±14.75b	52.00±5.76ab
FPR (days)	7.34±0.39a	8.98 ± 0.28a	17.30±1.37bc	18.88±1.10b	14.62±1.15c
ENGWT (g)	0.052±0.001a	0.057±0.001a	0.036±0.002b	0.037±0.002b	0.041 ±0.001 b
MPR (days)	23.97±0.23a	27.24 ± 0.16b	22.73+0.36C	24.02±0.43a	22.28±0.19c

*Data were analysed using analysis of variance and SNK test
Means in the same row not followed by the same letter are significantly different ($P < 0.05$)

Table: 8 Means and variations of the major biological parameters of *A. variegatum* nymphs after repeated infestations on low tick-resistant Boran cattle

Biological parameters (Means±SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	77.50±4.99ab	87.50±4.27a	55.75±7.12b	62.25±11.57b	55.00±2.41 b
PMLTD (%)	92.17±4.23a	95.37±0.91a	57.63±3.38b	78.75±8.80a	96.27±0.93a
PUMLTD (%)	6.64±4.32a	4.63±0.91a	41.30±4.40b	26.55±15.08b	3.73±0.93a
PDEAD (%)	22.25±4.75ac	14.00±3.67a	64.75±8.04b	48.25±12.26bc	46.75±2.84bc
FPR (days)	7.77±0.442a	8.69±0.54a	16.67±0.00bc	17.75±2.36c	13.09±0.71b
ENGWT (g)	0.053±0.00a	0.059±0.002a	0.036±0.002b	0.037±0.003b	0.042±0.000b
MPR (days)	23.76±0.12a	26.60±0.67b	22.57±0.21 a	23.79±0.69a	22.18±0.26a

*Data were analysed by analysis of variances and SNK test
Means in the same row followed by the same letter are not significantly different (P > 0.05)

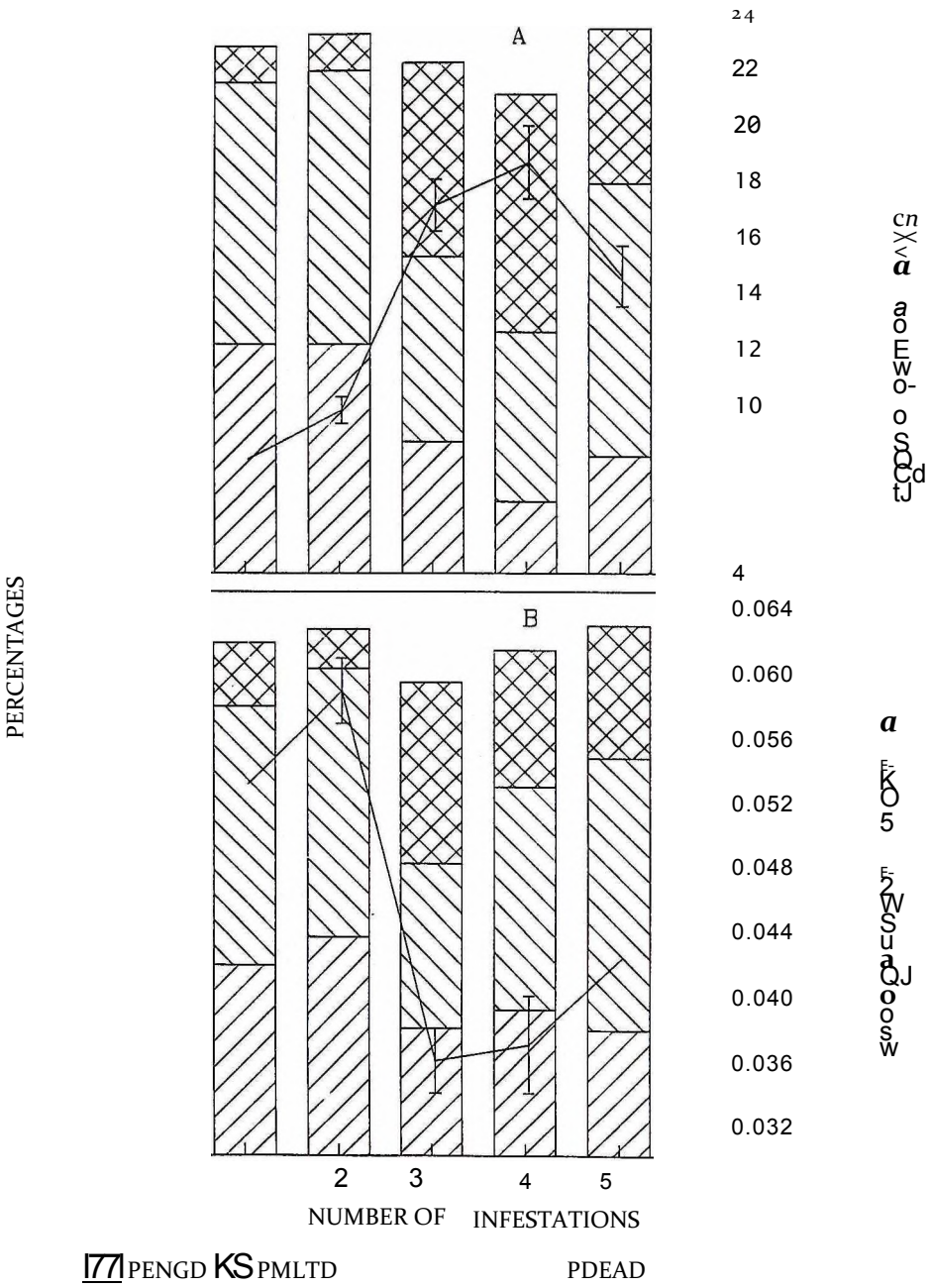

Fig. 2 Means and variations of some biological parameters of A *variegatum* nymphs after repeated infestations on Boran cattle.

B = Low-resistant group

4.1.2 Infestations with adult stages

On Table 9 are shown the different biological parameters used in the assessment of Boran steer resistance to the adult stage. The variations of the means of the different biological parameters are shown in Table 10. Axes with eigenvalues greater than 0.8 were retained for each infestation. As with the nymphs, the first four axes were retained. They explained respectively from infestation 1 to 5 about 85%, 82%, 80%, 88% and 81% of the total variations from infestations 1 to 5 respectively (Table 11). Throughout the infestations the high loads in absolute terms were mostly on PENGD, PDEAD and POVIP when axis 1 was considered. Axis 2 was consistently made up by ENGWT and FPR. There was no consistency in the make up of axes 3 and 4 which was shared by PHATCH and POVPR (Table 12). As in the same way with the nymphal stage, values of Prin 1 were plotted against values of Prin 2. The diagrammatic plot of the first infestation results, (Fig.3a) showed that M and B belong to different groups. The grouping of the remaining animals was not clear Fig.3b. The plot of Prin 1 versus Prin 2 showed that H is quite distinct from N, F, A, I, Q, T and from the others R, S, P, B and K. After the third infestation (Fig.3c), animal G made itself conspicuous from the bulk of the other animals.

Table : 9 Abbreviations given to the biological parameters of *Amblyomma variegatum* adults used for the assessment of acquired resistance in Boran cattle

Variables	Biological parameters
PENGD	Percentage of adults engorged out 20
PDEAD	Percentage of adults which died before and after feeding
POVIP	Percentage of adults which oviposited after feeding
PEACWT	Percentage of adults which engorged above the critical weight
PHATCH	Hatchability of eggs which were laid by the harvested ticks
FPR	Feeding period
POVPR	Preoviposition period
ENGWT	Engorgement weight
EGGMASS	Eggmass

Table : 10 Means and variations of different biological parameters of tick *Amblyomma variegatum* adults after repeated infestations on Boran cattle

Parameters (Means±Sd)	Infestations				
	I	II	III	IV	V
PENGD (%)	59.35±22.52	88.11 ±21.33	85.86±11.73	75.34±14.94	72.87±18.97
PDEAD (%)	40.65±22.52	8.12±11.74	14.14±11.73	24.69±14.98	27.13± 18.97
POVIP (%)	50.71 ±18.89	80.18±16.48	76.90±12.19	70.97±14.54	63.26±18.57
FPR (days)	21,24±6.7	16.85±6.26	17.49±7.43	23.36±9.38	25.38±9.52
ENGWT (g)	2.00±1.11	2.57±1.28	2.34±1.16	2.65±1.076	2.23±1.01
EGGMASS(g)	1.13±0.55	1,58±0.48	1,34±0.48	1.31 ±0.53	1.07±0.44
PEACWT (%)	30.89±21.57	66.19±20.69	55.58±14.35	55.38±17.94	41.10±14.46
PHATCH (%)	46.68±30.47	48.16±27.09	18.92±16.60	12.20±14.54	23.93±21.16
POVPR (days)	12.01 ±5.64	12.52±5.31	10.04±3.77	12.57±4.73	10.97±4.48

Fig. 3 Configuration of the resistance status of Boran breed cattle repeatedly infested with *A. variegatum* adults under axes 1 and 2.

Table : 11 Eigenvalues of the selected axes by infestation using the adult stage

Infestation	Axes	Eigenvalue	% Variation accounted for	Cumulative percentage
I	I	3.43598	38.18	38.18
	II	2.24294	24.92	63.09
	III	1.09436	12.15	75.25
	IV	0.87309	09.70	84.96
II	I	3.48915	38.76	38.76
	II	2.00153	22.23	61.00
	III	1.06732	11.85	72.86
	IV	0.83073	09.23	82.09
III	I	3.04357	33.81	33.81
	II	2.02189	22.46	56.28
	III	1.11631	12.40	68.68
	IV	1.01532	11.28	79.96
IV	I	4.04403	44.93	44.93
	II	2.14302	23.81	68.74
	III	0.87511	09.72	78.46
	IV	0.86243	09.58	88.05
V	I	3.07579	34.17	34.17
	II	2.13573	23.73	57.90
	III	1.10555	12.28	70.19
	IV	0.96963	10.77	80.96

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Table : 12 Scores of the major parameters on the selected axes after successive infestations with the adult stage

Axis	Infestations				
	I	II	III	IV	V
I	Pengd(.470) Pdead(-.470) Povip (.521) Peacwt(.454)	Pdead(-.426) Povip(.470) Peacwt(.477)	Pengd(.534) Pdead(-.534) Povip(.543)	Pengd(.471) Pdead(-.471) Povip(.456) Peacwt(.467)	Pengd(.548) Pdead (-.548) Povip(484)
II	Engwt(.549) Fpr(-.412) Eggmass(.461)	Engwt(.401) Fpr(-.544) Povpr(.527)	Engwt(.593) Fpr(-.440) Eggmass(.522)	Engwt(.490) Fpr(-.522) "	Engwt(.536) Fpr(-.499) Eggmass (.450)
III	Phatch(.748) Povpr(.542)	Eggmass (.785)	Povpr(.778) Eggmass(-.453)	Phatch(.403) Povpr(.850) "	Phatch(.412) Povpr(.539) "
IV	Phatch(.584) Povpr(.692) Engwt(-.402)	Phatch(.790)	Phatch(.885)	Phatch(-.78) Engwt(.400)	Phatch(-.601) Povpr(.755)

The results of the fourth infestation in Fig. 3d, showed that B and G belong to one group which is opposite to the group formed by I, L, P, and O. When we examined Fig. 3e, cattle I, C, L, O, and N, belong to different groups.

The same way Fastclus analysis produced 3 groups following infestations with the adults. The different groupings obtained after successive infestations are displayed in Table 13.

Summary of the ranks attributed to each animal after each infestation with the nymphal or adult stage and the final rank of the corresponding animal are respectively shown in Tables 14 and Table 15. After infestation with the adults, animals, B, H, E, R, S, were finally ranked in group I while G, D, I, P and U were allocated the intermediate position. Group III was consisted of cattle A, L, O, Q and C.

Results of the challenge with adult stage over the infestations showed that PENGD and the PEACWT decreased from the 4th infestation with the high resistance group (Table 16) and Fig. 4. There was also an increase in the FPR and the PDEAD from the 4th infestation. Results of the moderate and low resistance group are in Tables (17 and 18).

Table: 13 Resistance level of animals by infestation with adult stage and group composition

Groups (Clusters)	Infestations				
	I	II	III	IV	V
I Highly-resistant animals	B, E, I.O.R,	E,H,P,R,S	G	B, C, D, E, H, S	D, E, G, I, P, Q, R, S
II Moderately -resistant animals	D, H, S, L, U, N, P	B	A, B, C, D, H, N, U	A, G, N, Q, R, U	H, U, B
III Least-resistant animals	A, C, G, Q	A,C, D, G, I, L, N, Q, 0, U	E, I, L, P 0, Q, R, S	I, L, 0, P	A, 0, C, L, N

Table : 14 Rank of animals according to their resistance status after repeated nymphal infestations

Animal Number	Rank /Infestations					
	I	II	III	IV	V	Mean Cluster
A	1	2	2	3	2	2
B	1	2		2	1	1.4
C	3	3		2	2	2.2
D	3	3		3	1	2.2
E	3	3	3	3	2	2.8
G	3	3	1	3	2	2.4
H	1	3	1	2	2	2
I	3	3	1	2	1	1.46
L	1	2	1	3	2	1.8
N	2	2	1	1	2	1.6
0	1	3	3	3	3	2.6
P	3	3	2	1	2	2.2
Q	1	3	3	3	1	2.2
R	3	3	1	2	2	2.2
S	3	3	3	2	2	2.6
U	1	1	3	1	1	1.4

1 —–> 1.67] = Group I
1.67 ---> 2.34]= Group II
2.34 --> 3.0] = Group III

Table: 15 Ranks of animals according to their resistance status after repeated infestations with adult ticks

Animal Number	Rank per infestation					
	I	II	III	IV	V	Final Rank
A	3	3	2	2	3	2.6
B	1	2	2	1	2	1.6
C	3	3	2	1	3	2.4
D	2	3	2	1	1	1.8
E	1	1	3	1	1	1.4
G	3	3	1	2	1	2
H	2	1	2	1	2	1.5
I	1	3	3	3	1	2.2
L	2	3	3	3	3	2.8
N	2	3	2	2	3	2.4
0	1	3	3	3	3	2.6
P	2	1	3	3	1	2
Q	3	3	3	2	1	2.4
R	1	1	3	2	1	1.6
S	2	1	3	1	1	1.6
U	2	3	2	2	2	2.2

