HOST-PARASITE RELATIONSHIP OF *Amblyomma variegatum* (Ixodidae) (Fabricius,1794) AND TWO BREEDS OF SHEEP, AND THE PROSPECTS OF IMMUNIZATION AGAINST TICK INFESTATION

BY

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DECLARATION

This is to certify that I, Jonathan B. D. George carried out the work reported in this Thesis in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, under the supervision of Professor James O. Adekeye, Dr. Jacob J.K.P. Kwaga and Dr. Joseph O. Ajanusi, all of the Faculty of Veterinary Medicine, A.B.U., Zaria.

The work of other investigators in the areas covered by this work have been duly acknowledged and appropriately quoted.

No part of this thesis has been submitted elsewhere for the award of a diploma or degree.

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CERTIFICATION

This project report / thesis entitled **Host-Parasite relationship of *Amblyomma variegatum* ,(Ixodidae) (Fabricius, 1794) and Two Breeds of sheep, and the prospects of immunization against tick infestation** by Jonathan B.D. George meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contributions to knowledge and literary presentations.

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DEDICATION

To my Wife, Mother, my Brothers and Sisters, Relatives and Friends whose shared concern and wish was to see me finally graduate. Most of all, this Thesis is dedicated to those countless messengers of Peace and Love of our Lord **Jesus Christ.**

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ABSTRACT

Three experiments were performed:

In the first experiment, eight sheep of different breeds (Yankasa and Uda) were randomly selected into groups groups A and B. Group A animals were infested with 50 (40 females and 10 nymphs) ticks , according to the method of Theis and Budwiser (1974) while the group B animals were not infested. Skin biopsies using a 5mm biopsy punch were taken daily from day 2 to 8 of infestation. Histopathological sections were prepared from the biopsies and examined microscopically.

Secondly, an anti-tick vaccine produced according to the method of Johnston *et al,*(1986), using the midgut of *Amblyomma variegatum* was inoculated parentarally into four comparatively tick-naïve Yankasa sheep. PBS was inoculated into four control animals . After booster doses, the animals were each challenged with 30 nymphal and 20 adult ticks. Detached ticks were counted and weighed daily. They were also examined for physical or immunological damage. Apparently healthy ones were incubated for the purpose of egg laying or ecdysis, as the case may be.

Thirdly, anti-tick vaccination was repeated using three groups of rabbits, each group inoculated with a different quantity of the midgut protein of *A. variegatum* but now semi-purified using ion-exchange chromatography. A 4th group of rabbits (control) was inoculated using PBS at pH 7.2. All the four groups were then challenged with 100 nymphs of *A. variegatum* per rabbit on the ears , with the aid of prepared ear bags. A second tick challenge was instituted 4 weeks after the end of the first challenge. During both challenges, the ticks were monitored as was done during the sheep challenge.

A developing erythromatous feeding lesion measuring between 2-3mm in diameter within the first two days of tick attachment were observed. This increased to about 4mm in diameter within the next two days, forming a cone-

like structure. Few neutrophils and lymphocytes were seen within the focal lesion. There were also eosinophils and basophils in tissues surrounding the tick mouthparts. In places were the dermis broke, there were cloudy, swollen cells and extensive damage to blood capillaries and collagen fibres, resulting in necrosis and liquefaction necrosis by day 4. The lesions increased in size to between 5-6mm in diameter by days 5 and 6. There was an accumulation of cement material secreted by the ticks around their mouthparts. In places were more than one tick attached, there was a coalition of lesions which measured between 7-10mm in diameter. This was more pronounced in the Uda breed of sheep where lesions became degenerative, oedematous and exudative. The liquefied, necrotizing tissues became reduced, leaving behind a developing vacuolar site with erythrocytes, lymphocytes and other polymorphonuclear cells proliferating. Epithelial tissues began to develop by day 6 in the Yankasa sheep. This did not occur in the Uda , rather there was deterioration and expansion of the lesions. Most ticks detached by days 7 and 8, leaving behind ulcers which completed healed within 14 days in the Yankasa , and much later in the Uda after chemotherapy. Detached ticks were counted and weighed . Apparently healthy ticks were then incubated at 28°C and 85%RH for the purpose of egg laying or ecdysis as the case may be.

Results showed significant reductions ($P<0.05$) in engorgement weights , number of detaching ticks prior to the attainment of the Crititical Engorgement Weight (CEW) (Dipeolu *et al,* 1991)., percentage of immunologically damaged and dead ticks, mean egg mass and percentage of successfully ecdysed nymphs (into adults) of ticks fed on vaccinated and control sheep. These effects were more pronounced in ticks fed on vaccinated animals during the secondary infestation.

In an attempt to semi-purify the crude antigen used in the inoculation of sheep, it was subjected to the process of ion-exchange chromatographical

separation. Eluents collected separately were freez-dried and later reconstituted in PBS pH 7.2 before use in inoculating three geoups of rabbits. Eluents with protein concentrations of 1.4mg/ml, 3.6mg/ml and 2.4mg/ml, as ,derived from the chromatography, were inoculated into three groups of rabbits respectively, while control rabbits were inoculated with 2ml of PBS (same volume used in the reconstitution of the proteins above). All the four groups were challenged with 100 nymphs of *A. variegatum* in each case, using ear bags as aids.

Unlike in the ticks fed on vaccinated sheep, there did not seem to be appreciable differences in the effects observed on ticks fed on vaccinated rabbits. This could be due to the comparatively lower concentrations of proteins used in the rabbit inoculations. There may have been denaturation of proteins used in this latter experiment due to processing during chromatography, thus may not have elicited enough challenge to the host rabbits to produce antibodies against the tick proteins.

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LIST OF ABBREVIATIONS

AAP = Aggregation Attachment Pheromone

ANOVA = Analysis of Variance

BHC = Benzene Hydrochloride

Contrl. = Control animals

DD = Dead ticks

DDT = Dichlorodiphenyl-trichloroethane

DEAE = Diethylaminoethyl

DM = Damaged (immunologically) ticks

DNA = Diribonucleic acid

DPX = Dibutylphalate and Xylol

EDTA = Ethylenediaminetetracetic acid

EC = Ecdysis

ICIPE = International Center for Insect Physiology and Ecology

IPM = Integrated Pest Management

ITM = Integrated Tick Management

MWt = Mean engorgement weight

MMs = Mean egg mass

NAPRI = National Animal Production and Research Institute

NITR = Nigerian Institute for Trypanosomiasis Research

NVRI = National Veterinary Research Institute

PBS = Phosphate buffered saline

PI = Post infestation

PMSF = Phenylmethylsulphonylfluoride

SE	=	Standard error
Ecdy.	=	Successfully ecdysed
Tno	=	Total number
TTBD	=	Ticks and tick-borne diseases
Twt	=	Total weight
Vacc.	=	Vaccinated
WP	=	Wettable powder
%Ecdy.	=	Percentage ecdysis

CHAPTER ONE

1.0 INTRODUCTION

Ticks, perhaps more than any other group of parasites, are well suited for transmission of disease-causing agents in livestock. They are vectors of a variety of protozoa, rickettsiae, virus and bacteria to different vertebrates throughout most parts of the world (Cupp,1991). Hard ticks rank in first position as vectors of diseases to non-human vertebrates (Balashov Yu,1972) , and are second only to mosquitoes as vectors of human and animal diseases (Sonenshine,1991).

Ticks are potent vectors because they are widely distributed, do not have many natural predators or parasites and are able to evade host defense mechanisms while feeding (Kocan,1995). Ticks can maintain dense populations in suitable environments because of their enormous reproductive capabilities (Friedhoff,1990).

Animal diseases in general, and ticks and tick-borne diseases(TTBD) in particular are among the many factors which directly hamper the growth of the livestock sector. They also affect the whole agricultural sector because livestock serve as a source of food and income through generation of employment, delivery of energy (dung and biogas), fertilizer, weed control,use of marginal lands, investments and savings as well as transport (Sansoucy, 1995; Willadsen, 1997a) are all affected negatively.

Failure to control TTBD is therefore a major factor limiting animal production world-wide, especially in Africa where they are endemic (Kocan, 1995). Worldwide economic losses are estimated at billions of dollars annually due to diseases like cowdriosis , theileriosis, anaplasmosis and babesiosis (Sonenshine,1991). Damage done to hides and skins as a result of tick infestation is computed to result in

significant economic losses (Petney *et al*, 1987; Dipeolu,1991). In Africa, theileriosis, dermatophilosis and cowdriosis are regarded as the most important tick-borne diseases in cattle and other livestock, while anaplasmosis and babesiosis may be more important in certain regions only (de-Castro, 1997). An integrated approach to control of TTBD may be necessary due to shortcomings of the use of single methods, which in most cases, breeds resistant ticks to most chemical acaricides as reported worldwide (Jongejan, 1990; Uilenberg, 1990; Dipeolu,1991; Kocan, 1995; Perry *et al*, 1998). Similarly, the widespread use of chemotherapy in treating infected animals, except in cases where enzootic stability is disrupted, may breed early resistance to drugs by parasites and cause a change in virulence of the parasites, depending on whether they are transmitted mechanically via contaminated blood or biologically by the ticks (Kocan, 1995).

Current control strategies for TTBD depend on individual circumstances that are influenced by financial resources, degree of acaricide resistance, development of immunity to tick infestation and / or availability of vaccines against tick-borne pathogens and the ticks themselves (Kocan, 1995). Control has relied largely on two techniques: the use of chemicals (acaricides) to directly control ticks and the use of animal (cattle) breeds with an ability to acquire high levels of immunologically mediated tick resistance. Other techniques such as pasture spelling have played a more minor role in tick control. Recently, the use of anti-tick vaccines against cattle ticks has introduced a new phenomenon in tick control strategies (Willadsen, 1997b).

Acaricides are expensive, require stringent application regimen and management strategies, often contributing to environmental pollution and when used extensively, give rise to acaricide resistance by ticks. Young animals are particularly susceptible to the toxic effects of acaricides (Norval *et al*, 1989; Kocan, 1995). The disruption of

acaricidal tick control often results in disease outbreaks since the population of susceptible animals increases also (Young *et al*, 1988).

A variety of biological control measures for ticks , including use of predators and pathogens, tick-killing plants and their extracts, habitat modification and use of tick resistant animals have been tried with little success (Young *et al*, 1988; Mwangi *et al*, 1991; Malonza *et al*, 1992). The immune potentials of tick-exposed animals has been examined with a view to its use as a natural control measure (Amin-Babjee and Riek, 1986; Kemp *et al*, 1986; Johnston *et al*, 1986; Adamson *et al*, 1991; Fiwaz et al, 1991). It was observed that tick antigenic material was transferred into the host during tick feeding (Morrison, 1989) which elicited host immune mechanisms involving cellular and antibody mediation, coupled with complement-dependent factors and effector mechanisms all geared towards interruption of tick feeding (Willadsen, 1987,1988; Opdebeeck *et al*, 1988; Wikel *et al*, 1992) . This fact stimulated tremendous interest in the possibility of the production of an anti-tick vaccine which will not only control ticks, but also the parasites they transmit.

Vaccination against ticks, using concealed antigens was first proposed by Galun, (1975), and first demonstrated by Johnston *et al*, (1986) against *Boophilus microplus* , a one host, temperate climate hard tick, using crude extracts derived from adult female ticks. A protective antigen Bm 86 was subsequently identified from the gut walls of semi-engorged female *B. microplus* (Willadsen, 1987; Willadsen and Kemp,1988; Willadsen *et al*, 1989). The antigen was further synthesized using recombinant DNA technology involving the Polymerase Chain Reaction (PCR) technique (Cobon and Willadsen, 1990; Willadsen,1990). Field trials were conducted to evaluate the efficacy of the vaccine (Floyd et al, 1995).

Currently, this vaccine containing a single recombinant antigen Bm 86 in an oil-based adjuvant is on commercial sale in Australia and a few other countries where *B. microplus* is endemic. It is traded as TickGARD® , and the antigen is expressed in *Escherichia coli* (Tellam *et al*, 1992) or in *Pichia pastori* (Rodriguez *et al*,1995). The antigen is a membrane-bound glycoprotein which binds the digest cells (surface) of the tick gut walls, causing lysis of the epithelium and leakage of gut contents into tick haemolymph (Gough and Kemp, 1993). The potentials of this vaccine in the control of other ticks species, especially the two and three host ticks is not known (Willadsen, 1997a) . This is why the present work was designed to study the protective effects of mid-gut extracts derived from female *Amblyomma variegatum* . when used to immunize sheep or rabbits.

The objectives of this study include:

1. to study the tick-bite sites of female *Amblyomma variegatum* fed on Yankasa and Uda breeds of sheep with a view to understanding the pathological and cellular reactions taking place during tick feeding

2. to compare tick-bite lesions in the two breeds of sheep with a view to finding out if breed or ecological factors have an effect on the host - parasite relationship

3 to produce and test a crude anti-tick antigen vaccine using the midguts of partially fed female *A. variegatum* ticks in Yankasa sheep. This is in an attempt to control ticks by exploiting host immune capabilities

4 to partially purify the crude tick-derived antigen vaccine by separating component proteins through the use of the ion-exchange chromatography , and to test the derived components for anti-tick reactions using rabbits as hosts.