1 -—> 1.67] Group I
1.67 —> 2.34] Group II
2.34 —-> 3.0] Group III

Table: 16 Means and variations of major biological parameters of tick *A. variegatum* adults after repeated infestations on high tick-resistant Boran cattle

Biological parameters (Means±SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	27.50±8.54a	72.50±10.10a	85.00±6.12b	67.50±8.78b	67.5±7.77b
PDEAD (%)	63.91 ±12.98a	27.50±10.10ab	15.00±6.12b	32.50±8.78ab	32.5±7.77ab
POVIP (%)	95.00±5.00	80.26±7.31	90.55±3.19a	87.69±1.92	85.76±6.95
FPR (days)	21.38+1.55ab	16.97±1,29b	16.31±0.52b	23.22+3.50ab	28.95±3.65a
ENGWT (g)	2.27±0.38a	2.02±0.18a	2.69±0.23a	2.74±0.24a	2.21 ±0.05a
EGGMASS(g)	1.11±0.38a	1.30±0.11a	1.58+0.19a	1.23±0.18a	0.89+0.06a
PEACWT (%)	48.33±21.15a	53.43±6.36a	81.80±6.27a	69.63±7.56a	51,23±2.69a

*Data were analysed by analysis of variance and Student-Newman-Keuls test (SNK)
Means in the same row followed by the same letter are not statistically different (P > 0.05)

Table: 17 Means and variations of the major biological parameters of *A. variegatum* adults afte repeated infestations on moderate tick resistant Boran cattle

Biological parameters (Means±SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	45.00 ± 9.57a	85.00 ± 6.12a	73.75 + 7.74a	61.46 ± 10.07a	50.00 ± 11.73a
PDEAD (%)	55.00 ± 9.57a	15.00 ± 6.12a	26.25 ± 7.74a	38.75 ± 10.07a	50.00 ± 11.73a
POVIP (%)	96.43 ± 3.57a	90.55 ± 3.19a	88.13 ± 5.44a	97.16 ± 2.41a	90.83 ± 5.33a
FPR (days)	18.56 ± 1.62a	16.31 ± 0.52a	17.87 ± 1.24a	24.13 ± 2.33b	25.65 ± 0.52b
ENGWT (g)	2.23 ± 0.36a	2.60 ± 0.32a	2.07 ± 0.19a	2.53 ± 0.12a	1.85 ± 0.24a
EGGMASS(g)	1.28 ± 0.23a	1.58 ± 0.21a	1.17 ± 0.13a	1.10 ± 0.07a	0.98 ± 0.18a
PEACWT (%)	71.19 ± 14a	69.15 ± 11.5a	57.80 ± 7.40a	61.27 ± 6.29a	40.70 ± 10.55

*Data were analysed by analysis of variance and SNK
Means followed by the same letters in the same row are not statistically different (P > 0.05)

ible : 18 Means and variations of the major biological parameters of *A. variegatum* adults after repeated infestations on low tick-resistant Boran cattle

Biological parameters (Means+SE)*	Infestations				
	1	2	3	4	5
PENGD (%)	73.75 + 8.51a	96.25 ± 3.75a	88.75 ± 3.14a	77.5 ± 6.29a	76.25 ± 14.20a
PDEAD (%)	25.00 ± 8.66a	6.40 ± 6.40a	11.25 ± 3.14a	22.5 ± 6.29a	23.75 ± 14.20a
POVIP (%)	88.54 ± 7.86a	94.56 ± 2.41a	86.08 ± 3.50a	91.84 ± 1.56a	93.17 ± 2.49a
FPR (days)	22.71 ± 0.30a	15.82 ± 0.54b	18.80 ± 1.47ab	21.23 ± 1.85ab	24.31 ± 2.21a
ENGWT (g)	1.91 ± 0.28a	2.70 ± 0.20a	2.05 ± 0.16a	2.60 ± 0.26a	2.30 ± 0.33a
EGGMASS(g)	1.08 ± 0.08a	1.71 ± 0.17b	1.22 ± 0.10a	1.47 ± 0.04ab	1.08 ± 0.14a
PEACWT (%)	52.74 ± 13.20a	78.90 ± 4.17a	56.02 ± 8.44a	70.49 ± 9.97a	55.72 ± 12.94a

*Data were analysed by analysis of variance and SNK test.
Means in the same row followed by the same letter are not statistically different (P > 0.05).

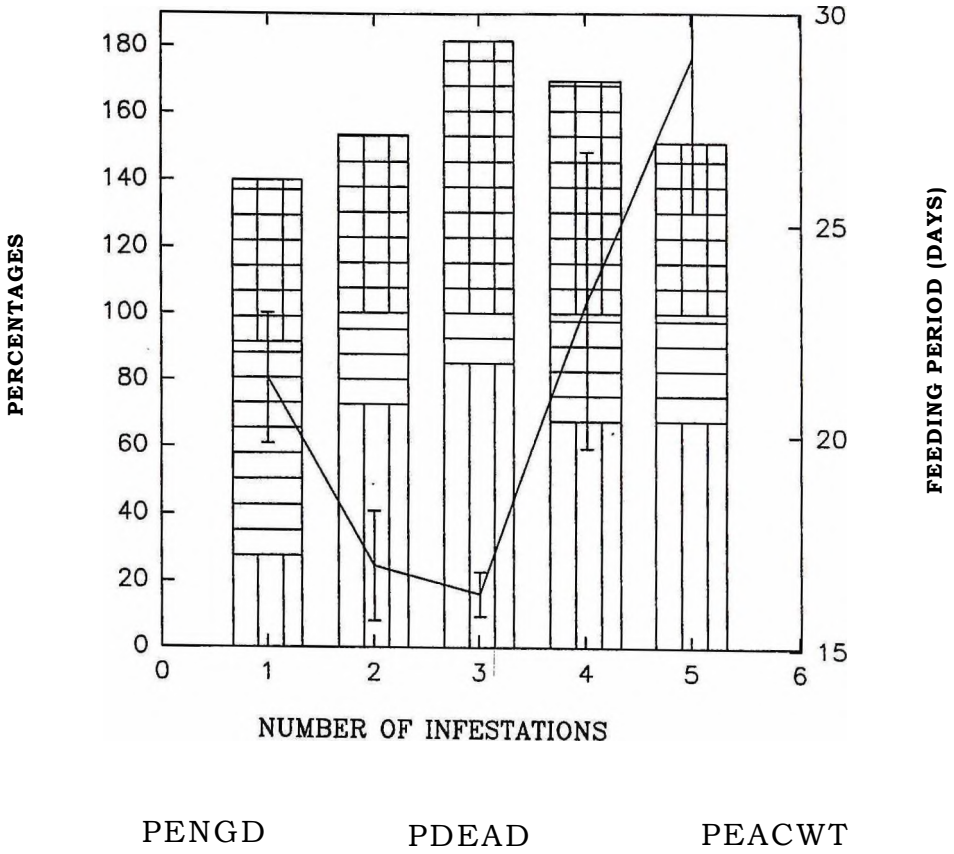


Fig. 4 Means and variations of some biological parameters of *A. variegatum* adults after repeated infestations on high tick-resistant Boran cattle.

4.1.3 Effects of parasitism on the tick-host interactions

4.1.3.1 Effects of parasitism on the biology of the ticks.

Two steers belonging to group I and III (high and low-tick resistance) died after 25 days following trypanosome-infections before ticks were fed on them. However, a significant reduction ($P < 0.05$) in the PENGD and the ENGVVT of the ticks fed on *T. congolense*-infected cattle was noticeable in all the 3 groups. PMLTD after trypanosome-infections was lower though not significantly different from that observed after the 5th infestation. In the high-resistance group, for example, Table 19 and Fig. 5A, the figures were respectively 21% ± 3.00, 70% ± 4.166 and 0.0347g ± 0.002 as compared to the values before trypanosome infection. There was also a significant increase ($P < 0.05$) in the FPR and the PDEAD in all trypanosome-infected groups. The trend observed when adult ticks were fed on the infected animals was the same (Table 22). PENGD, POVIP and PEACWT were significantly reduced ($P < 0.05$) while FPR and PDEAD were significantly increased. Tables 20, 21, 23, 24 and Fig. 5B, display the results obtained from the moderate and low resistance group.

When *B.* 6/gem/na-infected blood was inoculated to the tick-immune steers we were not able to detect the parasites in the animals through

able: 19 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* nymphs fed on high tick-resistant Boran cattle

Parameters (Means±SE)*	Infestations						
	1	2	3	4	5	6	
						Post Trypano-somosis	Post *Babesia* infection
PENGD (%)	87.5±2.10a	87.5+1.04	54.75±2.25b	34.5±1.85cb	49.75+11.09b	21.00±3.00c	32.5±8.5cb
PMLTD (%)	89.09±2.98a	93.00+5.17a	62.35+3.14b	57.16+7.37b	92.32±2.63a	70.83+4.17ab	84.50+11.33a
PUMLTD (%)	7.13±0.92a	6.99±5.17a	38.63±3.25b	43.01+7.73b	7.67±2.63a	20.83+4.17a	15.5+11.33a
PDEAD (%)	12.5±2.10a	12.5+1.04a	66.00±1.41cb	80.5±2.33cd	52.25±9.51b	85.00+3. OOd	73.5±3.5cd
ENGWT (g)	0.051 ±0.001 a	0.059±0.001b	0.039±0.001 cd	0.032±0.008e	0.043±0.006c	0.035±0.002de	0.032±0,003e
FPR (days)	8.05+0.31 a	9.80±0.47a	17.09±0.93cb	18.63+1.30b	14.56+1.08c	30.60±0.18d	31.6±3.22d
MPR (days)	23.45±0.19a	27.90+0.27b	22.57+0.21 ac	23.58±0.24a	22.07±0.47e	23.88+0.87a	25.56+ 0.99d

*Dala were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different at (P > 0.05)

able: 20 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* nymphs fed on fed on moderate tick-resistant Boran cattle

	Infestations						
Parameters (Means±SE)*	1	2	3	4	5	6	
						Post Trypano-somosis	Post *Babesia* infection
PENGD (%)	82.00±2.31a	81.33±8,41a	62.67±6.96ac	43.75±15.70bc	52.5+6.70ac	9.50±0.5d	13.5+3.5bd
PMLTD (%)	95.87+1,90a	95.13±3.73a	60.89+10.96b	62.64±12.11	86.27±4.05ab	68.33± 1.67b	61.47±8.53b
PUMLTD (%)	4.56±2.30a	4.85+3.73a	39.11±10.96b	34.53±10.33bc	13.03±4.02ac	31.67±1.67bc	38.53±8.53b
PDEAD (%)	23.33±7.42a	18.67± 8.41a	62.67+6.36b	67.25±14.76bd	52.00+5.76b	93.50±0.50c	92.00±1.00cd
ENGWT (g)	0.052±0.00ae	0.057+0.001 a	0.036+0.002bd	0.037±0.002bd	0.040±0.001be	0.031 ±0.003cd	0.024±0.003c
FPR (days)	7.34±04a	8.98±0.28a	17.30±1.37b	18.88±1.1b	14.62+1.1 Sab	30.55±10.11c	38.72±0.48d
MPR (days)	23.97±0.23a	27.24+0.16b	22.73±0.36c	24.02±0.43a	22.28±0.19c	28.37±0.12d	26,49+0.29b

*Data were analysed by analysis of variance and /-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

able: 21 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* nymphs fed on fed on low tick-resistant Boran cattle

	Infestations						
S Parameters (Means±SE)*	1	2	3	4	5	6	
						Post trypano-somosis	Post *Babesia* infection
PENGD (%)	77.50+4.99a	87.5± 4.27a	55.75±7.12abc	62.25±11.57ab	55.00±2.41 abc	24.00± 8.00c	27.5± 24.5bc
PMLTD (%)	92.17± 17ae	95.37± 0.91a	57.63+3.38cd	78.75±8.80be	96.27±0.93a	68.75± 6.25bd	44.55±11.22c
PUMLTD (%)	6.64± 4.32a	4.63+ 0.91a	41.30±4.40ab	26.55+15.08ab	3.73+ 0.93a	18.75+ 18.75a	55.45± 11.22b
PDEAD(%)	22.25± 4.75a	14.00± 3.67a	64.75±8.04bc	48.25±12.26b	46.75± 2.84b	83.00+ 7.00c	84.5± 13.50c
ENGWT (g)	0.053+0.001 a	0.059±0.003a	0.036+0.002bc	0.037±0.003bc	0.042+0.0006b	0.027± 0.001c	0.027± 0.0003c
FPR (days)	7.77± 0.44a	8.69±0.54ab	16.67±0.74cd	17.75± 2.36d	13.09±0.71bc	37.97± 4.03e	31,26± 1.74f
MPR (days)	23.76±0.12ab	26.60+0.67a	22.54± 0.07b	23.79±0.69ab	22.18±0.03b	22.40+ 2.03	25.72± 1.94a

*Data were analysed by Analysis of Variance and f-test
Means followed by the letters in the same row are not significantly different (P > 0.05)

Table: 22 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* adults fed on high tick-resistant Boran cattle

Parameters (Means±SE)*	Infestations						
	1	2	3	4	5	6	
						Post Trypano-somosis	Post Babesiosis
PENGD (%)	27.5±8.54a	72.5+10.1 Ob	85.00+6.12b	67.5±8.75bc	67.5±7.77bc	20±0a	37.5±12.5acb
PDEAD (%)	63.91±12.97bd	27.5±10.10ac	15.00+6.12dac	32.5+8,78dac	32.5± 7.77dac	80±0b	62.5+12.5abd
POVIP (%)	95.00±5.00ab	80.26+7.31 b	90.55±3.19ab	87.69±1.92ab	85.76±6.95ab	25 ± 0c	100±0a
PEACWT (%)	48.33±21.15abc	53.43±6.36abc	81,80±6.27b	69.63±7.56bc	51.23±2.69cab	25a	35.0+5. Oac
PHATCH (%)	45.64+10.03ac	63.88± 11.63a	16.60±3.13b	12.04±4.10b	34.13±11.23bc	0b	6.93±5.27b
FPR (days)	21.37+1.55a	16.97+1.29a	16,31±0.52a	23.22+3.50a	28.95±3.65a	56.75b	55.3±3.30b
POVPR (days)	12.42±0.94ac	16.51±0.57b	10.56±0.07a	13.31 ±1.15c	11,66±0.35ac	13ac	14.3+0.3 cb
ENGWT (g)	2.27+0.38ab	2.02+0.143ab	2.69±0.23ab	2.74±0.25a	2.21±0.05ab	0.87c	1.84+0.03b
EGGMASS (g)	1.11±0.38ab	1.30±0.115a	1.58±0.19	1.23±0.19ab	0.89±0.06ab	0.43b	0.49±0.08b

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 23 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* adults fed on moderate tick-resistant Boran cattle

Parameters (Means±SE)*	Infestations						
	1	2	3	4	5	6	
						Post Trypa-nosomosis	Post *Babesia* infection
PENGD (%)	45.0±9.57bc	83.75±4.27a	73.75±7.74ac	61.46±10.07abc	50.0±11.73bc	52.5±2.5bc	42.5+2.5b
PDEAD (%)	5.0±9.57ac	18.59±5.44b	26.25±7.74bc	38.75+10.08abc	50.0±11.73ac	47.5+2.5cab	57.5±2.5a
POVIP (%)	96,43±3.57a	85.99±12.32ab	88.13±5.44ab	97.16±2.41a	90.83+5.33a	65.91 ±15.91b	94.44±5.55a
PEACWT (%)	71.19±13.97a	69.15+11.54a	57.80±7.40ac	61.27±6.29ac	40.70+10.55abc	19.09+0.91 b	34.72±9.72bc
PHATCH (%)	48.39+5.81 a	38.31 ±12.35ac	20.35±6.54b	9.32±2.21b	16.59±5.80bc	4.75±2.75b	0± 0b
FPR (days)	18.56±1.62ae	16.79+2.14a	17.87+1.24ac	24.13±2,33ce	25.65±0.52c	48.10±2.10b	57.62±1.49d
POVPR (days)	13.27±0.74abc	14.18±0.60bc	11.02±0.22a	13.77±0.19abc	11.42±0.42ac	14.80±0.69b	15.35±2.22b
ENGWT (g)	2.23±0.36cb	2.60±0.32bc	2.07±0.19cab	2.53±0.12bc	1.85±0.24cab	1.33±0.34a	1.70±0.20ab
EGGMASS (g)	1.28±0.23ac	1.58 +0.21 a	1.17± 0.13ac	1.10± 0.07ac	0.98± 0.18bc	0.528± 0.08b	0.43± 0.03b

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 24 Effects of trypanosome or *Babesia* infection on the biological parameters of *A. variegatum* adults fed on low-tick resistant Boran cattle

	Infestations						
Parameters (Means±SE)*	1	2	3	4	5	6	
						Post try-panosomosis	Post babe-siosis
PENGD (%)	73.75+8.51ac	96.25±3.75a	88.75±3.15a	77.5±6.29a	76.25+14.2ac	47.5±7.5bc	20.0±20b
PDEAD (%)	25.00±8.66ac	6.40+6.397a	11.25+3.15ac	22.5±6.3ac	23.75±14.2ac	52.5+7.5bc	80+20.0b
POVIP (%)	88.50±7.86a	94.56+2.41 a	86.08±3.5a	91.84±1.6a	93.17+2.49a	51.14±23.86b	50.0±0b
PEACWT (%)	52.74±13.21	78.90±4.17	56.02±8.44	70.49±9.97	55.72±12.94	0	12.5±0
PHATCH(%)	38.13±9.49ac	48.22+2.90a	22.09±7.48cab	16.61±3.73cab	30.25±6.60abc	7.5±7.5bc	0b
FPR (days)	22.71 ±0.30a	15.82±0.54b	18.80±1.47ab	21.23±1.86ab	24.31 ±2.21 a	51.48±4.60c	60.75±0d
POVPR (days)	13.73±0.47a	. 13.92±0.42a	11,44±0.27a	12.74±0.58a	11.99+0.53a	23.33±7.66b	18.75+0b
ENGWT (g)	1.91+0.28a	2.70+0.54a	2.05±0.16a	2.60±0.26a	2.30±0.33a	0.77±0.02b	0.87+0b
EGGMASS (g)	1.08+0.09a	1.71+0.18c	1.22±0.10ac	1.47±0.04ac	1.08+0.14a	0.14±0.07b	0.36±0b

*Data were analysed by analysis of variance and f-test
Means with same letters in the same row are not significantly different (P > 0.05)

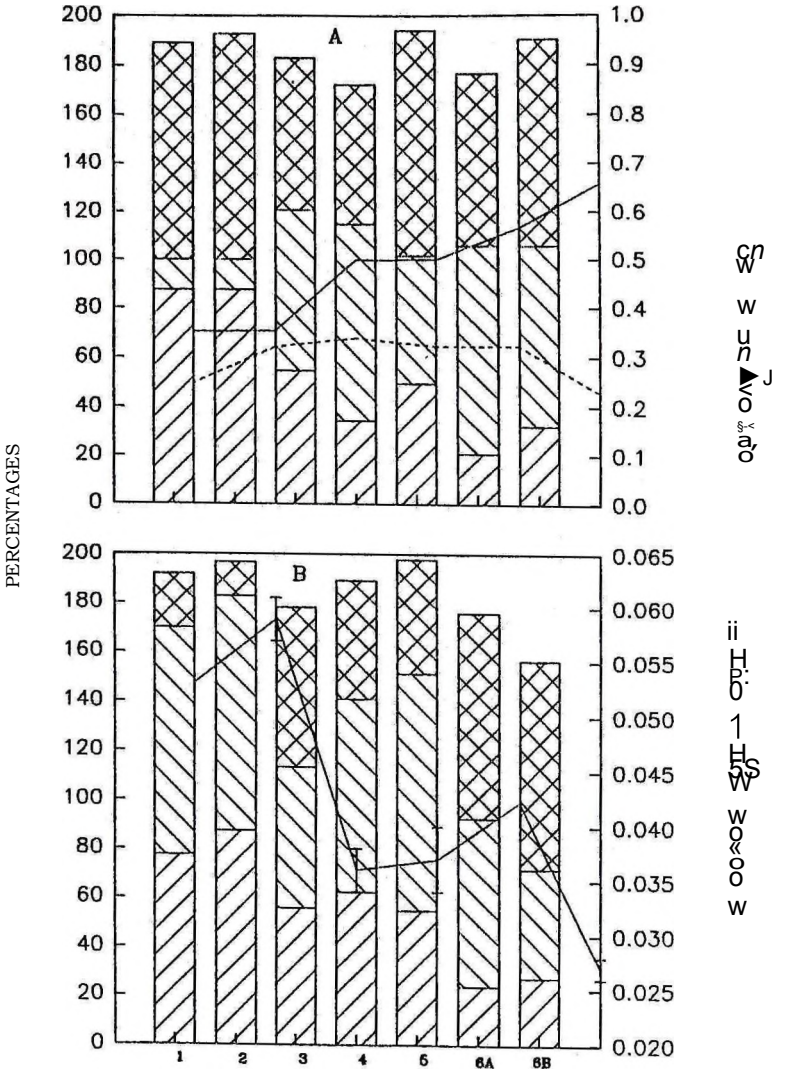


Fig. 5 Effects of haemoparasitism on the acquired immunity due to *A. variegatum* nymph infestations.

A = High-resistant group B = Low-resistant group

6A. Chart shows effects in tryps-infected cattle.

6B. Chart shows effects in Dafaes/a-inoculatod cattle.

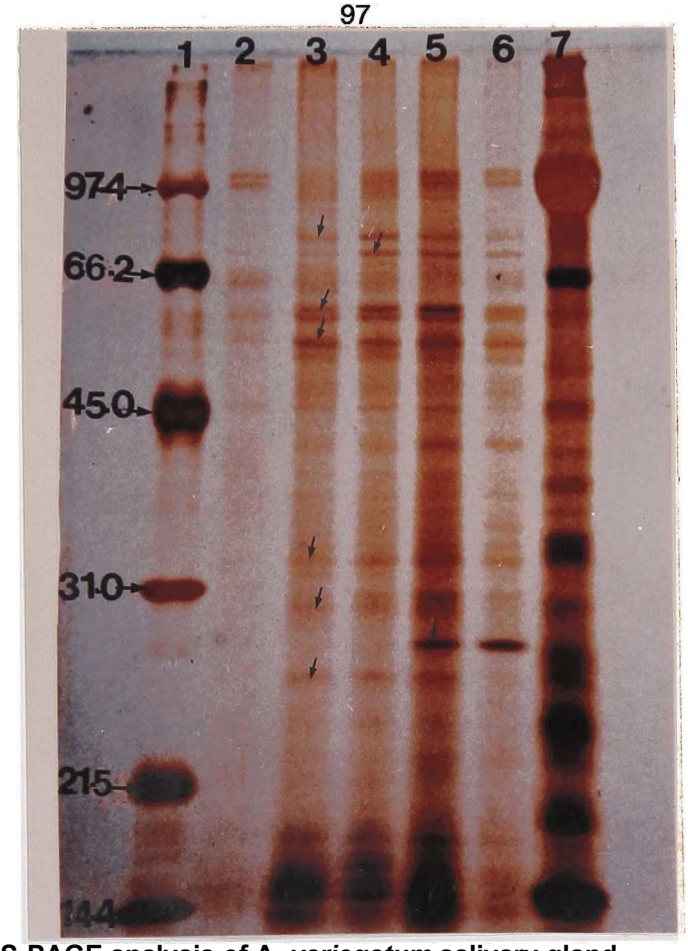


Fig. 6 SDS-PAGE analysis of A. *variegatum* salivary gland homogenates sequentially prepared at different feeding time interval and saliva using silver-staining. New bands are shown by an arrow.

Lane 1 = Molecular weight Standards (kDs);

Lane 2 = Day 0; Lane 3 = Day 3; Lane 4 = Day = 6

Lane 5 = Day 9; Lane 6 = Day 12; Lane 7 = Saliva

Giemsa-stained blood smears but we observed a rise in temperature for two days. The animals became lethargic and lost their stoutness and plumpiness. A significant reduction ($P < 0.05$) in the PENGD, the PMLTD, the ENGWT of the ticks fed on *Babesia* parasite-inoculated tick-immune steers was remarkable in groups I, II and III animals.

4.2 Immune responses to the tick infesl.ai.ions

4.2.1 SDS-PAGE analysis of salivary gland homogenates (SGA), Saliva and immunological detection of stage-specific proteins

Examination of the silver-stained proteins in the various homogenates and the saliva resolved under denaturing conditions (Fig.6) showed that

different polypeptides were synthesized while other disappeared during attachment and in the course of feeding. Protein bands of molecular weights (MW) 72.4, 63.0, 54.0, 48.0, 47.0, 33.0, 25.0, 17.0 and 16.5 kiloDaltons (kD), appeared in day 3 homogenate. The day 6 homogenate showed 3 additional proteins of 71.0, 38.5 and 33.0 kD. However it was the Day 9 homogenate which showed comparatively more proteins than the others with prominent band proteins of 87.0, 86.0, 72.4, 71.0, 63.0, 61.0 and 58.0 kD. Most of the protein bands found in the Day 9 extracts, were either faintly seen or absent in the Day 12 extracts.

Table: 25 Major protein changes in salivary gland homogenates and in saliva of *A. variegatum* during attachment and feeding

Band No	Esimated MW (Kd)	Day 0	Day 3	Day 6	Day 9	Day 12	Saliva
1	160.0			-	-	-	+
2	87.0	++	++	++	+++	+	++
3	86.0	++	++	++	+++	+	++
4	72.4		++	++	+++	+	±
5	71.0	±	+	+	+++	+	±
6	67.5				±		++
7	66.0	+	-				
8	63.0		+	+	+++	++	+
9	61.0	±	+	+	+++	++	+
10	58.0	+	+	+	+++	++	±
11	55.0						++
12	54.0		+	+	+	+	+++
13	49.5						+ i
14	48.0		+	+	+	+	+++
15	47.0		+	+	+	+	+++
16	44.0		+	±	+	+	+
17	38.5			+	+	+	+
18	33.0		±	+	+	+	
19	29.5	-	+	±	+	+	+
20	27.0	-			+	+	
21	25.0		+	+	+		
22	18.0	-	-				+
23	17.0	-	+	+	+	±	
24	16.5		+	+	+	-	+
25	15	±	+	+	+		++

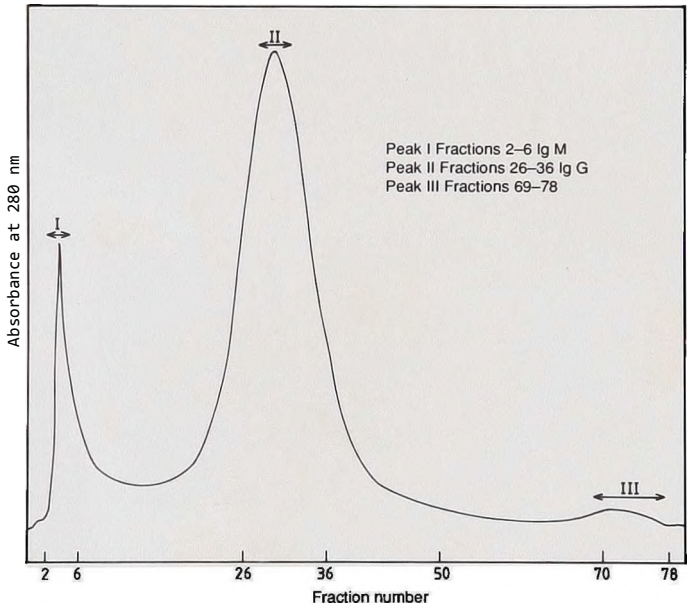


Fig. 7 Gel filtration elution profile of tick immune bovine serum through Bio-Gel^{R} A 1.5M.

The profile of the SDS-PAGE of the saliva, showed three major protein bands of 54.0, 48.0 and 47.0 kD The sequential changes in the protein synthesis is shown in Table 25. Fig. 6 showed that most of the proteins synthesised by the tick during feeding were present in the Day 9 homogenate. Consequently, the Day 9 homogenate was used.

4.2.2 Preparation of antibodies by gel filtration chromatography

Fig. 7 showed the elution profile of the serum precipitated with saturated ammonium sulfate and fractionated through Bio-Gel^{R}. According to the conditions used three peaks were obtained. The first, the void volume contains the IgM, the second, the IgGs and the third, the albumin.