5 to make recommendations on the way forward based on the results obtained from this work

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CHAPTER TWO

2.0 LITERATRURE REVIEW

2.1 Ixodid ticks as parasites of Livestock in Nigeria:

Ixodid ticks are obligatory blood sucking parasites of vertebrate and invertebrate animals world-wide. They are also important vectors of livestock diseases including cowdriosis, babesiosis, anaplasmosis and theileriosis and certain bacteria and viral diseases. Female ixodid ticks can suck blood up to twenty times their unengorged weight, thus causing anemia through exsanguination in infested hosts.

Balashov Yu (1972) classified the family ixodidae into two sub-families as Ixodinae and Amblyomminae. The ixodinae is comprised of two genera, *Ixodes* and *Ceratixodes*, both of which do not occur in Nigeria (Morel,1958; Strickland, 1961; Mohammed, 1974; George, 1987). The sub-family Amblyomminae comprise of eight genera, *Amblyomma, Aponoma, Hyalomma, Boophilus, Haemaphysalis, Rhipicephalus, Rhipicentor* and *Dermacentor*. All but the last two genera have been reported in Nigeria (Hoogstraal, 1956).

Amblyomma variegatum is the only species of the genus *Amblyomma* in Nigeria. It is the vector for *Cowdria ruminantium*, the causative agent of cowdriosis, an often fatal disease of cattle, sheep and goats in Nigeria (Hemobade, 1977). *Aponoma exornatum* is also the only species of *Aponoma* reported in Nigeria (Simpson, 1912). It has rarely been seen or reported in livestock, but may occasionally be found infesting snakes. There are about five species of *Hyalomma* in Nigeria and most of them infest cattle (*H. impeltatum, H. impressum, H. rufipes, H. truncatum)* and camels *(H. dromedarii)*. Most of them are vectors of *Theileria mutans* (cattle) or *T. annulata*

(Camels). The genus *Boophilus* comprises three species in Nigeria, inccluding *B. annulatus, B. decoloratus* and *B. geigyi* . They are one host ticks incriminated in the transmission of babesiosis and anaplasmosis in ruminants. There are only two species of *Haemaphysalis* in Nigeria. These are *H. aciculafer* and *H. leachi leachi* (Strickland, 1961). They are mainly parasites of wild carnivores and rodents but are occasionally found on domestic dogs and cattle (accidental hosts). There are about seven species of *Rhipicephalus* in Nigeria. The most common on cattle and sheep are *R. capensis, R .simus* and *R. evertsi* (Mohammed, 1974;'George, 1987). The common brown dog ear tick *R. sanguineus* is about the most important ectoparasite of dogs in Nigeria. *R. muhsamae, R. tricuspis* and *R. compositus* are less common on domestic animals in Nigeria (Strickland, 1961). Most of these tick species show strong affinity for host preference (*R.sanguineus)* while a few are ubiquitous (*Amblyomma).*

2.2 *Tick mouthparts and the Feeding process*: The tick mouthpart is situated in a heavily sclerotized structure called the capitulum. This structure joins the mouthparts (gnasthosoma) to the tick body (idiosama). The gnasthosoma is comprised of a number of appendages which, together , are used as instruments of feeding and attachment to host animals. The chelicerae are a pair of leg-like, sclerotized and rigid cuticular tubes which are provided with cutting digits in the form of recurved teeth (Kemp *et al*, 1982). The digits are provided with flexor and extensor muscles which pass to the distended base of the cheliceral shaft. Host skin is penetrated by the cutting action of the digits. Deeper lesions are formed when the digits are inserted by a forward movement of the whole chelicerae. The chelicerae could bend from the membranous cuticle at the base (Arthur, 1960). Short cheliceral sheaths cover small portions of the shaft in ixodid ticks.

The hypostome is a club-shaped, barbed extrusion placed between the pair of chelicerae. One of the important functions of this organ is to aid anchorage of the feeding tick by embedding in incised host tissue. The hypostome is further grounded by the secretion of cement material in ticks which are cement secreting. The apposition of the hypostome to the pair of chelicerae forms the food channel through which imbibed blood from the host passes. It also allows the passage of saliva into host tissue during blood feeding. Separation of the two fluids occur at the pharyngeal orifice where the salivary secretions flow out from the dorsal salivarum , and host blood via the ventral salivarium.

The short labrum of ixodid ticks is supported by a cuticular rod which projects backwards between walls of the pharynx and salivarum (Kemp, *et al*, 1982). Paired salivary ducts enter the salivarum at its posterior end in the buccal cavity. The pharynx and oesophagus are tubular exetnsions of the midgut. They are supplied with powerful, serially arranged dilator and circular muscles which enable them to function as a pump. Nerves to both sets of muscles originate from the stomatal pons (Obenchain and Oliver, 1976). The oesophagus terminates at the entrance of the midgut in a plug of cells or proventricular folds which act as a weak valve (Gregson, 1960).

The salivary glands are made up of a complex variety of cell types and their derivatives. The glands are made up of extensive rough endoplasmic reticulum and various types of secretory granules. There are acini types I and IV , each composed of different cell types (Binnington, 1978) in *the* slivary glands of hard ticks. The shape and size of some of these cells change during feeding, resulting in different functions during the process of digestion. A large number of granules are released from granulocytes during this process. The granules contain different enzymes such as

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glycoproteins, esterases (acetylcholinesterase, type-B carboxyl esterase), protease, aminopeptidase, monoamine oxides, phosphatases etc. Some of these enzymes are present in the cytoplasm of cells (Kemp *et al*, 1982) . The salivary glands of female ixodid ticks is thought to contain two types of granular acini ie II & III , with a third type IV found in males. Attachment of ticks to the host tends to trigger the production of granules in some cells and in others, secretion of granules. Two cell types in *B. microplus* (C2 & f) show marked increase in granule production during the first 48 hrs. after attachment while cell types a, d and e (cement precursors) secrete their granules by 72 hrs post attachment (Binnington, 1978; Kemp *et al*, 1982). The enzymes released are thought to be digestive in function while other secretions during tick feeding may be responsible for dilation of skin capillaries, development of extensive haemorrhage , tissue destruction and prevention of blood clotting in the feeding lesion. Tick salivary secretions encourages immune autolysis of host tissue (Tatchell and Moorhouse, 1968). Neutrophils infiltrating the feeding lesion destroy collagen fibres and cause mast cell degranulation (Allen *et al*,1977). Agents causing mast cell degranulation and leucocyte infiltration may originate in the tick salivary glands which could set a train of host immune reactions. The introduction of salivary gland antigens is likely to be the potent mediator of host reactions, leading to oedema , haemorrhage, leucocyte degranulation, tissue damage and prevention of blood clotting (Tatchell and Moorhouse, 1970; Allen *et al*, 1977).

There have been reports documenting up to nine cell types in the salivary gland of ixodid ticks which has been attributed to the complexity in morphological changes which occur during feeding. Histological differences between these cells are however minor (Binnington, 1978).

2.3 Tick Attachment and Detachment;

In order to attach to the host, the ixodid tick grips it with its legs, lifts up its body at an angle to the skin surface and applies the tip of the chelicerae , commencing an outward cutting movement with its teeth-like digits. This creates a lesion and the insertion of the chelicerae and hypostome into the wound is followed by the secretion of cement material. Cement flows into the wound and the surrounding epithelial layers and gradually climbs to the mouthparts , embedding them (Gregson, 1960). Secretion of cement starts within the first 5 to 30 min. of attachment and hardens as it flows to form an inner cone (internum). Deposition of the outer cone cement commences and hardens within 24 hours to form the cortex. Additional cement continues to be deposited 48 to 72 hours later (Kemp *et al*, 1982).

The cement material is composed of about 18% lipid and 82% protein in *B. microplus* , although the cortical parts contain some carbohydrate. Some of the lipids and carbohydrates are in the form of lipo- and glyco-proteins (Stone *et al*, 1977) respectively. Hardening of the cement material is probably the function of dehydration and denaturation by air (Kemp *et al*, 1982).

The cement is primarily a holdfast mechanism for those tick species with short mouthparts (brevirostrata) and possess a superficial form of attachment to the skin (eg *Rhipicephalus* spp). although certain hard ticks with long mouthparts (longirostrata) like members of the sub-family Amblyomminae also secrete cement (Arthur, 1973). The process of tick detachment is not well known.

2.4 Tick Control

A number of methods for the control of the menace of ticks and tick-borne diseases have evolved. These include chemical, biological and ecological control methods. The use of pheromones as attractants and growth regulators in life stages of

pest arthropods have all been tried with varying degrees of success. More recently, the immune mechanism of host animals have been stimulated through exposure to parasite antigen to build up resistance, leading to rejection of the parasite (Allen and Humphreys, 1979; Johnston *et al*,1986; Opdebeeck *et al*, 1988; Willadsen, 1997). Merits and demerits of each method used can be assessed by the degree to which the parasite population subjected to control is reduced or even eliminated.

2.4.1 Chemical Tick Control:

Chemical agents used in the control of ticks and mites (acarines) are called acaricides. Major acaricide compounds include organochlorides, organophosphates, carbamates and pyrethroids. Ivermectin is derived from Avermectin (plant) and is used as a systemic parasiticide, in this case, against ticks and mites infestations. Acaricides are generally toxic compounds which, if handled carelessly, can lead to toxicity in animals and man. Reliance on the use of acaricides for the purpose of tick control over prolonged periods of time has led to a number of important tick species developing resistance to certain of the chemical compounds. There is also the problem of environmental contamination due to persistent and careless use and disposal of spent chemicals. In certain cases, animals treated wrongly with acaricides harbour residues in their tissues and milk products. This is passed on to man through consumption of contaminated products of animal origin. Non-target animals and useful arthropods are often victims of uncontrolled use of acaricides. Exposed animals suffer chemical poisoning which result in morbidity and mortality, contributing to loss of production (Norval *et al*, 1992).

The development of tick resistance is a major drawback to sustained use of acaricides in tick control (Norval *et al*, 1992; 1996). One-host-ticks like *Boophilus*

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spp. develop resistance more rapidly than do other types because of their shorter generation time and continual exposure of the same tick stages to acaricide while on the host. The continued use of acaricides over prolonged periods of time on animal flocks/herds interferes with the development of endemic stability to the tick parasites, which is often seen in animals not exposed to artificial treatments. In these animal populations there is, thus, the risk of disease outbreaks when acaricide treatments are interrupted (Norval *et al*, 1991). The high cost of producing acaricides and high cost of purchase, coupled with the manpower requirement during tick control programmes makes its use costly. Dipping of animals may not necessarily reduce tick populations appreciably in the field as other factors such as temperature and humidity, vegetation cover and tick-carrying wild animals and birds come into focus (Kocan, 1995; Floyd *et al*,1995;Nari,1995;de-Castro,1997;Willadsen,1997a).

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2.4.2. Application of Acaricides:

The most commonly used methods for the application of acaricides on animals are spraying with and dipping in acaricides. Animals are normally driven through the vat in such a way that ensures complete immersion of all body parts. This method is more effective in controlling ticks than the use of hand or spray-races (Norval *et al*, 1992). Ticks are also controlled by the use of pyrethroids such as flumethrin and cypermethrin, as pour-on or spot-on, applied to limited surface areas of animals for a duration of time. Their short residual life span when applied makes them ideal for use without too much worry of environmental contamination. This contrasts directly with the organochloride and organophosphate acaricides often used in dips. Organochlorides such as Dichlorodiphenyl-trichloroethane (DDT), Benzene hexachloride (BHC) and Dieldrin are the most persistent in the environment long after

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application while organophosphates such as Asuntol®, Supona®, Ectigal® are less so although could be very toxic if used inappropriately. Slow-release acaricide boluses, and acaricide-impregnated ear or tail tags are used only in limited cases. Systemic acaricides like ivermectin are effective against ticks when administered to infested animal hosts as demonstrated by studies carried out in Africa and elsewhere (Wilson, 1993). Such treatments may be carried out as trans-cutaneous (using high pressure jet or spray), sub-cutaneous (injectables) or through the oral route (Norval *et al*, 1992).

The construction of a dip requires capital expenditure because a large space, large number of experienced staff and much quantity of acaricides and water are required. The risk of accidental ingestion of acaricides by dipped animals, and also their proness to fractures (of limbs) and other injuries are important limiting factors (Morel, 1989). Strict precaution is paramount in the use of most acaricides in dips and spray races to prevent toxicity.

The use of spray-races may not be as expensive except where fixed ramps or mobile races are required. Toxicity is also less of a problem and most acaricides can be re-used. Less stable chemical compounds like the pyrethroids are best suited for use in spray races.

The age, size and physiological conditions (pregnant and lactating mothers) of animals must be considered before they are subjected to dipping or spraying. Fatigued animals should not be dipped or sprayed until after a rest period when the chances of absorbtion of acaricides through dilated pores in the skin is minimal. Animals that are to undergo treatment must first be properly fed and watered in order to avoid ingestion of the solution. The dipped or sprayed animals should be dried as fast as possible in order to reduce increased absorbtion through the skin (Morel, 1989).