4.2.3 Fractionation of the different IgGs isotypes by the gel affinity chromatography with Protein A-Sepharose 4B

The elution profile of fractions of the IgGs through affinity column packed with Protein A gel is displayed in Fig 8. Under the conditions employed two different peaks were obtained. The first one (the unbound), consisted of the IgG2 fraction while the second one, the bound fraction, was the IgG1. The silver-stained SDS-PAGE profile showing the purity of the two components could be seen in Fig 9. Two major bands respectively with average MW of 54 and 29 kD, corresponding to the heavy and light chains could be identified. In addition to those two chains, another polypeptide could be seen on the SDS-PAGE profile (Fig. 9).

4.2.4 Immunological detection of stage-specific proteins

4.2.4.1 The immunoblot test

The results of the immunoblotting experiment (Fig.10a and 10b) revealed that Day 9 SGA, NA and the SA, contained several proteins which were reactive to the post-infestation sera and their fractions. The whole serum after reaction with SGA showed more than 15 bands while 9 bands were recognized by the SA and fewer protein bands (5) picked by the NA. The unbound fraction (IgG2) bound poorly the antigens contained in the different extracts. The eluted fraction IgG1 reacted against 10 polypeptides from the SGA and 4 from the SA. There was no detectable reaction with the NA. The control serum did not show any reactive band under the conditions of the experiments (Fig. 10b) .

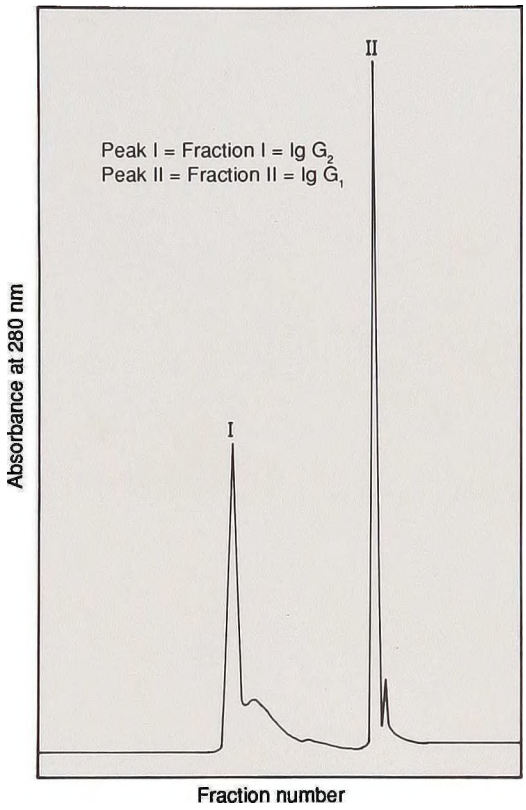


Fig. 8 **Gel affinity elution profile of tick-immune bovine serum through Protein A-Sepharose 4B[B].**

SDS-PAGE analysis of affinity-purified lgG1 and !gG2 using silver-staining.

Lane A = Whole serum; Lane B = lgM;
Lane C = lgGt; Lane D = lgG2;
Molecular weight are in kD.

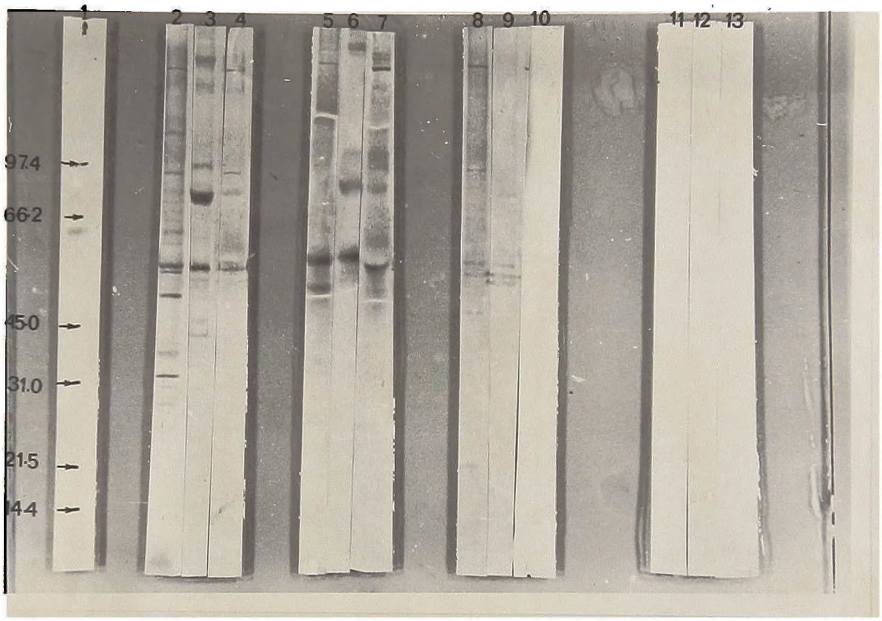


Fig 10a Immunoblot analysis of SGA, Saliva and Nymphal extract with whole bovine tick-immune serum, purified IgG1 IgG2.

Lane 1 = Molecular weight markers;
Lanes 2, 3, 4, respectively represent SGA, SA and NA strips probed with whole tick-immune serum;
Lanes 5, 6, 7, respectively represent SGA, SA and NA strips probed with tick-immune IgG1;
Lanes 8, 9,10 respectively represent SGA, SA and NA probed with tick-immune IgG2;
Lanes 11,12 and 13 were treated with tick-naive serum.

Fig. 10b Immunobiot analysis of SGA, Saliva and Nymphal extract with bovine anti-tick IgM.

Lane B = SGA probed with tick-immune IgM;
Lane C = SA probed with tick-immune IgM;
Lane D = NA probed with tick-immune IgM.

4.2.4.2 Quantification of antibody responses to the tick infestations

4.2.4.2.1 Kinetics of anti-tick IgG1 and IgG2 synthesis in tick immune Boran cattle and in parasitized-animals to *A. variegatum*

Repeated infestations of Boran cattle by simultaneously feeding nymphs and adults elicited synthesis of antibodies of IgG1 type detectable by ELISA test after the first infestation. A second tick challenge performed 30 days after the first, stimulated a higher secondary immune response in all the groups of animals as summarised in Tables 26, 27 and Fig. 5A. The third and fourth infestations raised the antibody synthesis level. By the fifth infestation, the ELISA reading had increased by two to three times when compared to the pre-infestation serum (Control 1) and to that of a tick-free bovine serum's (Control 2) optical density (O.D). A higher tick-specific IgG1 synthesis was detected in the serum after the 6th infestation when cattle were either carrying trypanosomes or injected with *Babesia* parasites (Fig.5A). However, cattle n° P in group II infected with *Babesia* showed a lower specific anti tick-specific antibody. There was no detectable tick-specific IgG2 in the tick immune animals before and alter trypanosome or babesia infections.

tble: 26 Serum antibody responses in ticMmmune and in *T. congolense* or *Babesia*-infected tick-immune Boran cattle to *A. variegatum*

i iroups	ELISA Mean 0. Ds by infestation at 450nm:							
animal Jode	Preinfestation Control 1	Post infestation 1	Post infestation 2	Post infestation 3	Post infestation 4	Post infestation 5	Post infesta-tion 6***	Control 2
I (79 + 294)	0.216* 0.256**	0.351 0.247	0.352 0.32	0.499 0.338	0.500 0.323	0.567 0.323	0.658 0.228	0.245 0.253
II (293 +96)	0.239 0.316	0.44 0.32	0.399 0.307	0.537 0.348	0.592 0.312	0.619 0.33	0.787 0.278	0.245 0.253
III (78 + 300)	0.214 0.248	0.341 0.237	0.342 0.257	0.477 0.264	0.474 0.291	0.514 0.3	0.648 0.232	0.245 0.253

* First row = optical density developed by anti-tick lgG1 in samples diluted 100 times
** Second row = optical density developed by anti-tick IgM in undiluted samples
*** Infestation 6: Ticks were allowed to feed on *T. congolense* or Ba&es/a-infected animals to repletion

able: 27 Serum antibody responses in tick-immune and in *T. congolense* or *Babesia*-infected tick-immune Boran cattle to *A. variegatum*

I (306+307)	0.235	0.341	0.492	0.644	0.657	0.624	0.913	0.245
	0.213	0.255	0.262	0.266	0.265	0.256	0.162	0.253
II (304+309)	0.232	0.429	0.425	0.476	0.499	0.538	0.314	0.245
	0.217	0.263	0.26	0.253	0.276	0.253	0.159	0.253
III (305 + 92)	0.241	0.448	0.55	0.645	0.755	0.796	0.669	0.245
	0.281	0.295	0.282	0.296	0.26	0.283	0.226	0.253

* First row = optical density developed by anti-tick lgG1 in samples diluted 100 times
** Second row = optical density developed by anti-tick IgM in undiluted samples
*** Infestation 6: Ticks were allowed to feed on *T.congolense* or Babes/as-infected animals to repletion

4.2,4.2.2 Kinetics of anti-tick IgM synthesis in tick-immune Boran cattle and in haemoparasitized-animals to *A. variegatum*

The results summarised in Fig. 5A and Tables 26 and 27, showed the absorbance of the serum IgM content at 450 nm of sera collected from the Boran cattle before tick challenges and after repeated infestations. There was a slight rise in the IgM synthesis from the first to the second infestation. That synthesis then stagnated up to the fifth infestation of the experiments. A slight decrease in the serum IgM content was noticed after the 6th infestation when ticks were fed on cattle carrying either trypanosome or *Babesia* parasites.

4.3 Correlation between Immunoglobulin G1 and acquired immunity by the Boran cattlb.

There were respectively a significant negative correlation (r=-0.52) after infestation 1 and a positive correlation (r = 0.565) after infestation 6 between PUMLTD and FPR and immunoglobulin G1 as revealed by the O.D (Table 28).

No significant correlation was found when infestations with adult stage and immunoglobulin G1 level were considered (Table 29).

Tabie: 28 Correlation matrix of nymphal *Amblyomma variegatum* biological parameters and serum IgG1 after repeated infestations of Boran cattle

Biological Parameters	Infestations/ Correlation coefficients and Probabilities at P < 0.05					
	1	2	3	4	5	6
PENGD	-0.1382 0.6234	0.2234 0.4234	0.0489 0.8625	0.0082 0.9767	-0.0227 0.9357	0.5014 0.0678
PMLTD	0.226 0.4179	0.0921 0.744	-0.0109 0.9692	-0.0102 0.971	0.0478 0.8656	0.3113 0.2785
PUMLTD	-0.5208 0.0465*	0.1842 0.511	0.074 0.7931	0.0637 0.8215	-0.3396 0.2155	0.5274 0.0526
PDEAD	-0.2011 0.4723	0.2345 0.4	0.2401 0.3887	0.0888 0.7528	0.0513 0.8557	-0.1329 0.6505
FPR	-0.3524 0.1976	0.1809 0.5187	-0.082 0.7712	0.4165 0.1225	-0.2962 0.2837	-0.1564 0.5932
ENGWT	-0.3203 0.2444	0.0518 0.8543	0.1067 0.7209	-0.1895 0.4987	0.3225 0.2409	0.5653* 0.0351
MPR	0.3687 0.1762	0.1166 0.679	0.3055 0.2681	0.3846 0.1569	0.1659 0.5545	-0.0876 0.7659

* Significant coefficient correlation

Table: 29 **Correlation matrix of adult *Amblyomma variegatum* biological parameters and serum !gG1 after repeated infestations**

Biological parameters	Infestations/Correlation coefficients/Probabilities P < 0.05					
	1	2	3	4	5	6
PENGD	0.2869 0.2998	0.0942 0.7382	0.4906 0.0633	-0.1985 0.4981	-0.2129 0.446	0.1787 0.541
ENGWT	-0.2267 0.4164	-0.2138 0.4442	-0.2333 0.4026	-0.2818 0.3089	0.292 0.2909	0.0345 0.9066
FPR	0.0518 0.8543	0.045 0.8734	0.0425 0.8804	-0.1389 0.6214	-0.2758 0.3196	-0.3189 0.2664
POVPR	0.1636 0.56	0.0982 0.7276	-0.0229 0.9354	-0.043 0.879	0.0173 0.951	-0.169 0.5635
EGGMASS	0.0113 0.9679	-0.1579 0.5739	-0.2272 0.4153	0.2254 0.4191	0.2986 0.2796	-0.0007 0.9979
PHATCH	-0.2217 0.427	0.0257 0.9273	-0.2058 0.4618	-0.2605 0.3483	0.1541 0.5834	0.4665 0.092
PDEAD	-0.1402 0.6181	-0.0942 0.7342	-0.4304 0.1093	0.1985 0.4781	-0.0162 0.9542	-0.2281 0.4327
POVIP	0.1784 0.5246	-0.756 0.7887	0.3416 0.2126	-0.3055 0.2682	-0.0367 0.8966	0.1317 0.6334
PEACWT	-0.1412 0.6156	-0.2901 0.2925	0.1753 0.5318	-0.3987 0.1409	0.1771 0.5276	0.0346 0.9063

4.4 Repeated infestations of Ayrshire breed cattle with *A. variegatum* nymphs and adults

Ayrshire steer 58 died accidentally after the third challenge. The results on nymphal infestations are shown in Tables 30.1 to 30.4 and Figs. 11 while those on adult ticks are mentioned on Tables 31.1 to 31.4 and Fig. 12. It could be observed with most of the animals that the PENGD, the PHATCH, the PEACWT, the POVIP and the ENGWT decreased after the second infestation with the infestations while the PDEAD, the PUMLTD and the FPR in Tables 30.1 to 30.4 increased significantly with the infestations. Animals 57 and 67 in Tables 31.1 and 31.2 had relatively the lowest value of PENGD, PEACWT, POVIP, PHATCH and the highest PDEAD and PUMLTD as compared to those of ticks fed on the steers 68 and 86 over the 4 infestations.

4.4.1 Kinetics of anti-IgGI and anti-IgM synthesis in Ayrshire cattle repeatedly infested with *A. variegatum* nymphs and adults

Sera collected from animals 67 and 86 showed an increase in the IgG1 and IgM contents from infestation one to four (Table 32) with a breakdown after the third tick challenge but the O.D values remained very

low as compared to those observed with the Boran cattle. Anti-tick IgG1 and IgM serum content in the Ayrshire steer 57, fluctuated after the second infestation without displaying any constant trend. See fig. 11

4.5 **Repeated infestations of crossbred Boran** X **Friesian cattle with *A. variegatum***

The experiment commenced with 5 steers but two of them died in the process of meteorization. The results of the experiment are shown in Tables 33.1 to 34.3 and Fig. 13. The PENGD, PMLTD, PDEAD, FPR after the 5th infestation of steer 77 were respectively 20%, 12%, 92% and 16.6 days. The PENGD, PMLTD, PDEAD, and FPR after the 5th infestation on steer 91, were respectively 52%, 39%, 61% and 13.5 days while PEACWT in Table (34.2) was 10%. Steer 95 in Tables 33.3 and 34.3 carried through more ticks as compared to the other members of its breed. PEACWT (15%) and PENGD with nymphs and adults (62% versus 45%) were the highest while PDEAD was the lowest in both stages. The engorgement weight of the ticks fed on the crossbred cattle were significantly reduced ($P < 0.05$) during the infestations.

Table: 30.1 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on Ayrshire cattle 57

		Infestations			
Animal Code	Biological parameters (Means±SE)*	1	2	3	4
57	PENGD (%)	79.0a	80.0b	37.0c	31.0d
	PDEAD (%)	22.0a	22.0a	63.0a	71.0c
	PMLTD (%)	78.0a	78.0a	27.0b	29.0c
	PUMLTD (%)	1.0a	2.0b	10.0c	2.0b
	FPR (days)	10.276 ± 0.254a	12.333 ± 0.304a	16.973 ± 0.336b	17.645 ± 0.284b
	ENGWT (g)	0.0504 ± 0.0015a	0.0426 ± 0.001b	0.0386 ± 0.002b	0.0382 ± 0.0019b
	MPR (days)	17.921 ± 0.150a	21.25 ± 0.134b	23.567 ± 0.082c	24.322 ± 0.242c

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P> 0.05)

Table : 30.2 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on Ayrshire cattle 67

		Infestations			
Animal Code	Biological parameters (Means±SE)*	1	2	3	4
67	PENGD (%)	78.0a	76.0b	44.0c	37.0d
	PDEAD (%)	24.0a	24.0a	75.432b	66.0c
	PMLTD (%)	76.0a	76.0a	24.340b	34.0c
	PUMLTD (%)	2a	0b	20.090c	3.0d
	FPR (days)	9.722 ± 0.161a	11.512 + 0.382b	17.182 ± 0.686c	16.027 ± 0.579c
	ENGWT (g)	0.0452 ± 0.156a	0.0363 ± 0.001b	0.0252 ± 0.001c	0.0278 ± 0.002c
	MPR (days)	18.430 + 0.0835a	21.474 + 0.172b	23.674 ± 0.184c	23.864 ± 0.209c

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 30.3 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on Ayrshire cattle 68

		Infestations			
Animal Code	Biological parameters (Means±SE)*	1	2	3	4
68	PENGD (%)	76.0a	76.0a	63.0b	52.0c
	PDEAD (%)	24a	26.931a	51.0b	61.0c
	PMLTD (%)	76.0a	73.0b	39.0c	39.0c
	PUMLTD (%)	0a	3b	24c	13d
	FPR (days)	11.014 ± 0.276a	10.643 ± 0.299a	19.145 ± 0.567b	17.961 ± 0.504c
	ENGWT (g)	0.048 ± 0.0016a	0.0496 ± 0.0015a	0.0283 ± 0.001b	0.0272 ± 0.001b
	MPR (days)	18.780 ± 0.169a	18.780 ± 0.167a	21.581 ± 0.179b	21.529 ± 0.184b

*Data were analysed by analysis of variance and ***t***-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 30.4 Observations on the biological parameters of A *variegatum* nymphs after repeated infestations on Ayrshire cattle 86

		Infestations			
Animal Code	Biological parameters (MeanstSE)*	1	2	3	4
86	PENGD (%)	91.0a	67.0b	48.0c	42.0d
	PDEAD (%)	26.0a	36.0b	42.0c	58.0d
	PMLTD (%)	74.0a	64.0b	36.0c	41.0d
	PUMLTD (%)	17.0a	2.0b	12.0c	1.0d
	FPR (days)	11.529 ± 0.37a	12.119 ± 0.414a	19.870 ± 0.662b	18.929 ± 0.7970b
	ENGWT (g)	0.0518 ±0.001 a	0.0420 ± 0.0014b	0.0243 ± 0.002c	0.0256 ± 0.0018c
	MPR (days)	18.39 ± 0.152a	21.560 ± 0.187b	22.956 ± 0.256c	23.333 ± 0.258c

*Data were analysed by Analysis of Variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

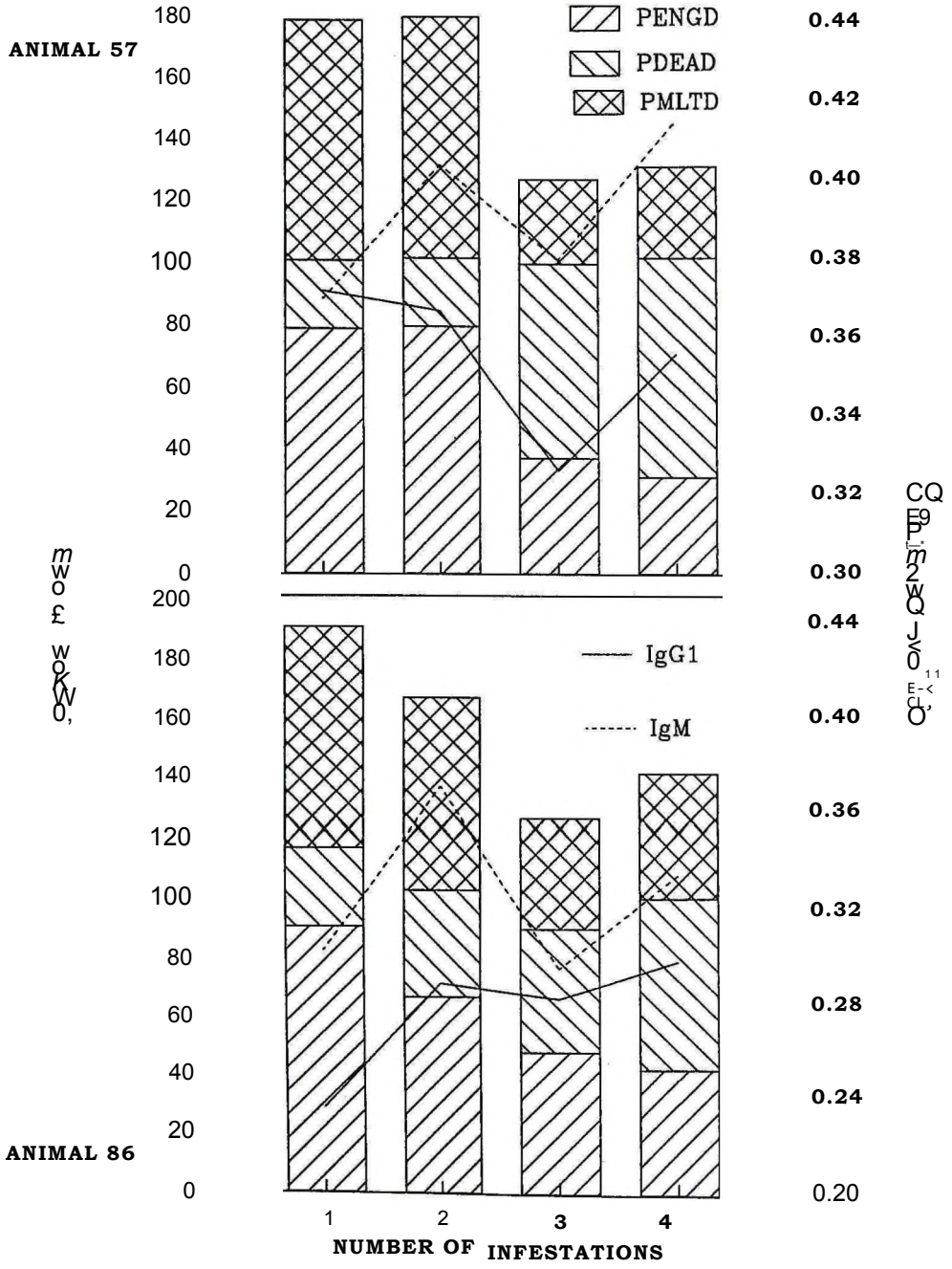


Fig. 11 Means and variations of some biological parameters of *A. variegatum* nymphs after repeated infestations on Ayrshire cattle.

able: 31.1 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on Ayrshire cattle 57

		Infestations			
Knimal Dode	Biological parameters (Means±SE)*	1	2	3	4
57	Percentage of adult ticks which engorged (PENGD)	55a	100b	5c	15d
	Percentage of adults which died before and after engorgement (PDEAD)	45a	0b	95c	85d
	Percentage of adults which engorged above critical weight (PEACWT)	40a	35b	0c	5d
	Hatchability of eggs which were laid (PHATCH)	80.83 ± 9.167a	1.625 ± 1.117b	15b	12b
	Feeding period (days) (FPR)	29.73 ± 3.272a	33.3 ± 1.556a	36.0a	34 ± 4.509a
	Pre Oviposition period (days) (POVPR)	13.9 ± 0.887a	15.95 ± 2.667a	25.0b	13 ± 2.0a
	Engorgement weight (ENGWT)	2.625 ± 0.294a	1.879 ± 0.154a	0.680b	1.400 ± 0.259a
	Egg mass (EGGMASS)	1.41 ± 0.22a	0.597 ± 0.084b	-	0.724c
	Percentage of adults which oviposited (POVIP)	50.0a	90.0b	5.0c	10.0d

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 31.2 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on Ayrshire cattle 67

		Infestations			
Animal Code	Biological parameters (Means±SE)*	1	2	3	4
67	PENGD (%)	30a	100b	50c	45d
	PDEAD (%)	70a	0b	50c	55d
	PEACWT (%)	5a	50b	15c	10d
	PHATCH (%)	66.667 ± 12.018a	8.9 ± 7.930b	17.325 ± 9.945b	3.162 ± 2.021b
	FPR (days)	30.0 ± 3.669a	32.850 ± 2.550a	34.50 ± 2.671a	33.77 ± 3.139a
	POVPR (days)	11.833 ± 1.249a	11.588 ± 0.454a	13.111 ± 0.978b	12.875 ± 0.97b
	ENGWT (g)	1.596 ± 0.266a	2.145 ± 0.294a	1.470 ± 0.212a	1.322 ± 0.242a
	EGGMASS (g)	0.461 ± 0.162a	1.054 ± 0.252a	0.851 ± 0.156a	0.613 ± 0.207a
	POVIP (%)	30a	85b	30a	40c

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 31.3 Observations on the biological parameters of A *variegatum* adults after repeated infestations on Ayrshire cattle 68

		Infestations			
Animal Code	Biological parameters (Means±SE)*	1	2	3	4
68	PENGD (%)	15a	70b	75c	80d
	PDEAD (%)	85a	30b	25c	20d
	PEACWT (%)	10a	25b	40c	30d
	PHATCH(%)	80 ± 0.164a	12.571 ± 0.169b	12.20 ± 0.204b	8.0 ± 0.182b
	FPR (days)	35.667 ± 5.044a	29.214 ± 2.514b	38.20 ± 1,598a	39.437 ± 1.316a
	POVPR (days)	14.333 ± 2.404a	10.071 ± 0.438b	12.786 ± 0.859c	11.929 ± 0.675c
	ENGWT (g)	2.0394 ± 0.377a	1.906 ± 0.200a	2.0395 ± 0.208a	1.821 ± 0.212a
	EGGMASS (g)	1.0967 ± 0.164a	0.613 ± 0.169a	0.730 ± 0.204a	1.013 ± 0.182a
	POVIP (%)	14.333a	10.071b	12.785c	11,929b

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

able: 31.4 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on Ayrshire cattle 86

		Infestations			
Animal lode	Biological parameters (Means±SE)*	1	2	3	4
86	PENGD (%)	45a	80b	85c	95d
	PDEAD (%)	55a	20b	15c	5d
	PEACWT (%)	20a	10b	35c	35c
	PHATCH (%)	78.75 ± 740a	1.444 ± 1.119c	5.962 ± 1.397cb	17.6 ± 6.650b
	FPR (days)	33.111 ± 3.335a	41.00 ± 1.974b	30.00 ± 2.338a	32.588 ± 2.782a
	POVPR (days)	12.889 ± 0.455a	13.750 ± 1.743a	13.312 ± 1.082a	13.071 ± 0.597a
	ENGWT (g)	2.0689 ± 0.213a	1.598 ± 0.145b	2.226 ± 0.243a	2.238 ± 0.249a
	EGGMASS (g)	0.720 ± 0.183ab	0.382 ± 0.110b	1.0069 ± 0.161a	1.0696 ± 0.200a
	POVIP (%)	45.0a	80.0b	80.0c	80.0c

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different ($P < 0.05$)

Table: 32 Serum antibody responses of Ayrshire cattle repeatedly infested with *A. variegatum*

Animal Code	ELISA Mean 0 Ds by infestation at 450 nm					
	Preinfestation (Control 1)	Post infestation 1	Post infestation 2	Post infestation 3	Post infestation 4	Control2
57	0.25*	0.371	0.366	0.325	0.355	0.245
	0.35**	0.369	0.403	0.379	0.414	0.254
67	0.244	0.295	0.402	0.392	0.396	0.245
	0.323	0.365	0.363	0.235	0.3	0.254
86	0.185	0.234	0.288	0.281	0.297	0.245
	0.202	0.302	0.369	0.294	0.333	0.254

First row = optical density developed by anti-tick IgG1 in samples diluted 100 times
Second row = optical density developed by anti-tick IgM in undiluted samples