2.4.3 Mode of Action of Chemical Acaricides:

2.4.3.1. Chlorinated_Hydrocarbons: These compounds are all liposoluble, thus permeate the lipids of insect cuticle leading to nervous symptoms. They slowly inhibit the activity of cytochrome oxidase, exerting their effects on the central nervous system. The toxic effects of organochlorides are seen in ticks within 12 hours of contact. There is permanent contraction of the dorsiventral muscles, causing deep longitudinal grooves on the body which appears flattened (Morel, 1989); death follows within 24 hours.

The most active acaricide within this group is lindane, the gamma isomer of benzene hexachloride (BHC). Dieldrin is also active but very toxic; toxaphene is similarly active against ticks but more persistent in the environment. Dichlorodiphenyl-trichloroethane (DDT) is not recommended as an acaricide because of its very long residual life after application in the environment and in warm blooded animals. A variable amount of the insecticide is retained and fixed in the adipose tissue from where it is gradually released into the circulatory system. Chronic poisoning may then occur as cumulative treatments are continued without a commensurate elimination of the chemicals imbibed. The chemicals are also passed into the milk and tissue of animals (Norval *et al*, 1992), building up a chain of contamination through consumption of affected animal products by man. Most organochlorides are contraindicated for use as acaricides presently.

2.4.3.2 Phosphated Hydrocarbons: Organophosphate acaricides are more acceptable in tick control programmes as they are less toxic and persistent compared to organochlorides. They are highly liposoluble compounds (and slightly water soluble) which penetrate arthropods more by ingestion than via the cuticle.

Haematophagus arthropods are most affected when oral, parenteral or transcutaneous routes of administration of the chemicals are used in host animals. Organophosphates have low stability in animal tissues and are rapidly eliminated within one week of administration. The constituent phosphorus enters into the general circulatory system and is metabolised. There is no accumulation of the chemical in the host tissues. Some examples of organophosphate acaricides include chlorfenvinphos (Supona®) coumapnos (Asuntol®), diazinon (Ectigal®) (Morel, 1989).

2.4.3.3 Carbamates: The most important compound in this group is carbaryl (Sevin), which often comes as a wettable powder (WP). The mode of action is similar to the organophosphates in which arthropods are killed more by ingestion than contamination. Both groups of acaricides act directly on tick enzymes or indirectly on the metabolic system. Toxicity is achieved through the inhibition of several enzymes, particularly cholinesterase, and the toxin remains in the enzyme system until a nervous sympton is manifest. Oogenesis and oviposition are disrupted in female ticks and eggs are inhibited from further development due to desiccation as the porous area glands which secrete the egg shell are also inhibited. As with the organochlorides, organophosphates and carbamates have been resisted by certain tick species due to prolonged usage (Stendel, 1986).

2.4.3.4 Other Acaricides; These include pyrethroids and amidines, which are less toxic and more user friendly. Common pyrethroids include deltamethrin (Butox), cypermethrin (Ectomine), flumethrin (Bayticol pour-on;) amidines comprise armitraz (Tritrix), cymiazole (Tigatol). These chemical compounds have a fast knock-down property as they act on the nervous system of arthropods. They are often used in

places where tick resistance to organochlorides and organophosphates have developed (Nolan *et al*, 1979; Liebisch, 1986; Stendel, 1986).

2.5 Tick Resistance to Acaricides:

Most reports of tick resistance to acaricides come from tropical and sub-tropical countries where intensive dipping programmes are enforced for the control of ticks.

Arsenic, one of the earliest acaricides; was also one of the first compounds to be resisted by hard ticks (Smirnova,1978). The most liable to becoming resistant were the one-host Boophilid ticks, followed by certain Rhipicephalid species (Morel, 1989). Certain *Amblyomma* spp and *Ixodes* spp. were also reported to have developed resistance (Smirnova, 1978; Crampton and Gichanga, 1979; Mathewson *et al*, 1980).

Acaricide resistance involves different mechanisms in ticks. Certain ticks have the capability to inactivate certain acaricides by combination with other biochemical substances which block fixation of the toxin to specific enzymes, thereby preventing activation. Organochlorides and organophosphates may be detoxified by the tick through enzymatic dehydrochlorination and hydroxylation. Changes in the toxic action sites will promote resistance to the acaricides and will require an increase in the toxic concentration of the acaricide to be effective. Cross resistance to DDT, the pyrethroids and other pyrethrins indicate the same mechanism in action (Morel, 1989).

Regular use of acaricides causes mutation of genes in tick populations. This leads to the development of heterozygote mutants with low sensitivity to the acaricides. This stage which is further enhanced by vigorous use of acaricides leads to the development of highly resistant homozygotes. This type of resistance is easily

induced by arbitrary increases in the concentration of acaricides in dips and sprays. Mutation of a single autosomal gene could lead to the appearance of resistance (Morel, 1989). This also depends on increased concentration of enzymes that are not normally abundant or typical in ticks. Schnitzerling *et al*, (1982) reported widespread resistance to organophosphates due to decreased sensitivity of the target enzyme, acetylcholinesterase, and suggested that the change in enzymatic behaviour could be due to an increased distance between catalytic sites. They observed lowered acylation of enzymes in resistant ticks than in the susceptible ones. The cuticle of resistant ticks contained 4 to 6 times more wax than those of susceptible ones, thus decreasing sensitivity (Smirnova, 1978). Tick resistance therefore remains one of the major problems in the development of viable livestock industries, especially in the tropics and sub-tropics (Nolan, 1990).

2.6. Alternative Methods of Tick Control:

2.6.1. Ecological Control Method:

Pasture modification of tick-suitable environments through bush clearing or burning will be of limited effect on the many species of ticks present in the wild. This is more so in the tropical and sub-tropical regions of the world with vast expanse of forest and grassland. These ecological areas are vast and almost impossible to modify, the inhabitants and various government policies inclusive. The destruction of wildlife in order to control hard ticks is not only detestable but difficult to carry out, especially in the light of opposition by conservationists and environmentalists.

A few plants and grasses have been identified as having anti-tick properties which kill and discourage ticks in areas where they grow. *Stylosanthes spp* (Surtherst *et al*, 1982) and *Gynandropsis gynandra* (Malonza *et al*, 1992) have been tried with

limited success. The impact of such control methods are certainly not far reaching considering the size of the areas and fauna in question.

Pasture rotation is a method of tick control extensively practiced in Australia (Morel,1989). The vast, mainly unoccupied grassland areas and the relatively few wild animals may be the reasons for the success of this system of tick control in Australia. The general principle was established on the basis of the length of time it will take for desiccated, starved ticks to die before returning the animals to the pasture. Herds are rotated between two or three pasture lots in the course of one year. Another rotation plan is based on the length of embryogenesis of tick eggs. This can be practiced by rotating the animals through four plots. The period spent in each plot depending on the development period of the eggs (Morel, 1989). Cattle were made to move from small disinfested plots to large ones, their stay being longer than the duration of their parasitic phases but shorter than the time required for the eggs to hatch. This method of tick control is effective mainly where species have short life cycles with one or two parasitic phases. In Nigeria, only *Boophilus* spp have shorter life cycles while other species are multi-host and with longer life cycles. This method of rotating animals from one feed lot to the other may therefore be impracticable in this country.

2.6.2. Biological Control Methods

The term biological control describes situations when biological, live agents such as viruses, bacteria, fungi, protozoans, turbellarians, nematodes, insects, copepods and mites are manipulated and used by man as antagonists to parasitic arthropods, protozoans and helminths. Only very few of these antagonists have shown promising qualities as biological control agents within veterinary science

(Gronvold *et al*, 1996). Few attempts have been made to control ticks with a degree of success (Sonenshine, 1993). One example is the chalcid wasp *Hunterellus hookeri* which was released to control the American dog tick *Dermacentor variabilis*, with an inconclusive assessment of success or failure (Gronvold *et al*, 1996; Mwangi *et al*, 1991. This parasitoid has been reported in a number of tropical countries where it parasitizes a number of different tick species (Mwangi *et al*, 1991). Engorging ticks are killed when the parasitoid pierces through the cuticle and lays eggs inside the haemocoel. Larvae of this parasitoid develop into adults which bore out of the now dead tick.

Ixodiphagus spp. are hymenopterans which, like *Hunterellus* sp. lay their eggs in engorging ticks, killing them. Bowman *et al* (1986) reported infestation of nymphs of *D. andersoni* and *D. variabilis* with *Hunterellus hookeri* which became distended with the developing stages 8 to 14 days post infestation. The irregular stripes of developing parasitoids were visible through the tick cuticles. About ten or more parasitoids often emerged from a parasitized tick (Mwangi *et al*, 1991). *Amblyomma* spp. were not suitable for control with parasitoids as they were consistently not attacked by the parasitoids under experimental condition (Mwangi *et al*, 1991).

Predators of ticks are many but birds appear to be most commonly observed. The red-billed oxpecker (*Buphagus erythrorhynchus*) and the yellow-billed oxpecker (*B. africanus*) prey on parasitic ticks on hosts such as cattle, sheep, horses, and wild herbivores like elephants and hippopotamus. The cattle egret (*Argeola ibis*), the African wagtail (*Motacilia agwimp*) and even the local domestic chicken (*Gallus* spp) are effective predators of hard ticks (Hassan,*et al* 1991; Mwangi *et al*, 1991). Ants, spiders, lizards, rodents, shrews etc are also predators of ticks (Mwangi *et al* 1991; Gronvold *et al*, 1996).

Microbial control involves the use of bacteria, viruses, rickettsiae, protozoans and fungi or their metabolites in the control of tick vectors (Carlsberg, 1986). A few species of bacteria have been isolated from dying ticks although none was specifically identified as the aetiological agent. Some of these include *Serratia marcescens*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus* spp, *Escherichia coli* and *Pseudomonas* spp. It was observed that all infected ticks lost their original morphologic colour from greyish to black, prior to their death after a few days (Hendry and Rechav, 1981).

Fungi have also been isolated from infected ticks (Lipa, 1971). Some of these are *Aspergillus fumigatus*, *Beuveria bassiana* and *Penecillium insectivora*. It is doubtful if any of these known pathogens of ticks could control tick infestation in livestock independently. Even if tick control will benefit from biological methods, an integrated programme involving biological, chemical and even immunological methods may be necessary in order to effectively control ticks and the diseases they transmit. Ultimately, the intention is to lower tick populations to an equilibrium position below the clinical and economic threshold levels (Gronvold,*et al* 1996).

2.6.3. Pheromone-mediated Control

The use of mating pheromones or externally relaseased pheromones, to lure pests to traps treated with insecticides is used for some crop pests. Ticks posses mating and, in the case of *Amblyomma* spp, aggregating pheromones also which can be used to lure them into traps or acaricide impregnated sites on hosts. Sex pheromones are important in attracting and aggregating female ticks at male feeding sites and during mating on vertebrate hosts (Sonenshine, 1991; Norval *et al*, 1991; Sonenshine *et al*,1992). An aggregation/attachment pheromone (AAP) emitted by

males of *Amblyomma* spp was observed to enhance aggregation of nymphs and adults on hosts (Norval *et al*, 1992). A pheromone-like synthetic compound (dichlorophenol) attracts ticks to objects on which it is applied. This can be used in conjunction with an acaricide to control tick populations (Norval *et al*, 1992; Allan *et al* , 1998).

2.6.4. Immunological Tick Control

There has been extensive studies of the cutaneous reactions of cattle to tick attachment by scientists in Australia and elsewhere, using the only economically important tick species, *Boophilus microplus* as the parasite (Johnston *et al*, 1986; Kemp *et al*, 1986). They observed, at the point on tick attachment, a developing vesicle surrounded by a patch of oedema the size of which depended on the level of the animal's resistance to ticks. Susceptible animals did not show signs of irritation and the oedema was slight; these reactions were extensive in resistant animals, indicating a high degree of resistance. They also observed that the blood histamine levels rose within 48 hours of tick attachment in resistant animals and fell gradually from the 4^{th} to the 7^{th} days. There was no change in the histamine levels in receptive animals.

Tick resistance in animals may be spontaneous or acquired, following infestation with ticks. Postulates as the length of hair coat, number of sebaceous glands or mast cells per area of skin, thickness of skin, depth of hair follicle, number of circulating lymphocytes and diet of the host have been introduced as possible reasons for differences in infestation rates in different animal hosts (Brown, 1988). *Bos indicus* cattle exhibit, to a greater extent, more innate resistance to ticks than *Bos taurus*; and the greater the percentage of *Bos indicus* blood in an offspring, the greater

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is its resistance. The mechanism of the innate resistance in animals is yet to be well understood, although this character can be exploited in order to partially control ticks (Brown, 1988; Johnston *et al*, 1986; Dipeolu *et al*, 1992). However, considerable immunological work was done in an attempt to understand the mechanisms of this acquired resistance in *Bos taurus* and *Bos indicus* breeds of cattle where a number of correlates were found between levels of acquired resistance and a variety of parameters, including mast cell density, eosinophil migration, total skin histamine and , in particular, correlations between skin sensitivity to specific tick allergens and acquired resistance which all suggested that the major mechanism of this resistance was allergic (Willadsen, 1980). Whether a strong allergic reaction which results in the removal of feeding ticks from an individual animal is beneficial to it is not clear, although the benefits of herd immunity are not questioned (Willadsen, 1997a). This form of tick control is only suitable in areas like Australia and South America where only one tick species (*B. microplus*) is of commercial concern, but will not be suitable in countries where there are several tick species (Wikel and Allen, 1982) as in Africa.

Acquired resistance consists of immune mechanisms that are acquired during the life of the animal ; and the ability to acquire the character is, to a large extent (between 40% and 80%) inherited (Wikel and Allen, 1976). In Africa, the Muturu and the Ndama breeds, and to a lesser extent, the Zebu exibit a degree of resistance to ticks. Exotic cattle like the Freisians and Herefords are very susceptible to tick infection (Dipeolu *et al*, 1992).

In *Bos indicus*, the mechanism responsible for acquired resistance to *Boophilus* ticks has been reported as a mast cell-dependent eosinophil hypersensitivity (Brown, 1988) based upon histological observations. However, the response of *Bos taurus* to *Amblyomma* spp., a three host tick, indicated a mixed

cellular response of neutrophils, eosinophils and basophils. This may indicate a difference in host reactions to tick feeding by one host ticks compared with the three host tick (Brown, 1988). Preliminary evidence suggests that resistance to ticks is acquired and therefore presumably immunological . Heritability is high and preliminary analysis indicates that the effect is either due to a single major gene or a small number of genes. The aquired resistance is strongest when infused into a resistant background , suggesting that the mechanism of allergic resistance may be different to or enhance each other (Willadsen, 1997a).

Whatever the mechanism of acquired or innate resistance, ticks feeding on resistant animals encounter hostile reception from the host's immune system. The observed hypersensitivity in bovines to tick feeding ,at attachment sites results in serious exudates within hours of infestations, which is associated with intensive grooming activity (Brown, 1988). The pruritis is probably due to mast cell degranulation, which releases vasoactive mediators, such as histamine at tick attachment sites, following interaction of cell-surface-bound , anti-tick antibody with tick-derived substances (Allen *et al*, 1977). Histamine and other mediators contribute to the inflamatory processes by altering the permeability of local blood vessels, resulting in oedema that could render the tick feeding environment unsuitable; the accumulation of effector cells that could act directly on the ticks or release other mediators that could adversely affect physiological processes crucial to tick feeding, ovipositional success and survival are other factors. The presence of antibodies in the immune reaction to tick infestation has also been reported (Brown and Knapp, 1980). The involvement of cell-mediated immunity (T-lymphocytes) in acquiring resistance to ticks in guinea pigs was demonstrated by passive transfer of lymph node cells and peritoneal exudate cells (Wikel and Allen, 1976; Brown, 1982). The

level of protection conferred ranged from 40% to 70%, as compared to the protection level of 20-40% resulting from passive transfer of serum. The immune response to tick infestation therefore involved both antibody and cell mediated responses.

Regardless of the host's immune reactions , ticks are able to remain attached to their hosts and, if let alone, will fully engorge before detaching. It has been suggested that the tick saliva contain immunosuppressive agents which react with host enzymes or other immunologic secretions to shield the feeding tick from hostile reactions from the host (Wikel and Allen, 1982). The effect of these immunosuppressants on the immune cells of the host causes a delayed type hypersensitivity reaction , enabling the tick to remain attached until repletion (Wikel, 1988). The mechanism of immunosuppression of host by ticks is not well understood but it has been attributed to the detrimental effect tick immunosuppressants have on the B-cell cell effector mechanisms in the host. There is a depletion of B-cell dependent areas as well as slight depletion of cells from T-cell dependent areas in guinea pigs injected with cyclophosphamide. This suppressed the population of B-cells which regulated the expression of classic delayed hypersensitivity and cutaneous basophil hypersensitivity reactions. This could be the type of reaction which takes place at the host/parasite interface during tick feeding (Turk, 1975; Turk *et al*,1972). There is evidence that effective immunosuppression induced by tick infestation through direct action of tick saliva and salivary gland components , and also through the cytokine network of host's immune system , leads to a non-protective response in the host. This tick-induced immunosuppression of the host is characterized by decreased primary antibody responses to T-cell- dependent antigens, decreased production of T helper cell type

1 cytokines and enhanced production of T helper cell type 2 cytokines (Wikel, 1996). These could be the mechanisms by which ticks remain and feed to repletion on susceptible , apparently, immunosuppressed hosts. Other factors which may induce immunosuppression in hosts could be pharmacological - as in the inhibition of blastogenic responses of bovine mononuclear cells by prostaglandin E2 contained in tick saliva , or the occurrence of high-affinity histamine-binding proteins in the saliva of *R. appendiculatus* capable of inhibiting the functions of immunocompetent components of the hosts (Wikel, 1996). The mechanism of immunosuppression in hosts by ticks is yet to be properly elucidated (Willadsen and Jongejan, 1999).

Detrimental effects on ticks feeding on immune hosts include reduced engorgement weights, delay in onset of oviposition and reduction in egg mass of female ticks, and reduction in viability of eggs and larvae (Allen and Humphreys, 1979; Ackerman *et al*,1980, McGowan *et al*, 1980; Willadsen and Jongejan, 1999).

The current approach to tick control involves the use of tick tissues as antigenic material for the development of anti-tick vaccines , which may be specific to tick species.

When ticks feed, they are exposed to immunoglobulins in the blood of an immunized animal. These will remain as active antibodies in the tick. If an antigenic protein of the tick such as a digestive protein is used as an antigen to vaccinate the animal, the animal will then develop antibodies against this protein. When ticks (of the same species) feed on the animal, antibodies in the blood will react with the antigenic protein in the tick and damage/kill the tick (Johnston *et al*, 1986; Kemp *et al* 1986; Opdebeeck *et al*, 1988). This is the principle behind the use of an anti-tick vaccine.

Tick feeding is interrupted by action of host antibodies against the tick species (Wikel, 1988, 1995; Willadsen and Kemp, 1988; Dhadialla *et al*, 1990; Willadsen and McKenna, 1991; Allen, 1992; Wikel *et al*, 1992). Antigens which are concealed and not normally exposed to the vertebrate host during feeding are preferred. This is because the concealed antigens will not normally be introduced into the host body during feeding, therefore hypersensitivity reactions will be avoided (Willadsen and Kemp, 1988, 1989; Wikel, 1992).

2.6.4.1. Immunisation against ticks:

Demonstration of the feasibility of immunisation falls within three stages. Firstly, it must be shown that resistance can be produced by vaccination with tick material. Secondly, a way must be found to produce tick antigens in commercial quantity either by tissue culture of appropriate tick cell lines or identification and characterization of antigens by biochemical and immunological techniques , followed by application of biotechnological or chemical synthesis to make synthetic antigens. Thirdly, the antigen must be incorporated into a practical vaccine after selection of suitable adjuvants and delivery systems (Willadsen, 1987).

Vaccines made with materials obtained from the gut and synganglion tissue of female *Boophilus microplus* gave good protection against heavy experimental infestation of larval *B. microplus* ticks (Opdebeeck *et al*, 1988, 1992). In similar experiments carried out by a number of workers, solubilized fractions of female tick gut membranes offered protection to cattle immunized against ticks (Willadsen and Kemp, 1988, 1989; Wong and Opdebeeck, 1989; Opdebeeck and Daley, 1990). Johnston *et al*, (1986) reported medium to good immune response to tick challenge in cattle vaccinated with whole extracts of female *Boophilus microplus ticks*. Affected

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female ticks showed progressive mortality before oviposition, and throughout the feeding period. There were about 60% of female ticks with damaged guts, and a delay in moulting of larval ticks was observed. Some of the female ticks failed to engorge (Johnston *et al*, 1986; Kemp *et al*, 1986). There were a number of male ticks which also suffered gut damage. Agbede and Kemp (1986) observed that within 24-28 hours of attachment, digest cells in the gut of affected ticks sloughed off into the lumen or were completely destroyed leaving only the basal lamina and muscle layer . Subsequent rupture of the gut allowed host leucocytes to enter the haemocoel and attack other tissues which caused a delay in the development of digest cells and gut basophilic cells. Host leucocytes in tick haemolymph further destroyed tick muscles and Malpighian tubules but not the salivary glands. In male ticks, the histopathology of the gut was similar to that in females but in addition, the accessary gland of the reproductive organ was also damaged by host leucocytes. They did not find these effects in ticks fed on control hosts, confirming that vaccination apparently stimulated a different mechanism of resistance to ticks than in naturally acquired immunity (Willadsen, 1987). Similar reactions were reported in guinea pigs which were immunized with extracts of *Amblyomma variegatum* larval ticks (Heller-Haupt *et al*, 1987). These effects can be produced in ticks fed on blood components from immunised bovine serum or plasma *in vitro*, showing that the effects are anti-body mediated (Willadsen, 1987). Essuman *et al*, (1991) immunized Boran cattle with a semi-purified fraction (F2) of solubilized membrane-bound antigens extracted from the midguts of female *Rhipicephalus appendiculatus*. They reported a good number of protected animals from those immunised as compared with the controls. As with other reports, they observed a decrease in the engorgement weight of female ticks and failure of replete larvae to moult. They also asserted that the main cause of tick

mortality was due to the excessive grooming activity of vaccinated hosts in which most of the attached ticks were licked off the skin. Animals which acquire resistance to ticks often carry less burden as ticks are rejected almost as soon as they attach. There is little or no apparent damage to them (Willadsen, 1980). The reduction in weight of engorged female ticks, reduced egg laying capability and egg viability were attributed to the toxic effects of the vaccine (Bowessidjaou *et al*, 1977) and antibody activity (Opdebeeck *et al*, 1988). Morrison, (1989) suggested that tick rejection involved both humoral and cellular responses since passive and adoptive transfer of immunity in laboratory animals to other tick species resulted in rejection. In rabbits, acquired immunity to ticks may be partially antibody mediated. This is because injecting cyclophosphamide into immunized guinea pigs blocked the expression of immunity (due to blockage of B-cells), which shows the involvement of antibody (Wikel and Allen, 1976; Willadsen, 1980; Brown, 1982). Willadsen *et al*, (1978) were able to measure antibody responses to purified tick antigens by direct haemaglutination in vaccinated cattle, which was absent in unexposed animals. The antiibody was adjudged specific although the concentration did not correlate with the degree of immunity conferred. This, however, confirms the involvement of antibodies in acquired immune responses in exposed animals, and it is on the basis of this that an anti-tick vaccine can be produced to control tick infestation in domestic animals.

In addition to studying the histopathology of tick bite sites in two breeds of sheep, the production and testing of a semi-purified, midgut-derived membrane-bound protein vaccine, using *Amblyomma variegatum*, will be attempted as part of this study. The objective is to protect or reduce the load of tick burden on sheep and cattle by minimal use of acarides after immunizing with anti-tick vaccine.

2.6.4.2. Vaccines Against Ticks:

Ticks have numerous cell membrane receptors and hormones that could serve as targets for vaccines (Kocan, 1995). A successful use of this approach depends on the identification and characterization of these internal receptors and cellular components which are essential and unique to the tick, and that could be accessible to and be affected by specific antibodies introduced when ticks feed on vertebrates (Kocan, 1995).

Three major classes of membrane receptors have been identified in the tick. These include the gate-ion channels, agonist-stimulated kinases and receptors that interact with CTP-binding proteins; targetting any of these might interrupt fluid secretions, inhibit turnover of plasma membranes or even block receptor-mediated endocytosis. The result will be an interference with vitellogenesis and uptake of lysed blood meals by digested cells in the tick midgut (Saur *et al*, 1994; Kocan, 1995). Hitherto, it has been demonstrated that ticks feeding on tick-sensitized cattle are rejected and damaged by host immune systems (Johnston *et al*, 1986; Kemp *et al*, 1986; Opdebeeck *et al*, 1988, 1992; Essuman *et al*, 1991; Rutti and Brossard, 1992). Furthermore, a recombinant vaccine named TickGARD has been produced in Australia. It is specifically targeted against a gut membrane protein (Bm86) in such a way that the antibody produced in the host and ingested by the tick during blood feeding binds to certain gut digest cells of the tick, on whose surface is located the antigen , inhibiting endocytosis of blood meal components leading eventually to lysis of the gut cells (Hamilton *et al*, 1991; Tellam *et al*, 1992; Willadsen, 1997a,b). The antigen has been sequenced and expressed in bacterial, yeast and insect cell expression systems (Willadsen *et al*,1989; Tellam *et al*,1992). This vaccine was

developed against *B.microplus* ticks in Australia and other temperate areas where the tick species exists. It was registered for commercial sale in Australia in 1994. No cross reactivity has been demonstrated between target proteins in this species and other tick species (Willadsen, 1997a) although a variety of tests of efficacy and safety has shown that the vaccine was completely safe for use and efficacious in a variety of geographical locations, while at the same time is effective against a wide range of ticks that were resistant to different classes of acaricides. However, there were differences in susceptibility of a number of tick isolates for reasons that are not known. It was further shown that a range of different cattle breeds responded to the vaccine in equivqlent ways, as measured by antibody responses following vaccination. Vaccination of cattle when tick population is very high was observed to be less effective. An improved version of this vaccine has already been produced. It is called TickGARD plus, and was registered in Australia in 1996. This improvement relies on an enhanced production of antibody to the existing Bm86 vaccine (Willadsen *et al*,1996). Considerable effort has gone into the identification of other antigens which could be added to the existing Bm86 vaccine to further improve its efficacy. An antigen Bm91 which is present in the gut but also predominantly in the salivary gland of *B. microplus* was considered a less suitable recombinant protein than the Bm86. Lee *et al*,, (1991) were able to identify eleven monoclonal antibodies which could precipitate tick gut membrane of *Boophilus microplus*. These could be important target proteins for the development of anti-tick vaccines.