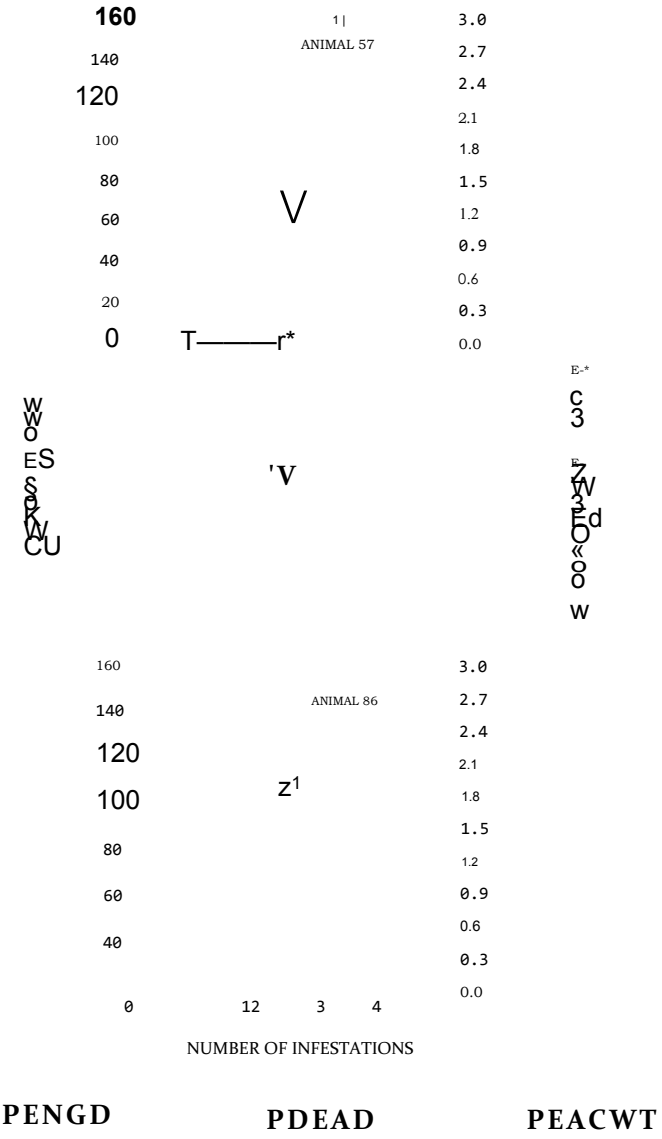


Fig. 12 **Means and variations of some biological parameters of A *variegatum* adults after repeated infestations on Ayrshire cattle.**

Table: 33.1 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on crossbred Boran x Friesian cattle 77

		Infestations				
Animal Code	Biological parameters (Means ± SE)*	1	2	3	4	5
77	PENGD (%)	16.0a	32.0b	48.0c	13.0d	20. Oe
	PDEAD (%)	91.0a	77.0b	53.0c	94.0d	92.0e
	PMLTD (%)	9a	24.0b	37.0c	6.0d	12.0e
	PUMLTD (%)	7a	76.0b	11.0c	7.0d	8.0e
	FPR (days)	20.81 ±1.46a	18.0+0.996b	10.17±0.39c	17.31 ±1.0b	16.6±1.01b
	ENGWT (g)	0.0243±0.00a	0.034+0.00ab	0.037±0.00b	0.024±0.00a	0.036±0.00ab
	MPR (days)	26.08±0.19a	23.0±0.547b	21,5± 0.220c	24.69± 0.33d	24.05±0.407d

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 33.2 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on crossbred Boran x Friesian cattle 91

		Infestations				
Animal Code	Biological parameters (Means+SE)*	1	2	3	4	5
91	PENGD (%)	62.0a	61.0b	60.Oe	44.0d	52. Oe
	PDEAD (%)	54.0a	91.0b	70.0c	76.Od	61.0e
	PMLTD (%)	46.0a	45.0b	30.0c	24.0d	39.Oe
	PUNMLTD (%)	46.0a	45.0b	30.0c	24. Od	39.0e
	FPR (days)	17.85 ± 0.56a	11.0 ± 0.46b	11.56±0.47b	13.95±0.39c	13.5±0.37c
	ENGWT (g)	0.037±0.001a	0.043±0.00b	0.041 ±0.00ab	0.029±0.00c	0.029±0.99c
	MPR (days)	24.22±0.174a	23.63±0.09b	21,9±0.252c	22.51 ±0.23b	22.56 ±0.21 b

*Data were analysed by analysis of variance and ***t***-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 33.3 Observations on the biological parameters of *A. variegatum* nymphs after repeated infestations on crossbred Boran x Friesian cattle 95

		Infestations				
Animal Code	Biological parameters (Means±SE)*	1	2	3	4	5
95	PENGD (%)	9.0a	73.0b	65.0c	65.0c	62.0d
	PDEAD (%)	93.0a	95.0b	45.0c	53.0d	59.0e
	PMLTD (%)	7.0a	62.0b	55.0c	47.0d	41.0e
	PUNMLTD (%)	2.0a	38.0b	10.0c	18.0d	21.0e
	FPR (days)	22.22± 1.73a	11,36±0.42b	12.12±0.41c	15.17±0.38c	16.49±0.33d
	ENGWT (g)	0.025±0.003a	0.039±0.002b	0.042±0.002b	0.029±0.00a	0.029±0.001 a
	MPR (days)	24.0±0.5a	22.78+0.702a	21.384±0.204b	21.35±0.18b	20.82±0.170c

*Data were analysed by analysis of variance and Mest
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 34.1 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on crossbred Boran x Friesian cattle 77

		Infestations				
Animal Code	Biological Parameters (Means±SE)*	1	2	3	4	5
77	Percentage of adults which engorged (PENGD) %	60.0a	70.0b	20.0c	Od	10. Oe
	Percentage of adults which died before or after engorgement (PDEAD) %	40.0a	30.0b	86.0c	100d	90.0e
	Percentage of adults which engorged above critical weight (PEACWT) %	15.0a	20.0b	0.0c	0.0c	0.0c
	Hatchability of eggs which were laid (PHATCH) %	2.5±2.5a	5.06 ± 5.06a	15.0a	0a	0a
	Feeding period (FPR)(days)	30.17±3.70ab	36.71 ±2.14a	14.25±1.0bc	-	30.5±1.5ab
	Pre Oviposition period (POVPR) (days)	14.22±1.09a	12.73±0.63a	15.667±0.7a	-	11.0±1.0ab
	Engorgement weight(ENGWT) (g)	1.57±0.32a	1.53 ± 0.22a	1.5671±0.2a	-	1,37±0.39a
	Egg mass (EGGMASS) (g)	0.75±0.36a	0.74 ± 0.23a	0.5425±0.3a	-	0.8932a
	Percentage of adults which oviposited (POVIP) %	40.0a	55.0b	13.75c	Od	5.0e

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 34.2 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on crossbred Boran X Friesian cattle 91

		Infestations				
Animal Code	Biological Parameters (Means±SE)*	1	2	3	4	5
91	PENGD (%)	50a	35b	10c	50a	40d
	PDEAD (%)	45a	65b	90c	50d	60e
	PEACWT (%)	5a	15b	0c	10d	10e
	PHATCH (%)	10.5±5.33a	8.68±8.68ab	20b	0.2±0.2b	2.5±2.5ab
	FPR (days)	23.72±2.969a	32.5±2.04ab	39±7b	34.4±4.07ab	28.62±3.881
	POVPR (days)	14.33±1.632a	9.33±0.667b	13.0±3.00ab	12.57±0.84a	11.60±0.509a
	ENGWT (g)	1.419±0.209a	1.836±0.180a	1.250±0.321a	1,609±0.42a	1.383±0.26a
	EGGMASS (g)	0.452a	1,052a	0.929a	0.978a	0.919a
	POVIP (%)	40.0a	15b	10c	35d	25e

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 34.3 Observations on the biological parameters of *A. variegatum* adults after repeated infestations on crossbred Boran x Friesian cattle 95

		Infestations				
Animal Code	Biological Parameters (Means ± SE)*	1	2 ...	3	4	5
95	PENGD (%)	85a	85a	80b	80b	45c
	PDEAD (%)	15a	15b	20c	20c	55d
	PEACWT (%)	55a	60b	10c	20d	15e
	PHATCH (%)	15.125±8.027a	40.46+16.38a	9.28 ± 9.28a	10.0 ± 6.324a	10.0±5.773a
	FPR (days)	28.529±1.841 ab	23.052+2.04b	33.125±2.47a	32.25±2.263a	25.5±2.75b
	POVPR (days)	13.687±0.835a	11.588±0.62a	13.933±1.240	17.0±1,839a	14.625±1.85a
	ENGWT (g)	2.424±0.248a	2.489±0.194a	1.555±0.189b	1.741±0.235b	1.447±0.213b
	EGGMASS (g)	1.105+0.162a	1.259±0.209a	0.68±0.197a	0.972±0.190a	0.691 ±0.205a
	POVIP (%)	80a	85b	75c	50d	40e

*Data were analysed by analysis of variance and f-test
Means followed by the same letters in the same row are not significantly different (P > 0.05)

Table: 35 Serum antibody responses of crossbred Boran X Friesian cattle repeatedly infested with *A variegatum*

Animal	ELISA Mean O.Ds by infestation at 450 nm						
Code	Preinfestation Control 1	Post infestation 1	Post infestation 2	Post infestation 3	Post infestation 4	Post infestation 5	Control2
77	0.23*	0.398	0.391	0.522	0.543	0.657	0.245
	0.316**	0.341	0.352	0.364	0.323	0.337	0.254
91	0.178	0.383	0.323	0.37	0.465	0.586	0.245
	0.227	0.353	0.362	0.332	0.328	0.315	0.254
95	0.193	0.311	0.403	0.356	0.409	0.446	0.245
-	0.298	0.404	0.351	0.345	0.302	0.33	0.254

First row = optical densities developed by anti-tick IgG1 in samples diluted 100 times

Second row = optical densities developed by anti-tick IgM in undiluted samples

4.5.1 Kinetics of anti-tick IgG1 and IgM synthesis in crossbred Boran X Friesian cattle repeatedly infested by tick, *A. variegatum* nymphs and adults

The trend was similar to that observed in the Boran cattle. See Table (35). There was anti-tick-specific IgG1 synthesis elicited by the successive infestations. After the 5th infestation, serum from steer 77 showed the highest IgG1 content. The mean absorbance values of anti-tick IgM synthesised, plateaued after the first infestation and remained at that level throughout the experiment. There was also no detectable IgG2 activity.

4.6 Comparison of feeding and development performance of *A. variegatum* nymphs and adults after repeated infestations on Ayrshire, Boran and crossbreds Boran X Friesian cattle

The results of the experiments are summarized in Tables (36 and 37) and Fig. 14. After repeated nymphal challenges, the crosses allowed significantly fewer ($P < 0.05$) ticks to engorge and moult to adult after the first two infestations. The PDEAD and FPR (56.33% + 10.99 and 13.60 days ± 2.37) were significantly higher as compared to those of the Boran and Ayrshire cattle. From infestations 3 to 4 there was no clear cut difference between the breeds. However, the values of PENGD, PMLTD and that of the ENGWT, were relatively higher after the 3rd infestation while

PDEAD and FPR were relatively lower. After the 4^{lh} infestation PDEAD increased relatively while PM LTD and ENGWT decreased significantly (P< 0.05). Nymphs fed on Ayrshire and Boran seem to perform in the same manner under the conditions of our experiments (Table 36). The trend after repeated infestations with adults was the same as with the nymphs. The crossbred in general gave poorer PENGD eg. 15.312% ± 24.918 as compared to that of the Boran (68.819% ± 4.879), higher death rate (56.667% ± 23.333), lower percentage of ticks which oviposited (64.09% ± 5.909) after the 4th infestation. The PEACWT, the ENGWT and the EGGMASS were also lower (Table 37).

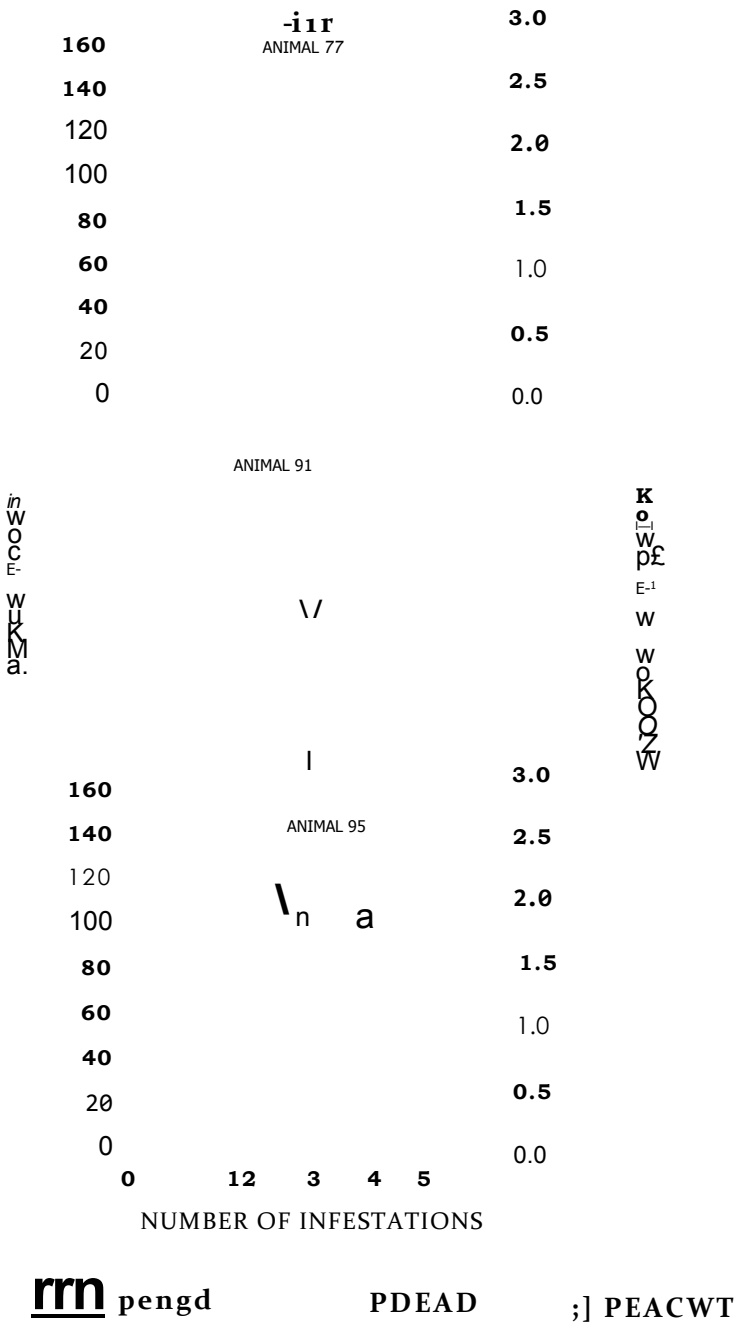


Fig. 13 Means and variations of some biological parameters of *variegatum* adults alter repeated infostedons on crossbred Boran x Friesian Calfle.