2.7. Histopathology of Tick Attachment Sites:

A histopathological examination of tick attachment sites revealed infiltration of the dermis by eosinophils and other polymorphonuclear cells, and lymphocytes (Wikel and Allen, 1982). Accumulation of these cells formed distinct pustles around tick mouthparts. On non-resistant cattle, only slight infiltration of these cells formed, with haemorrhagic pools around tick mouthparts. There is also accumulation and degranulation of eosinophils in epithelial vescicles at the attachment sites of ticks feeding on resistant cattle (Schleger *et al,* 1976). The reaction at tick-bite sites are more intense in more resistant animals, resulting in hypersensitivity reactions which hasten tick rejection (Wikel, 1982; Wikel and Allen, 1989). Furthermore, there is increased level of histamine (due to mast cell degranulation in the cutaneous reaction) and serous exudation with papular eruptions accompanying oedema in resistant animals (Willadsen and Kemp, 1989; Wikel, 1992, 1995). These immunological reactions could be passively transferred locally by serum from a donor to a recipient animal (Tatchell and Morehouse, 1968). Hypersentivity reactions at feeding sites elicit intense grooming activity by the sensitized host.

Theis and Budwiser (1974) gave an insight of sequential changes which took place at the bite site of *Rhipicephalus sanguineus* on the skin of dogs. There was a zone of erythromatous swelling 2-3mm in diameter around tick mouthparts 48 hours after attachment. The affected site was raised about 1mm above the surrounding tissues. The diameter of the erythromatous nodule increased to 4mm 72 hours after attachment; and then to 5mm from 96 to 120 hours after attachment. During this time, the tick mouthparts were completely embeded in the cement material secreted by the attached tick, as well as in the tissue. There was thickening of the affected epidermal tisues as a result of increase in the number of viable cells recruited from

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surrounding tissues into the inter- and intracellular spaces, constituting oedema. Cells in the stratum germinativum and the prickle-cell layers of the epidermis were enlarged compared to those outside the reaction areas, and their intercellular bridges were prominent due to the degree to which cells had been pushed apart by the accumulation of oedema fluid.

Destruction of collagen fibres in the dermal layers of the dog's skin began within 24 hours after attachment. A cavity measuring about 180µm in length and 210µm in depth had formed at this time. This gradually increased in size until the maximum size of 1,275µm in length and 600µm in depth were attained 24 hours before the tick detached. In contrast to the collagen, reticular fibres were not severely destroyed and a fine network of these fibres were seen throughout the reaction area. There was rupture of some of the smallest blood vessels.

There was no appreciable cellular infiltrates into the feeding lesion within the first 24 hours but within the next 24 hours, there was infiltration of mainly neutrophilic polymorphonuclear leukocytes. There was an intensive perivascular cuffing with neutrophils within 48 hours after attachment. This decreased as time went on, accumulating on destroyed collagen tissue instead. Many neutrophils and eosinophils accumulated in the formed cavity below the tick mouuthparts towards the final stages of tick engorgement. There were also erythrocytes and leukocytes in the cavity. Throughout the period of attachment, the polymorphonuclear cells were the most prominent in the lesion.

After the detachment of the tick, there was a gradual change in the components of the lesion. Large fibroblast cells, together with varying numbers of macrophages and a few plasma cells became the prominent cell types. Sometimes the macrophages contained small red cells that had haemorrhaged into the tick feeding

cavity. In the area of the lesion where leukocyte infiltrates were prominent, the mast cells were usually degranulated.

As part of the healing process after tick detachment, they observed increasing numbers of mononuclear cells in the lesions, many of which had ingested rbc's and fragments of broken down tissues. Fibroblasts and macrophages increased in population. Six to seven days after detachment, the epidermal cells in the germinal layers had begun to grow in from the edges of the lesion, although oedema of the epidermal cells persisted for days. There were numerous fibroblasts and the collagen fibres were beginning to form. There was healing by day 12 after detachment.

Histopathology of tick bite sites on tick-naïve animals differed markedly from similar sites in tick-exposed hosts. Allen (1973) observed slight changes in skin constitution of a tick bite site in tick-naïve guinea pigs during initial exposure. There was slight hyperplasia of the epidermis and a few granulocytes had infiltrated the dermis. During the second and subsequent exposures, he observed marked changes in the epidermis which included acanthosis, acantholysis, granulocyte infiltration, and the formation of vescicles which were filled with granulocytes, most of which were basophils. The other cells were eosinophils, macrophages, erythrocytes and lymphocytes. Wikel (1988) made similar observations as described by Allen (1973) and concluded that for the development of resistance to ticks, hosts needed to be exposed to infestation several times over a period of time. It has also been established that Langerhans cells trap salivary gland antigen in the epidermis of guinea pigs infested with ticks, suggesting that these cells may play a central role in the development of the resistance response (Allen and Humphreys,1979) which alters the feeding pattern of ticks on exposed animals (Wikel, 1982; Wikel and

Allen, 1982). A more efficient way of raising anti-tick response in animals may be the introduction of antigenic materials derived from ticks into host animals in the form of vaccination (Kocan, 1995).

CHAPTER THREE

SEQUENTIAL HISTOPATHOLOGY OF THE SKIN OF TWO BREEDS OF SHEEP EXPOSED TO THE BITES OF *Amblyomma variegatum*

3.1. INTRODUCTION

Amblyomma variegatum is a three -host tick species belonging to the order ixodidae. It is an economically important tick species infesting several species of domestic and wild animals including birds, reptiles and mammals. It is the vector for *Cowdria ruminantium* in Nigeria (Ilemobade, 1977), and has been incriminated in the transmission of dermatophilosis (Kirchi) in cattle (Mohammed, 1977). The feeding characteristics of this tick species is such that flat females can engorge as much as 20 times their weight of blood. Heavy infestations may therefore result in anaemia through blood loss, and damage to hides and skin as a result of their bites (Arthur, 1970, 1973; Tatchell and Binnington, 1973; Mohammed and Agbede, 1980; Kemp *et al*, 1982; Fabiyi, 1984).

The feeding process of ixodid ticks have been extensively studied in relation to disease transmission and host immunity (Balashov, 1972; Kemp *et al*, 1982). The structure of the feeding apparatus has also been properly elucidated (Gregson, 1960; Kemp and Tatchell, 1971; Balashov-Yu,1972). Most hard ticks are highly selective in their host species and their predilection sites. The sensory processes leading to attraction and attachment of ticks to host has also been well studied (Wikel, 1982; Waladde and Rice, 1982). The preference for particular parts of the body were attributed to a number of factors. Some of these include the thickness of the skin on a prefered site where it may be thinner; thickness of hair at the site, such as thinner and smaller hair at the perineum and genital areas; areas that are better protected from

adverse weather conditions such as the ventrum as opposed to the dorsum; as well as the stage of development of the parasitizing ticks (Callow, 1978; Bordeau, 1982).

Lesions created at tick feeding sites by active laceration of bovine host skin with the pair of chelicerae are further aggravated by secretion of saliva which contains active pharmacological agents aiding the process and resulting into ulcers (Kemp *et al*, 1982). Prostaglandin E is one of such substances which has been proved to cause dilation of blood capillaries, resulting in extensive haemorrhage, tissue damage and prevention of host blood coagulation. In the bovine, response leads to oedema, haemorrhage, leucocyte degranulation and further damage to tissues (Allen *et al*, 1977). Vascular permeability is increased due to the action of these agents at bite sites, especially just before final rapid engorgement in female ticks. Histologically, there is intense infiltration of basophils, eosinophils lymphocytes and other polymorphonuclear cells (Theis and Budwiser, 1974; Bagnal, 1975; Brown and Knapp, 1980).

Although there is abundant literature (Kemp and Tatchell, 1971;Theis and Budwiser, 1974; Brown and Knapp, 1980) on the histopathological lesions created by tick bites in cattle, there is paucity of information on the subject in the ovine, which may also serve as a model of tick infestation in cattle.

This experiment was therefore designed with the objective of studying the sequential histopathology at the bite sites of female *Amblyomma variegatum* on the skin of two local breeds of sheep in Nigeria.

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3.2 MATERIALS AND METHODS

3.2 1. Experimental Animals:

Six sheep aged between 6 and 8 months belonging to two breeds (3 each of Yankasa and Uda) were purchased from apparently tick-free sources in Tudun-Wada, Zaria and metropolitan Gusau in Zamfara State. The flocks from which they came were vitually zero-grazed all through the year as feeds were supplied to them in enclosures which restricted their movements.

The sheep were separated into two cubicles, according to breed, in an arthropod-proof pen in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Faecal and blood samples were examined for the presence of endo- and haemoparasites after which they were treated accordingly. Tick baths (using 0.25% Chlorfenvinphos (Supona) as emulsion were administered at two-week intervals before commencement of experiments, allowing four weeks between the last tick bath and tick infestation.

Animals were fed on leguminous plant residues including cowpea and groundnut, and on bales of grass hay purchased from the National Animal Production Research Institute (NAPRI) in Shika. Wheat and maize brans were also provided while salt lick blocks were placed for their use *ad libitum*. Clean drinking water was always present in plastic buckets.

3.2.2. Tick Source:

Fully engorged female *Amblyoma variegatum* were removed from their attachment sites on zebu cattle from a Fulani herd in Zaria. In the laboratory, they were washed in 10% ethanol and rinsed twice in distilled water before placing each one of them into a separate sample collection bottle (as in Johnston *et al*, 1986)

These were then tightly stoppered with tufts of cotton wool and maintained in an incubator (28°C, 85%RH) until oviposition was concluded.

The dead remains of oviposited ticks were removed from the bottles and discarded, while the eggs were placed back inside the incubator until larvae were produced. Nymphs were obtained from the larvae by feeding the latter on the ears of rabbits, using ear bags. Engorged nymphs were collected and again placed inside sample bottles which were properly stoppered before returning them into the incubator inorder to obtain adult ticks. The young adult ticks obtained were placed in the incubator at 25°C and 80%RH for a period not exceeding 4 weeks, until required for use in the infestation of experimental animals.

3.2.3. Tick Infestation:

All six animals were infested with 50 adult ticks made up in each case of 40 females and 10 males , considering the surface area of the sheep and an adequate number of male ticks that will induce the females to remain attached. Tick attachment was facilitated by shaving areas measuring about 15 X 15cm around the scapulo-thoracic regions of each animal and placing a white cotton cloth (shettin) of similar size using an adhesive agent (Evostik). For the purpose of inserting ticks, the top edge was left unglued until after the starved ticks (two weeks after ecdyses) were placed on the sheep (Johnston *et al*,1986). This method of infesting animals with ticks ensures uniformity in atttachment and feeding within a specified area of the host. Skin biopsies of the attachment sites on the skin of infested sheep were taken from the second day after attachment of ticks until day 9 post-infestation (pi).

3.2.4. Skin Biopsy:

Skin biopsies of the tick attachment sites were taken by the use of a 5mm diameter biopsy punch obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) in Kaduna.

In order to enhance the taking of skin snipes, a single tick from each animal, on daily basis, was cut at the region between the basis capituli and idiosoma, leaving the mouthpart *in situ*. This ensures that the punch is not unduely obstructed by the large idiosoma while taking the skin sample. The entire feeding site of the tick was then taken and immediately dropped inside a sample collection bottle containing 10% solution of formaldehyde. The bottle was properly labelled with regards to the date and time of collection and also the host from which the sample was collected.

3.2.5. Preparation of Histological Sections:

The samples which were fixed in 10% formalin (pH 7.0) were subjected to serial dehydration using methanol, after which Gurr's tropical ester wax was used to impregnate the tissues (Brown and Knapp, 1980). Blocked samples were arranged in cross and transverse sections to the mouthparts in the tissues. Thin sections of the skin samples were cut serially at about 5um/piece using a rotary microtome. Serial sections were stained using Haematoxalin and Eosin (H & E) and Giemsa stains for differential recognition of tissue and blood cells. Respective stained sections were serially mounted on properly labeled glass slides using Kirkpatrick's and Sandrum's mixture of distrene 80, dibutylphthalate and xylol (DPX). The glass slides were later read, interpreted and photographed , where appropriate.

The number of cells per unit area of tissue within the confines of the embedded mouthpart was estimated by the use of grid ocular micrometer mounted on a

compound binocular light microscope. Photographs were taken using a camera-mounted microscope with planatic objectives and ocular lenses.

3.3.1. Appearance of Feeding Lesions on Yankasa and Uda Sheep

Observations made grossly on the pathology of the tick feeding sites of female *A. variegatum* on the skins of the two breeds of sheep, as compared with normal, unifected host tissue collected prior to tick infestation are presented in Table 3.1 below; histopathological appearances of the same tissues are presented in Table 3.2.

Table 3.1 Macroscopic appearance of tick feeding sites in Yankasa and Uda sheep from day 2 post infestation to day 9

Days Post infestation	Yankasa	Uda
1-2	A developing erythromatous swelling measuring between 2-3mm in diameter and raised 1mm above the surrounding tissues was evident. A milky white secretion believed to be cement material secreted by the tick , accumulated at the distal end of the embeded mouthparts.	Similar observations were made at the tick attachment sites on Uda sheep.
3-4	Size of the erythromatous nodule had increased to about 4mm in diameter , and the cement material had built upwards on the mouthpart and outwards over some parts of the swelling , forming a cone-like structure. There was a flaky dried material around the tick bite sites suspected to be serum exudate from the host.	Similar observations were made from lesions observed in the Yankasa, although they were larger (5.5-6.5mm) in diameter . Greater damage appeared to have been done to the epithelial tissues.
5-6	The size of the lesions increased to between 5 to 6mm in diameter , with obvious signs of hyperplasia and erythema. There was also an increase in the amount of cement secreted around the mouthparts. The lesions had become larger in size and tended to coalesce on feeding sites where more than one female tick attached. In such places, the width of the bite	The mean diameter of lesions exceded 7mm, especially at sites where more than one tick attached. Lesions were ulcerative and coalesced, with massive necrotizing epithelial tissues.

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	lesions measured between 7 to 10mm in diameter, with visible caking of serum exudate around tick mouthparts. An oedematous, exudative lesion was observed by day 6.	
7-8	The lesions had become well demarcated , oedematous swellings with signs of necrosis and erosion of the epithelial tissues. An ulcerated, exudative , necrotizing lesion which appeared to hold the tick mouthparts loosely was evident by day 8 pi. A number of female ticks were observed to have detached at the end of day 8. Large sized exudative lesions were left behind by deatached ticks .	Most of the lesions had become purulent, with massive necrotizing tissues in and around the ulcers. At sites where more than one tick must have fed before detaching, the ulcers became very enlarged and purulent, exuding pus and serum.
9-14	Virtually all ticks had completely detached by day 8 , leaving behind open lesions which attracted flies (*Musca* spp.) . It was observed though that most of the bite sites on the animals had completely healed by day 14 after deatchment of ticks. The sites had become scarified , exfoliative and hardened.	Skin lesions in the Uda continued to enlarge , with purulent exudates and necrotizing tissues. They turned into sores which needed to be treated therapeutically before healing commenced.

Table 3.2 Histopathological appearance of tick feeding lesions in Yanakasa and Uda sheep : Microscopic appearance of bite sites.

Days Post infestation	Yankasa	Uda
1-2	Tick bite sites showed mild oedema and mild neutrophilic infiltration into the superficial dermal areas. This was seen as clear , focal necrosis with few neutrophils and lymphocytes . Very few eosinophils and basophils were observed in the bite lesions, especially around the mouthparts of ticks.	Observations made were similar to those in the Yankasa sheep.
3-4	There was a break in the dermal epithelium and some cloudy, swollen cells were seen around tick mouthparts. This developed into an epidermal abcess by day 4, and was	There was a massive increase in the number of neutrophils and eosinophils in the lesion. There was aslo massive

	marked by moderated parakeratosis with marked necrosis of collagen fibres of the superficial dermis. There was extensive damage to tissues and blood capillaries; with marked necrosis and erosion of the dermal tissues. Furthermore, there was eosinophilic peri-vascular cuffing around blood capillaries , and evidence of massive necrosis and liquefied tissues around tick mouthparts in the lesions. (Plate V)	damage to the epithelial cells at the bite sites, resulting in desquamation of the hyperkeratotic layers of the skin. Tissues were undergoing liquefaction necrosis , thereby creating deeper lesions. (Plate VI)
5-6	The mass of liquefied tissues had been substantially eroded below the tick mouthparts, leaving a developing vacuolar region with lots of erythrocytes, lymphocytes and other polymorphonuclear cells in the matrix. A one-cell thick layer of epidermal cells had begun to develop below the abcess by day 6, which continued subsequently until healing was achieved later . (Plates VII & VIII).	There was massive erosion and desquamation of the hyperkeratotic layer, allowing the inflow of tick cementing material in places. This must have resulted in massive damage to blood capillaries and haemorrhage as large number of erythrocytes and neutrophils were seen. (Plates IX & X).
7-8	Much of the developing epidermal layer became substantially obstructed by the cement material secreted by ticks. Fluid-filled cavities were produced due to massive erosion of tissues below the tick mouthparts. This was increased by the inflow of erythrocytes and lymphocytes from damaged capillaries. Large vacuolated cavities which had eroded walls were left behind by detached ticks . (Plates XI & XIV).	Large vacuolated areas below tick mouthparts had developed as a result of substantial removal of tissues by feeding female ticks. No tissue regeneration was observed , rather, there appeared to be degeneration of tissues inside and on the walls of the lesion. (Plates XII & XV).
9-14	A second epidermal layer had begun to develop below the parakeratotic layer of cells. Epithelial cells bordering the abcess were vesiculated and tended towards the formation of a feeding capsule. There was complete healing of the lesions with the formation of scar tissues 14 days after the termination of the infestation.	The lesions had deteriorated into completely eroded, purulent sores with festering flies. Only few fibres (muscles ?) were seen in the ulcers. (Plate XV).



Plate I: Typical Yankasa breed of sheep. These animals were used in the feeding female *A. variegatum* upto day 5



Plate II: Young, tick-naïve Yankasa and Uda breed of sheep used in the study of histopathological reactions of the skin to bites of female *A.variegatum.*



Plate III: Engorging adult female ticks on one of the young Yankasa sheep, as skin biopsies were taken at the time.

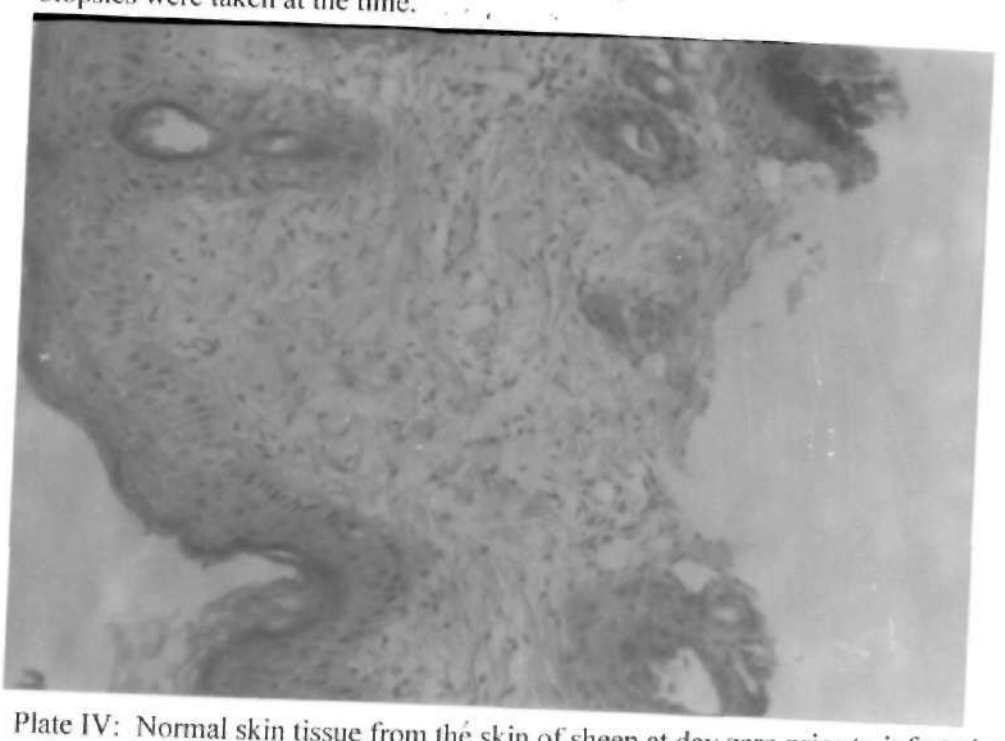


Plate IV: Normal skin tissue from the skin of sheep at day zero prior to infestation with females of *A.variegatum* x 200 (H&E stain)

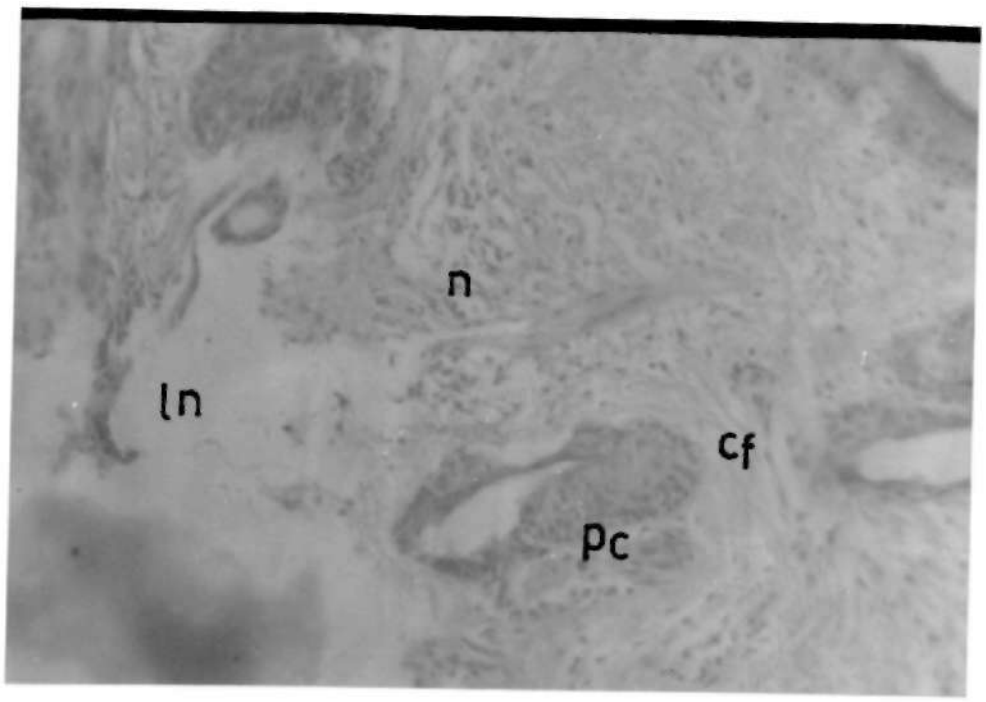


Plate V: Cross-section of tick bite site of the skin of Yankasa sheep on day 3 Pi. Damage to tissue and blood capillaries, with eosinophilic, peri-vascular cuffing (pc) and necrosis of collagen fibres (cf) are evident; so is liquefaction necrosis (ln) and allot of polymorphonuclear cells (neutrophils) (n) X 200.(H&E stain)

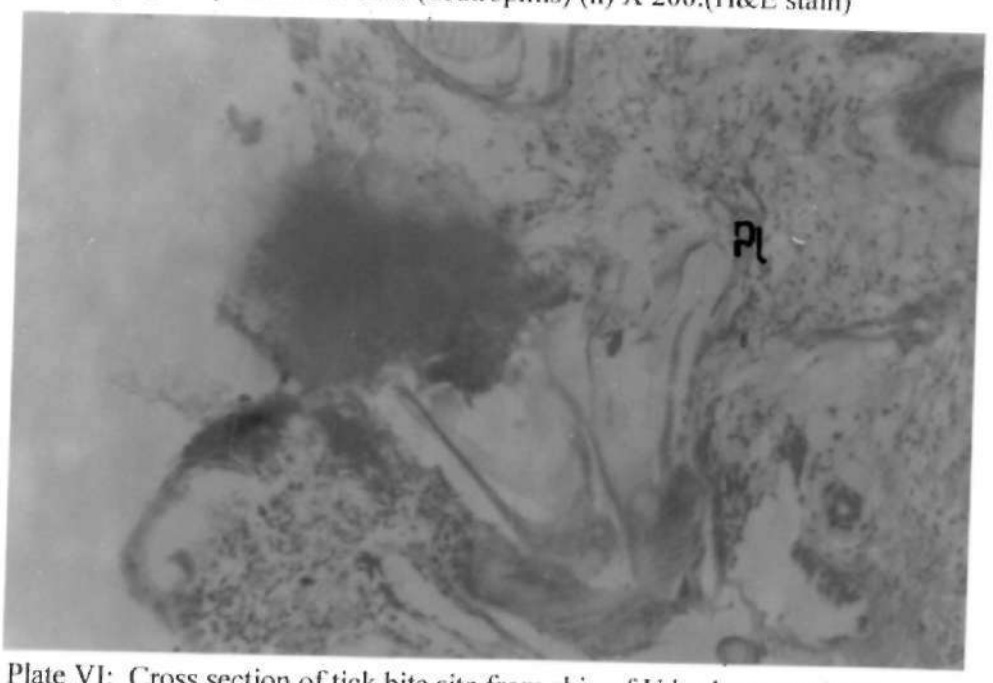


Plate VI: Cross section of tick bite site from skin of Uda sheep on day 4 pi. Similar histopathology was observed in the Yankasa sheep at day 3. Infiltrated polymorphs (pl) and extensive liquefaction necrosis are evident. X 200 (H&E stain)