Table: 36 Observations on the biological parameters of A *variegatum* nymphs after repeated infestations on different cattle breeds

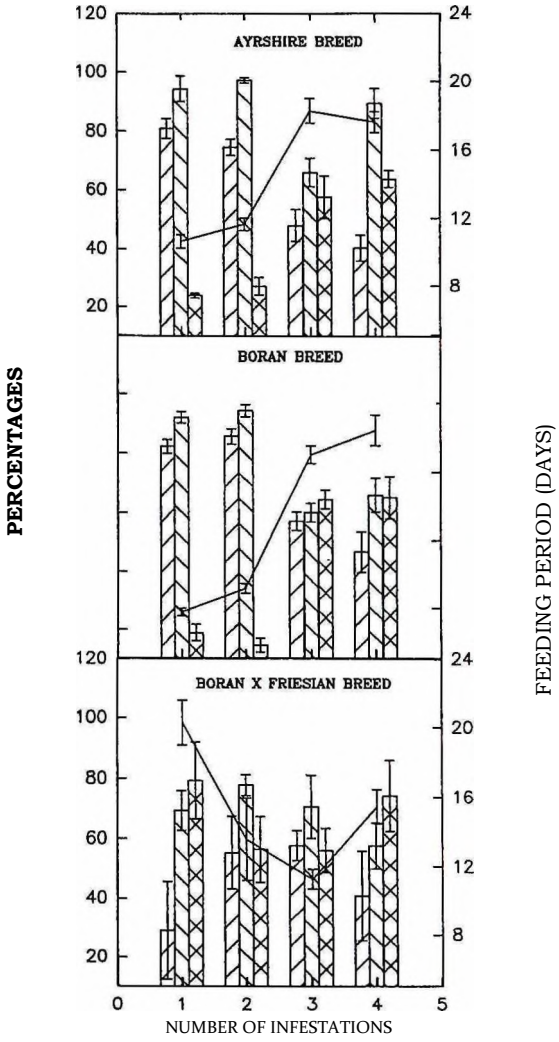
Biological parameters* Inf. Breeds	PENGD ± SE	PMLTD ±SE	PUMLTD ±SE	PDEAD ±SE	ENGWT ±SE	FPR ±SE	MPR ±SE
Ayrshire **1** Boran x Friesian Boran	81.0 +3.40a 29. Gt 16.62b 82.364±2.305a	94.38 ± 4.38a 69.41 ± 6.66b 92.06 ± 1.96a	5.63 ± 4.38a 30.59 ± 6.66b 6.25 ± 1,59a	24.0 ± 0.82a 79.33 ± 12.68b 19.0 ± 2.89a	0.05 ± 0.00a 0.03 ± 0.00b 0.05 ± 0.00a	10.63 + 0.40a 20.295 ± 1.288b 7.754 ± 0.220c	18.38 ± 0.18a 24.77 ± 0.66b 23.71 ± 0.11c
Ayrshire 2 Boran x Friesian Boran	74.75 ± 2.75a 55.33 + 12,17b 85.82 ± 2.59a	97.27 ± 1.00a 77.90 ± 3.53b 94.45 ± 1.97a	2.36 ± 0.84a 22.10 ± 3.53b 5.55 ± 1.97a	27.23 ± 3.09a 56.33 ± 10.99b 14.73 ± 2.42a	0.04 ± 0.00a 0.04 ± 0.00a 0.06 ± 0.00b	11.65 + 0.38a 13.60 ± 2.37a 9.17+ 0.29b	20.76 + 0.66a 23.15 + 0.25b 27.25 + 0.30c
Ayrshire 3 Boran x Friesian Boran	48.11 ± 5.47a 57.67 ± 5.04a 57.27 ± 3.14a	66.17 ± 4.76a 70.57 + 10.51a 60.24 ± 3.05a	33.84 ± 4.761a 29.43 ± 10.51a 39.73 ± 3.16a	57.86 ± 7.27a 56.00 ± 7.37a 64.64 ± 3.11a	0.029 ± 0.00a 0.039 ± 0.00b 0.037 + 0.00b	18.292 + 0.718a 11.283 ± 0.581b 16.998 ± 0.513a	22.95 ± 0.48a 21.59 ± 0.16b 22.60 ± 0.11a
Ayrshire **4** Boran x Friesian Boran	40.5 ± 4.444a 40.667 ± 15.103a 46.833 + 6.855a	89.515 +4.986a 57.669 ± 7.710b 66.182 ± 5.740ab	10.485 ± 4.986a 42.331 ± 7.710b 34.696 ± 6.320ba	64.0 + 2.858a 74.333 ± 11.865a 65.333 ± 7.061a	0.0297 ± 0.003a 0.0273 + 0.001 ba 0.0353 + 0.001a	17.640 + 0.603a 15.479 ± 0.979a 18.421 ± 0.890a	23.26 ± 0.61a 22.85 ± 0.98a 23.79 + 0.26a

•Data were analysed using analysis of variance and SNK-test
Means not followed by the same letter in the same column are significantly different (P <0.05)

able: 37 **Observations on the biological parameters of *A. variegatum* adults after repeated infestations on different breeds of cattle**

j 3iologicai oarameters* | Inf. Breeds	PENGD	PDEAD	POVIP	PEACWT	PHATCH	FPR	POVPR	ENGWT	EGGMASS
f Ayrshire 1 1 8oran xFriesian k Boran	36.25 ± 8.75a 66.667 ± 9.280a 48.75 ± 7.389a	63.75 ±8.75a 33.33 ± 9.280a 47.969 ± 7.459a	97.727±2.273a 80.867 ± 7.938b 93.323 ±3.182ab	50.126 ± 12.702a 32.932±16.537a 57.422 ±9.119a	76.562±3.326a 10.819 ±3.757b 44.058 ±4.704c	32.126 ± 1.408a 27.141 ± 1.717b 20.882 ±0.857c	13.239 ±0.0558a 13.576 ±0.529a 13.142 ±0.418a	2.082±0.211 a 1.805 ±0.313a 2.138±0.186a	0.922±0.208a 0.743 ± 0.204a 1.158 ±0.138a
Ayrshire 2 Boran xFriesian Baran	87.50 ± 7.50a 63.33 ± 14.813a 84.167± 4.557a	12.5±7.5a 33.33 ± 17.40a 17.497 ±4.742a	93.75 ±3.75a 73.81 ± 16.667a 86.937 ±4.724a	33.303 ± 7.747a 47.339 ±12.334ab 67.160±5.233b	6.135 ±2.76a 18.068 ±11.245a 50.138±6.082b	34.091 ± 2.478a 30.756 ± 4.039a 16.526±0.785b	12.839 ±1.282ab 11.216 ±0.997a 14.871 ±0.449b	1.883 ±0.111a 1.952±0.283a 2.442 ±0.156a	0.662±0.141a 1.018 ±0.150b 1.532±0.103b
Ayrshire 3 8oran xFriesian Boran	53.75 ±17.839ab 36.667±21.858b 82.5 ±3.667a	63.75*16.121a 65.417 ± 22.734a 17.5 ± 3.667b	83.529±8.895a 87.5 ± 9.547a 88.258 ± 2.244a	31.127±11.417a 4.167±4.167 65.211 ±5.254c	12.456 ±2.605a 14.762 ± 3.095a 19.682±3.217a	34.675 ± 1.734a 28.792±7.466a 17.663±0.675b	16.052 ± 2.984a 14.2±0.781ab 11.009±0.152b	1.604±0.347ab 1.457 ±0.104b 2.272±0.136a	0.82±0.098ab 0.720 ± 0.110a 1.326 ±0.095b
Avrsnire Boran xFriesian Boran	58.75± 17.955a 15.312±24.918a 68.819±4.879a	41.25 ± 17.955a 56.667±23.333a 31.25 ± 4.890a	81.816±5.144b 64.090 ± 5.909a 92.229± 1.565b	32.474±3.538a 21.636 ± 1.636a 67.129 ±4.406b	10.082 ± 2.973a 3.40±3.30a 12.859 ±2.015a	34.95 ± 1.527a 22.216 ± 11.125b 22.863 ± 1.434b	12.719±0.266a 9.857±5.091a 13.274 ±0.413a	1.695 ±0.212a l.117±0.560a 2.625 ±0.118b	0.855+0.110ab 0.650 ±0.325a 1.267 ±0.076b

'Data were analysed using analysis of variance and SNK-test
Means not followed by the same letter in the same column are significantly different ($P < 0.05$)



I7H PENGD [XI PMLTD [2J PDEAD

Comparison of acquired immunity between Ayrshire, Boran and crossbred Friesian x Boran cattie

CHAPTER FIVE

DISCUSSIONS

5.1 Acquisition of resistance to *A. variegatum* ticks by Boran cattle

These studies were aimed at investigating (1) the pattern of acquisition of immunity in Boran cattle following repeated infestations with the tick *A. variegatum,* using quantitative statistical methods (2) the effects of blood protozoan parasites *T. congolense* and *B. bigemina* on the acquired immunity and (3) compare the immunity level attained by the Boran breed cattle to that of the crossbreds Boran x Friesian and the Ayrshire cattle.

To assess the resistance levels in our study, we used the Principal Component Analysis (PCA) and FASTCLUS system to test for the quantitative variables which could characterize the resistance of purebred Boran cattle to *A. variegatum* and group animals with the same performances into the same group. Both PCA and clustering analysis agreed to a large extent. Cluster analysis allowed finer groupings by bringing closer individuals which showed the same effects on the ticks after challenge. These analytical methods have the advantage of taking into account concurrently the effects of all the variables on an animal as they discriminate between the main parameters to be chosen for the quantification of natural resistance against the ticks. Table 4, showed that Percent Engorged (PENGD), Percent Dead (PDEAD), Percent

Moulted (PMLTD) which consistently made up axis 1 were of paramount importance, followed by the PUMLTD and Feeding Period (FPR) which consistently made up axis 2 with the nymphal instar, and that they would be the most appropriate variables to be considered as far as resistance developed by cattle against *A. variegatum* nymphs is concerned.

The most important variables in the assessment of acquired resistance against A *variegatum* adults are PENGD, PDEAD, POVIP, ENGWT, FPR and EGGMASS which made up axis 1 and 2 (Tables 4 and 12). Results of our investigations which involved quantifying the importance of each variable in the assessment of host natural resistance confirmed those discussed by Dipeolu et aL (I992). We found that the use of several biological parameters resulting from the host-tick interaction and their simultaneous induction of immunity has allowed us to demonstrate that Boran cattle breed could be clustered into different groups of resistance. Animals with high resistance level are not significantly different from those of the medium group but they are different from those of the low group. The results were opposed to the findings of Rosenberg (1984) who considered only the engorgement weight of the ticks after four challenges and also to those of Norval (1978) who failed to develop resistance in sheep after repeated infestations with *A. hebraeum* by considering only the parameter, engorgement weight. This work also showed that a herd of animals artificially exposed to the tick *A. variegatum* infestations acquired immunity and that immunity was not uniform as it fluctuated from

infestation to infestation. However, animal B remained mostly in group I while animal O remained in the low resistance group throughout the experiment. These observations conform to those of Starchuski (1993), when he considered the tick pick up rates of zebu Gudali in the fields. Surprisingly, all the tick parameters for the second infestation (Tables 7, 9 and Tables 16 to 18) increased or decreased in such a manner as to favour the tick survival. This confirmed the findings of Barriga et a], (1991) with *A. americanum* on sheep that the engorgement weight recorded at the 2nd infestation was higher than that recorded from the first infestation. According to those authors, tick infestations cause immunodepression in the host. There are pre-existing antibodies in the host and therefore the inoculation of tick saliva during the first infestation triggers a secondary response of these pre-existing antibodies with the subsequent activation of the specific and non-specific suppressor mechanisms that are normal components of the immune response. These mechanisms in turn, inhibit the responses to the little immunogenic protective antigens below the protective and detectable levels. The protective effects are manifested when the accumulation of several infestations had raised slowly the response to the protective antigens to a higher level with its corresponding antibodies. But we think that after the first encounter, the host develop stronger defence mechanism but as the tick continued injecting immunosuppressive molecules in order to avoid its complete elimination, the host immune response declined after the 2nd infestation but peaked up from the 3rd challenge. The protective immunity to *A. variegatum* was manifest in

all the three groups against the nymphs through the FPR which significantly increased from infestation 3 with subsequent significant reduction in the PENGD, ENGWT and an increased PDEAD. The role of the significant reduction in the MPR following the acquisition of resistance remained to be explained. A breakdown of the resistance was observed from the 5^{lh} infestation. Immunity breakdown to *R. appendiculatus* has been observed by Dipeolu et al. (1992). Unlike the nymphs, the immunity to the adult tick was remarkable from the 4th infestation in the group of high resistance. These results might suggest that before stabilization, the animals should be under constant challenge of a specific tick load. Further investigations in the number of ticks to apply in order to induce a constant and stable resistance would be needed to confirm this idea.

5.2 Acquisition of resistance by crossbred Friesian x Boran and purebred Ayrshire cattle

We also compared the resistance of pure bred *Bos taurus* Ayrshire, a European dairy breed, acclimatized to the African, especially the Kenyan conditions and that of crossbred Boran x Friesian. During this investigation, we noticed that the resistance by animal within a breed and by breed, after successive infestations, varied from one another. In the Ayrshire breed, animals (57 and 67) developed stronger reactions to the nymphal and adult

stages. The acquisition of resistance was noticeable through the PENGD, the PEACWT and the POVIP which fell significantly in the case of the adults. There was also a significant decrease in the ENGWT, the PMLTD and a significant increase of the FPR and the PDEAD with the nymphal stage. With the crossbred, animals 77 and 91 (Tables 33.1 to 33.3, 34.1 to 34.3) stood out clearly due to the high level of resistance they developed to the tick feeding. By the fifth infestation, only 10% of adults and 20% of nymphs could feed to repletion on animal 77 respectively in Tables 34.1 and 33.1. In both breeds, the POVIP was significantly affected (Tables 31.1 to 31.4 and 34.1 to 34.3). Those results were in agreement with the findings of Utech et al. (I978b) who stated that *Bos taurus* Jersey heifers were 98% resistant to *B. microplus* compared to another *Bos taurus* breed Friesland heifers which were 85% resistant to the same tick. George et al. (I985) reported a case where pure bred *Bos indicus* Brahman and half-bred Brahman calves, artificially infested three times with *A. americanum* both yielded ticks with a reduction of 42% in engorgement weight after the 3rd infestation. Fivaz and Norval (I990) documented that in terms of recoveries of larvae and nymphs, *Bos taurus* Friesland proved to be significantly more resistant to *R. appendiculatus* than *Bos indicus.* They speculated that the actual status might be due to an early exposure of the Brahman group during their neonatal period to high dose of tick infestations. The animals became tolerant to the extent that immune response may have been suppressed. However, Latif (I984a) has shown that local crossbred (*Bos taurus x Bos indicus)* carried 4.5 times ticks *H.* a.

anatolicum more than the local *Bos indicus,* Kenana or Butana breed.