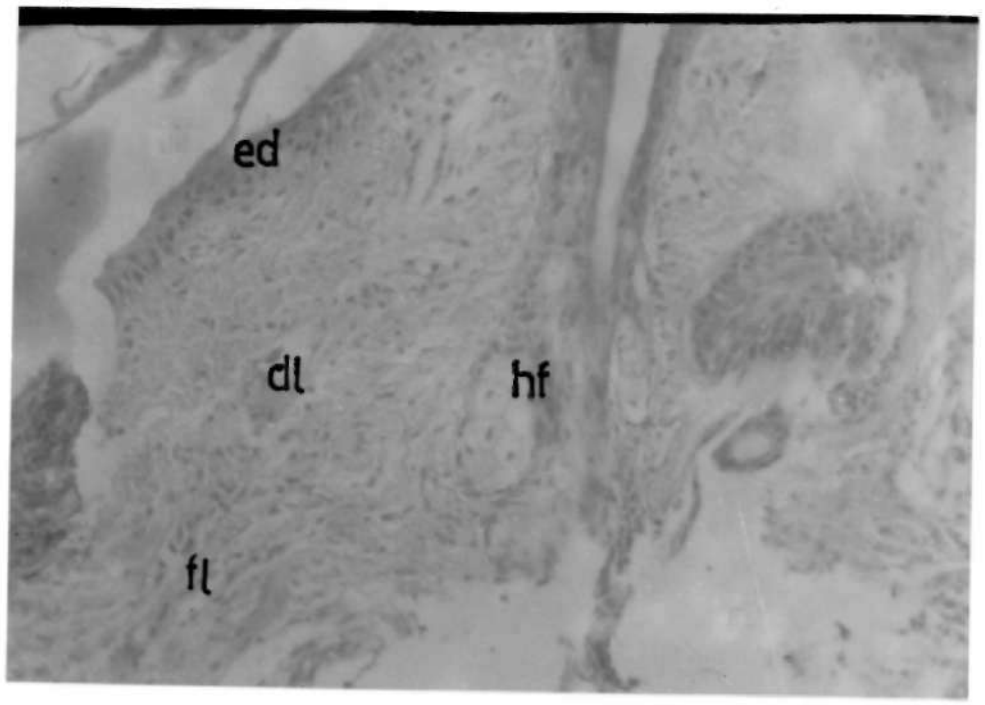


Plate VII: Vertical section of host skin showing erosion of the feeding lesion (fl) below the dermal layer (dl) at day 5. Epidermis (ed) and hair folicle (hf) are seen, with enhanced cellular infiltration into the lesion. X 200 (H&E stain)

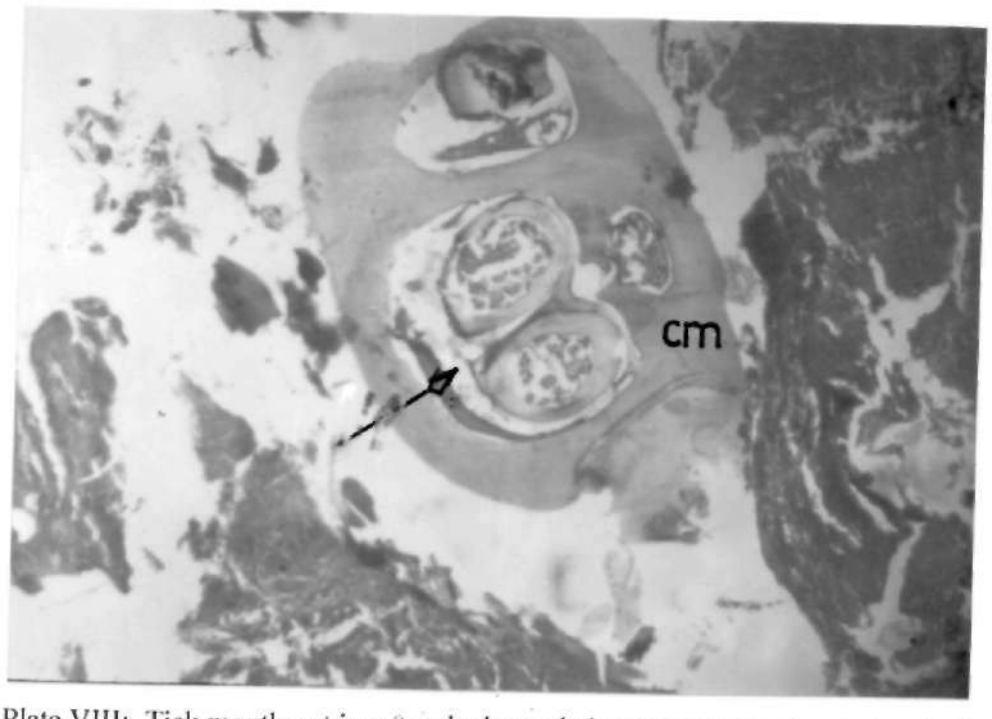


Plate VIII: Tick mouthpart in extensively eroded, necrotized feeding lesion in the skin of Yankasa sheep at day 6. Cement material secreted around the mouthparts can be seen (cm) bound to the feeding apparatus (arrow) which is immersed in the fluid-filled cavity (fc). X 100. (H&E stain)

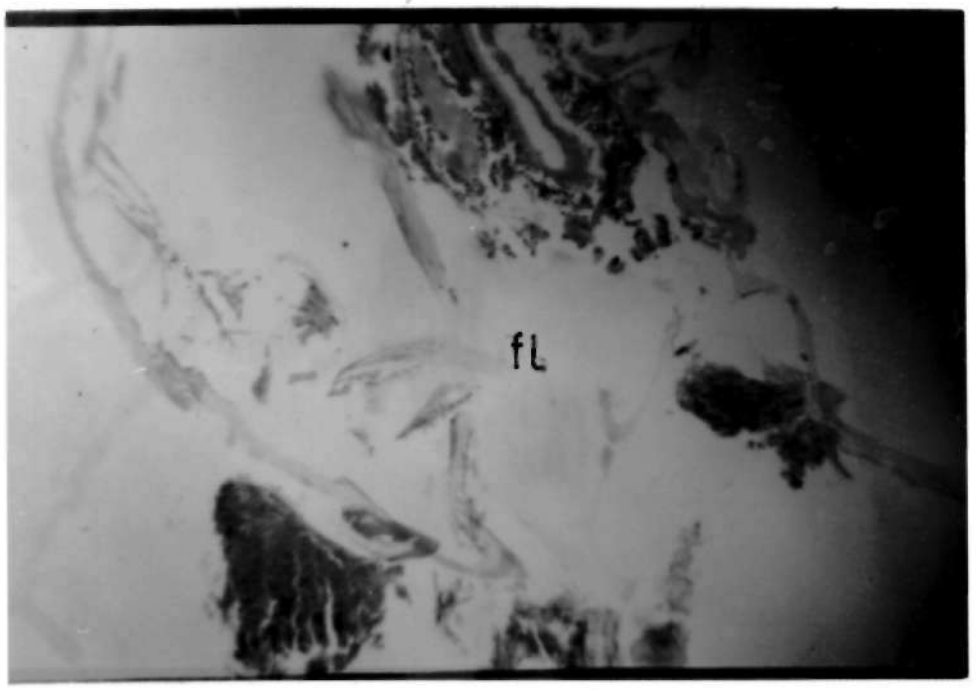


Plate IX: An extensively eroded, necrotizing feeding lesion (fl) in the skin of Uda at day 6. X 100 (H&E stain)

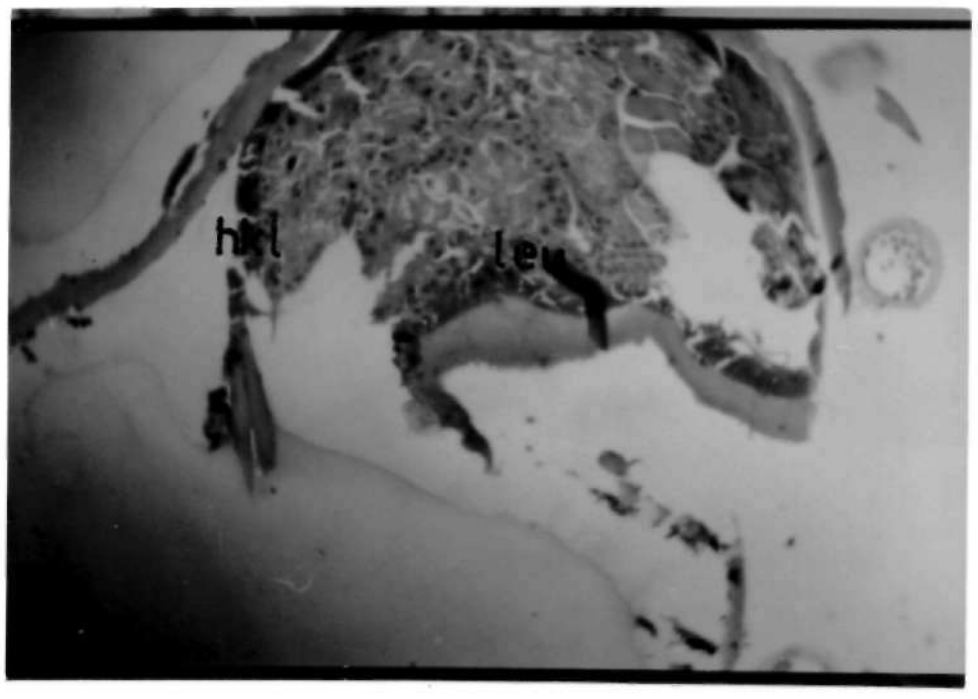


Plate X Massive damage to epithelial tissues and desquamation of the hyperkeratotic layer (hkl), with massive haemorrhage and infiltration of leukocytes (lymphocytes) (leu) due to destruction of capillary vessels in the skin of Uda breed at day 6. X 300 (H&E stain)

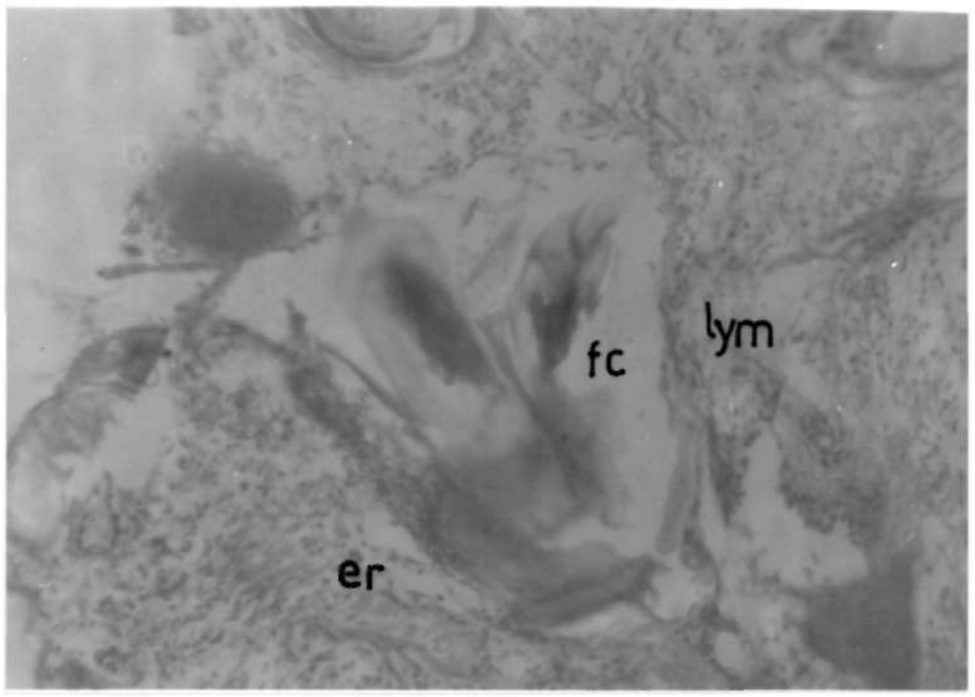


Plate XI:Fluid-filled cavities (fc) in which there was continued infiltration of erythrocytes and lymphocytes (er,lym) due to capillary damage and tissue destruction in the skin of Yankasa sheep at day 7. X 150 (H&E stain)