Comparison of resistance between breeds (Boran, Boran x Friesian and Ayrshire) showed that the crossbred were significantly more resistant than the purebred Boran cattle after the first two infestations. The exotic Ayrshire acquired the same resistance as that acquired by indigenous Boran cattle. Moreover, after the third and fourth infestation, the resistance acquired by the Ayrshire was not significantly different from that acquired by the Boran and the crossbred. These results contrast those recorded by other workers (Heweston, 1971; Seifert, I97I; Latif, I984a; I984b).

The selection of an appropriate site for tick attachment is a prerequisite for successful feeding (Walade and Rice, 1982).The selected feeding site for *A. variegatum* is on the sloping parts of the animal's body (Morel, I98I). So one could think that the apparently higher degree of immunity acquired by the crosses and the Ayrshire might also be due to the hairiness of the skin since the ticks have been fed on the ear for convenience. Whatever the case may be, the ticks were confined and their long mouthparts should have allowed them to engorge irrespective of their preferred feeding sites. The exceptional weight of 5g we recorded at certain instances with *A. variegatum* could suggest that feeding site had minimum effects during our experiments as the mean weight of an engorged female *A. variegatum,* on naive animal is 3g (Garris, 1984).

The less stronger response to *A. variegatum* infestations by Boran cattle could otherwise be explained by the fact that, the zebu cattle are longer established hosts than the more recently introduced European breeds. Tatchell and Moorhouse (I968) also found that zebu cattle developed less stronger response to *B. microplus* feeding. Randolph (1979) in her attempt to explain the host-parasite relationship, stated that the parasite adapts itself to the host for survival by reducing the amount of harm it causes to the host and if the remaining harm is insufficient to reduce the host's reproductive value, there may be little or no selective pressure for the natural host to evolve further defence mechanisms to counteract the parasite's adaptation for survival.

5.3 Humoral responses to the tick infestations

In addition to the use of biological parameters in the assessment of the acquired immunity, we have also considered the humoral responses associated with the acquisition of the resistance.

There have been numerous reports which showed that acquired immunity to ticks is an immune response mediated by antibodies, (Nyindo et a]., 1989; Essuman et al., 1991); complement, (Wikel and Allen, 1977) and T-cell-dependent mechanisms, associated with cutaneous hypersensitivity

reactions and increased grooming activity (Wikel, 1980; Binta and Cunnigham, 1984; Smith et al.. 1989). Shapiro et al. (1986); Brown (1988b), have shown that tick salivary gland homogenates were protective to further tick challenges in both laboratory and cattle. To date, studies on the characterization of *A. variegatum* salivary gland antigens responsible for the induction of host immune resistance in cattle have been very few apart from those made by Jongejan et al. (1989). In our studies, an attempt was made to characterize the different antigens that may be playing a role in the acquisition of the resistance and measure the antibody responses to those antigens in the different breeds of cattle at isotype level. The affinity purified lgG1 and lgG2 showed one heavy chain and one light with an additional chain as documented by Butler (1983).

During attachment and feeding, we found that several proteins were injected into the host as revealed by the silver-staining and the Western blot analysis of salivary gland homogenates obtained from different days of feeding on the Boran cattle. It was found that there was sequential synthesis of the proteins from the day of attachment and culminated in the Day 9 when most of the proteins were present. For example, in Table 25, the 72.4, 63.0 and 61.0 kD proteins were present from Day 3 and became more prominent in Day 9 before shading off in Day 12. Proteins of 71.0 and 38.5 kD, could be detected in Day 6 and more prominently in Day 9. The antigens which appeared during the course of feeding, may reflect changes in the salivary

gland cell which shift from granular to secretory of fluid (Kaufman, 1983). It could be therefore, assumed that all the proteins responsible for the induction of the recorded acquired resistance had been synthesized and were present in the salivary glands, 9 days after attachment.

Immunoblotting experiments carried out, showed that many antigens in the Day 9 extracts were recognized by the tick-immune sera.

According to Willadsen etaj. (1978); 1979); Wikel and Whelen (1986) and our investigations, results not included here, the cattle immune response to natural infestations is characterized by an antibody-mediated hypersensitivity to SGA injections.

Circulating antibodies in the Boran cattle, the crossbred and Ayrshire cattle to Day 9 SGA of *A, variegatum,* were detected by ELISA in the preinfestation as well as in the subsequent infestation sera. The presence of specific IgM antibody could not be demonstrated with the immunoblotting procedure, however, that class of immunoglobulins were revealed in very low quantities by the ELISA technique. The IgM absorbance obtained from the preinfestation sera were not very high when compared to those yielded by the successive infestation sera of all the different breeds. Maybe there had been an early IgM secretion which plateaued in the cattle when they were still young due to some acarine related arthropods infestations. The fact that that class of IgM antibody was higher in the Ayrshire cattle, almost on the same as IgG 1,

remains to be explained. The binding capacity of lgG2 to *A. variegatum* antigens responsible for the induction of antibody could be demonstrated by the immunoblotting test through the presence of 5 bands (Fig. 10a). The lgG2 fraction might have been contaminated by lgG1 during the purification experiment since we were unable to show that activity using the ELISA technique. lgG1 were abundantly synthesized during the successive infestations in all the breeds. The binding capacity of lgG1 could be demonstrated in Fig. 10a where many bands had been evidenced. It shows that most of the day 9 SGA components injected into the host induced antibody of lgG1 class and that in terms of protective antibodies of cattle to *A. variegatum*, the immunoglobulin G1 was probably the most impoprtant. Similar results were found by Brown et al. (1982) who stated that, acquired immunity to ticks in guinea pigs was mediated by a 7S lgG1.

Many workers have tempted to elucidate the mode of action of antibodies which inhibit the tick feeding and or cause death on or off hosts. That role of biopesticide played by host specific antibodies in the acquisition of resistance has been expressed by Nogge and Giannetti (1980), Ackerman etaL (1981) and Ben-Yakir et al (I987). Immunoglobulins have been found in the haemolymph of several genera of hard ticks and one genus of soft ticks (Ben-Yakir, 1989; Fujisaki et al., 1984 and Mbogo, 1992). Titre of antibodies in hard tick haemolymph was higher than that of soft ticks (Ben-Yakir, 1989). Previous exposure to ticks and development of host resistance increase the

prevalence of IgG in the haemolymph (Tracy-Patte et al., I987; Mbogo et al., 1992) and antibody titre in *B. microplus* is several times higher than that of a three-host-tick. Ingestion of the host antibodies after successive infestations may upset the tick physiological processes resulting in lower engorgement weights, lower oviposition rate, lower egg hatchability and longer feeding duration (Nogge and Giannetti, 1980). According to Willadsen (1987), the immunological reactions in host-6, *microplus* tick interactions and in an animal immunized with extracts from ticks were different from those observed after repeated infestations. The main target-cells in ticks fed on immunized animals, according to Agbede and Kemp (1986), were the digest-cells which were completely destroyed. A subsequent rupture of the gut-cells could allow host leukocytes to enter the haemocoel and attack other tissues. It was possible that the same reactions occurred in the nymphs during our studies from the third infestation since the nymphs collected were significantly smaller, wrinkled and brown, suggesting an eventual invasion of the tick haemocoel by the host blood components. However Ben-Yakir and Baker (I987) observed that the amount of anti-haemolymph antibodies which traversed the midgut of *D. variabilis* has no relation with the level of anti-haemolymph antibodies in the host rabbits. The mechanisms leading to the crossing of high molecular weight of proteins I50 kD is to be explained.

5.4 Immune responses in protozoan-infected tick immune cattle

In these studies which involved infecting Boran steers which had acquired resistance to *A. variegatum* through successive infestations, with *B. bigemina* or *T. congolense*, it was found that irrespective of the stage, the immune response was not impaired but was significantly boosted when compared to the last infestations results. The resistance irrespective of the protozoan infection was higher against the nymphs than the adults. All the parameters examined confirmed the enhancement of the resistance status. One would be tempted to agree with Sacks et a}. (I980) that trypanosome strains can have inherently different immunosuppressive activities. The immune depression, if there was any might have been partial and the long feeding period (average 56 days) was enough for the ticks to inject large amounts of antigens which boosted the earlier induced immune response via the memory cells. It might also be possible that the strain used did not affect the lymphoid system, allowing B- and T-cells to induce the antibody response obtained after the sixth tick infestation in this study; similar antibody response was observed by Rurangirwa et aL (I983) in cattle infected with *T. vivax* and *T. congolense* without concurrent tick infestations. In fact, MacMillan (1992) showed that in cattle, the suppression of immune responses in the course of African trypanosomosis, is mainly in the lymph nodes, while both blood and spleen cells would still be able to respond to a T-cell activation during infection. She also reported that the mechanism of the impairment is through the generation of suppressor macrophages which block the humoral and

cellular immunity. The immuno-depressive role could be achieved either by T-cell contact with macrophage-secreted prostaglandin or by contact with macrophages themselves. The macrophage-mediated immune suppression in cattle blocks both the expressions of receptors for interleukin 2 on the surface of T-cells and T-cell reaction of interleukin 2.

Contrary to the findings of Francis and Little (I964) and Francis (I966), Callow and Stewart (I978) showed that crossbred calves primed with *B. bovis* became more susceptible to *B. microplus.* Cox (I975); Phillips and Wakelin (I976), documented respectively that 6. *microti* enhanced *T. musculi* infections and *B. hylomysci* or *B. microti* delayed *Trichuris muris* normal expulsion in mice. The immune depression was marked when the parasitaemia was high. However, Purvis (I977) elaborated that the murine immunosuppression observed in babesia-infected mice was not a competitive one and that T-lymphocytes were still active. So the immune depression observed might be due to non-specific blastogenesis in B-lymphocytes leading to clonal exhaustion as pointed out by many workers in the trypanosome-induced immunosuppression. In situations where cattle are constantly exposed to the *Babesia* parasites, young animals become infected and develop immunity which protects them in adult life (Morrisson, I989). Inoculated with 150 ml of infective blood containing 10% of *B. bigemina* infected erythrocytes, the tick-immune Boran steers did not develop patent babesiosis. We only observed a rise in temperature for two days which could be due to the presence of

foreign bodies in the animals. The parasites might have been cleared by the spleen since the animals were two years old and unsplenectomized. The manifestations of the disease could be transient and symptomatically undetectable. Nevertheless when the animals were challenged one month after the infection with ticks, a significantly ($P < 0.05$) lower PENGD, PMLTD and ENGWT were recorded. The FPR and PDEAD significantly ($P < 0.05$) increased with both stages. There was a rise in the antibody synthesis as detected by ELISA when ticks were fed on animals injected with *B. bigemina.* IgG1 was the most important isotype detected in terms of protection of the cattle against the ticks. This shows that *Babesia* parasites might not be able to critically infect old tick-immune zebu cattle as stated by Francis (1966), hence could not have any effect on the acquisition of resistance in cattle to *B. bigemina.*

CONCLUSIONS

Our contribution has shown that a multivariate analysis could be used to assess the resistance of cattle to the tick *A. variegatum.* It has also proved that crossbred Boran x Friesian steers and *Bos taurus* Ayrshyre cattle could also develop resistance against the same tick.

The question which could arise is what role could the acquired resistance in cattle play in the control of tick and tick-borne diseases?

The answer to that question could be obtained through our study which showed a significant reduction in the tick yield, a reduction in the reproductive ability of the ticks which could lead to the reduction of the tick population after successive infestations. The latter could have been demonstrated if progeny ticks were continuously used for challenges. It is, in fact, what happens in nature.

The fact that tick-resistant zebu cattle have proved to be refractory to *Babesia* infections and that immunity acquired by cattle is not suppressed by trypanosomosis which is panzootic to Africa, could militate for the selection and use of tick-resistant animals. The rearing of crossbreds could be cost-effective especially in areas where milk production is outstandingly poor because of its adaptability to the African conditions and because of its ability to acquire high resistance to tick feeding.

Successful use of the phenomenon of acquired immunity in controlling cattle ticks resides in the design and implementation of a sound integrated tick management control programme as suggested by Dipeolu (1991) whereby natural resistance, immunology, ecology, husbandry practices and utilization of parasitoids will form the umbrella of an integrated tick control programme. Since host resistance to *R. appendiculatus* potentiates the deleterious effects of a vaccine (Essuman et al.. 1992) and that there is cross-protection between *A. variegatum* and *R. appendiculatus,* the most important ticks in Africa, as demonstrated by Kiara (1994) the exploitation of tick resistant cattle, seems to be a pragmatic approach to a cost-effective livestock production. An inter-governmental agreement could lead to the creation of a pan-African livestock development programme, spearheaded by the international institutes whose mandates include the improvement of livestock development in Africa. The focus could be on the Boran breed in eastern africa, the shorthorn *Bos taurus* N'dama in Western Africa as demonstrated by Claxton and Leppere (1991) and the zebu Africaner in Southern Africa. The parasites could be *A. variegatum* or *A. haebrum* and *R. appendiculatus.* That could constitute the skeleton of an integrated physiological, ecological and epidemiological studies on the host-parasite interactions. To avoid being trapped by the companies manufacturing chemicals, clear and rigorous government policies have to be defined for the strategic or opportunistic use of the acaricides when the need arises. The target should not be the eradication of the ticks, which is undoubtedly illusive, but to keep an economic threshold infestation whereby

hosts and parasites will be in complete balance. So we could, with one mind say as did George (1992) that "Wherever and whenever the control of ticks is considered worthwhile, the use of tick-resistant livestock should be considered a viable option among the many factors that are weighed when devising and evaluating a management strategy".

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