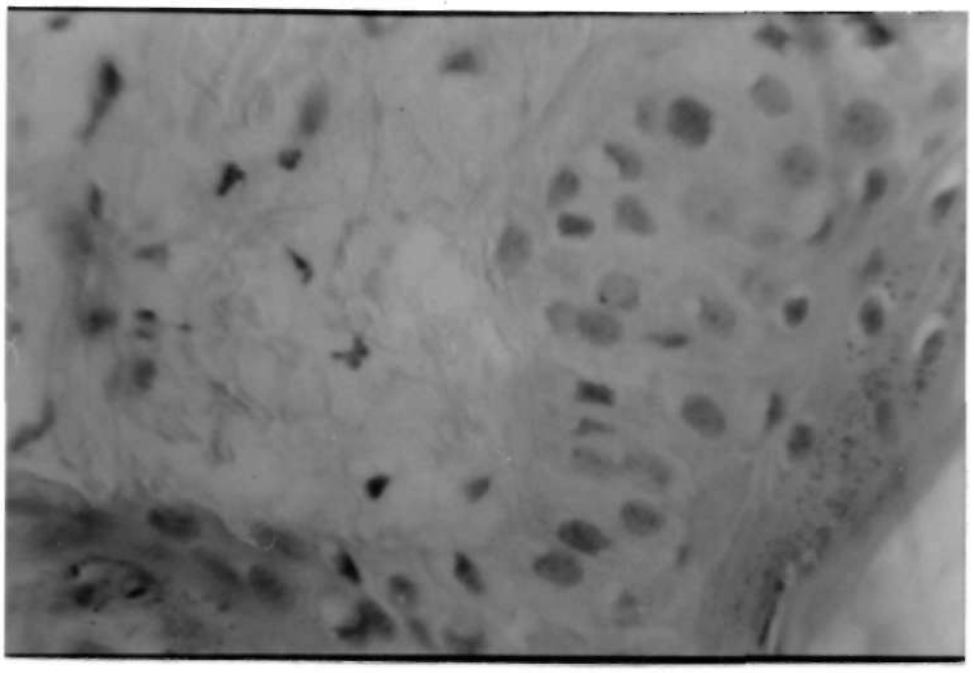


Plate XII:Massively eroded skin tissue in Uda sheep and lymphocytic peri-vascular cuffing at day 7. X 400 (H&E stain)

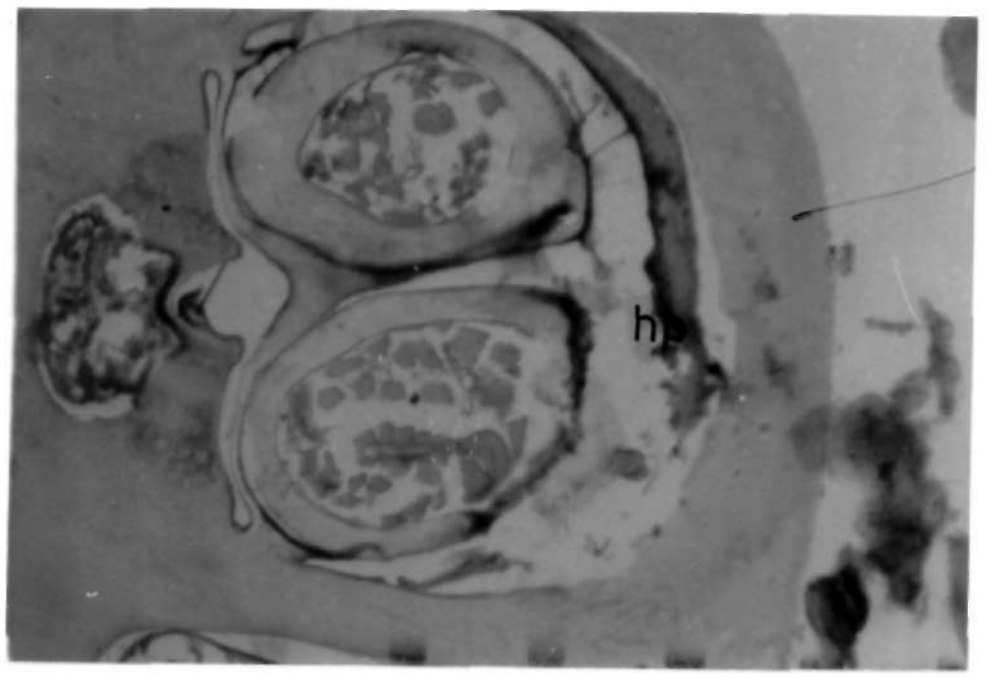


Plate XIII: Cross section of the mouthpart of female *A. variegatum* showing the pair of chelicerae (ch) (displaced), the salivarium (slv) dilator muscles (dms), the hypostome (hp) and the pharynx (ph) (or food channel) with its grinding organ (go) X 150.(H&E stain)

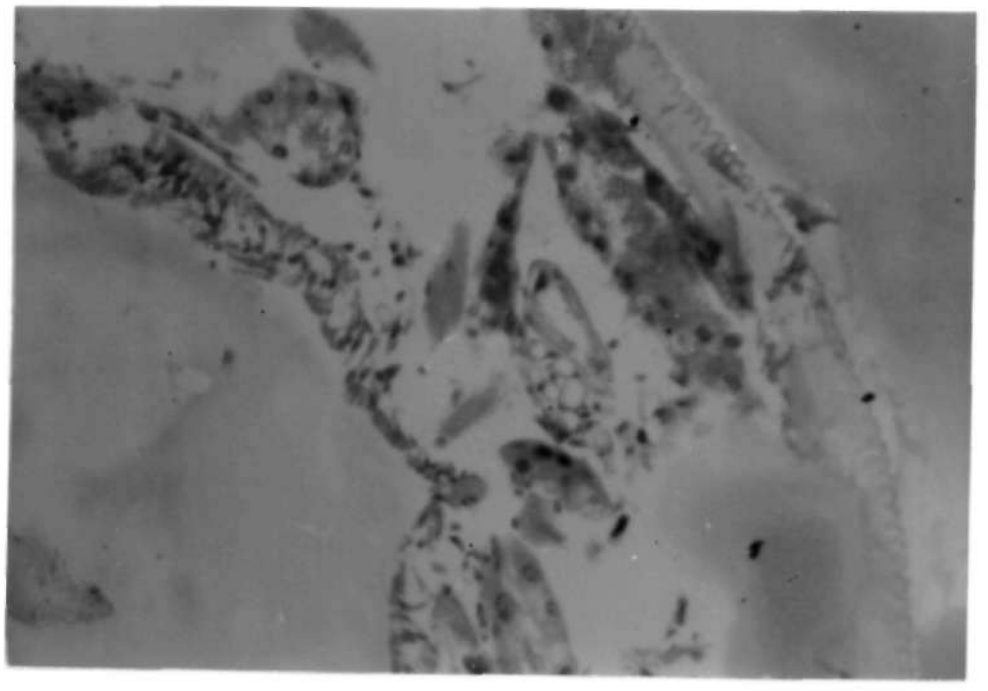


Plate XIV Regenerating epithelial layer (epl) in the skin of Yankasa sheep at day 8. Note the fluid-filled cavity at the centre of the lesion. X 200.(H&E stain)

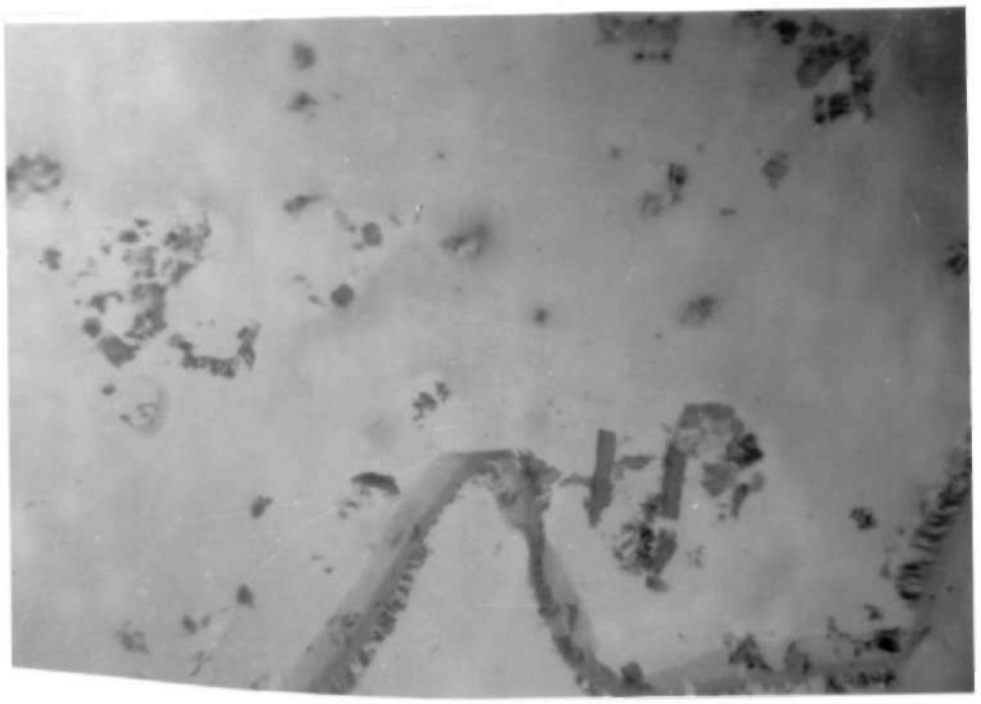


Plate XV. Completely eroded, fluid-filled cavity in the scapulo-thoracic skin of Uda breed after tick detachment at day 8. X200 (H&E stain)

3.4 DISCUSSION

The gross microscopic reactions of host skin to the bites of *A.variegatum* observed in both breeds of sheep in the present study is consistent with a typical inflammatory reaction mounted due to the presence of an invading parasitic organism (Gregson, 1967; Allen *et al*, 1977; Allen and Humphreys, 1979; Arthur, 1973; Brown & Knapp, 1980; Allen, 1992). The erythromatous/oedematous swelling observed on days 1 and 2 pi of tick attachment in this study may be due to massive infiltration of neutrophils and degranulating mast cells which released heparin and histamin, thus, preventing blood coagulation and, at the same time, increasing vascular permeability and tissue destruction (Allen *et al*, 1977; Wikel, 1995). The release of neutrophils into the bite lesions, as reported in the present study during early infestation, may have caused the destruction of collagen fibres between days 3 and 4 , serving to increase permeability and further tissue destruction. These observations are consistent with those reported by Tatchell and Moorhouse (1968) and Theis and Budwiser (1974) in their studies on the sequence of host tissue changes in the feeding process of the cattle tick *Boophilus microplus* on cattle, and the dog tick *Rhipicephalus sanguineus* on dogs respectively. The process of tissue damage may have been aggravated by certain components in the tick saliva which elicit the generation of a neutrophil-chemotactic factor from the complement component C5 (Wikel, 1995), causing sensitization leading to autolysis. The increased presence of eosinophils and basophils from days 1 and 2 to their levels in days 3 and 4 may be an indication of the increase in the inflammatory reactions taking place at tick bite sites, since both cell types are usually indicated in such processes. Theis and Budwiser (1974) and Essuman *et al* (1991) reported

similar reactions as occurring during early tick attachment to dogs and cattle respectively. The large presence of eosinophils around blood capillaries (peri-vascular cuffing) between days 3 and 4 may have aided the destruction of same, leading to the haemorrhage observed in the feeding lesions. This and other reactions could have led to the massive liquefaction necrosis observed between days 3 and 4. The presence, in substantial numbers, of erythrocytes, lymphocytes and other polymorphonuclear cells (neutrophils) in the latter part of the inflammatory process (days 5 to 8) was as expected in a protective, immunlogical reaction like the one mounted by the host body's defense systems, since these cells (neutrophils and lymphocytes) proliferate during advanced inflammatory reactions (Allen *et al*, 1977).

The development of feeding vacuole at tick bite sites, as observed in the present study, had earlier been reported by a number of authors from similar studies carried out on cattle, rabbits and guinea pigs (Johnston *et al*,1986; Kemp *et al*, 1986; Opdebeeck *et al*,1988; Essuman *et al*,1991; Allen and Humphreys, 1979; Brown and Knapp, 1980; McGowan *et al*,1980). This was the result of massive ingestion of liquefied tissue within the feeding lesion by engorging female ticks, which are known to dramatically increase the pace of feeding from relatively slow to many times faster during the latter (6-8) days of attachment (Kemp *et al*, 1982). The depth of penetration of the mouthparts of female *A.variegatum* into the skin of both breeds of sheep, which was well below the dermis as reported in this and the other cited studies, and the action of various tick salivary enzymes in the feeding lesion as reported by Tatchell and Moorhouse (1970) and Tatchell and Binnington (1973), could have elicited more host reactions resulting in the development of massive liquefaction necrosis and erosion of the walls of feeding lesions, leading to

the expansion of the feeding lesion. Similar observations had earlier been reported by Brown and Knapp, (1980); Kemp *et al.*(1982; 1986); and Johnston *et al.*(1986) in dogs and cattle respectively.

The change in the size of the feeding lesions as the infection progressed, and the infiltration of polymorphonuclear leucocytes into the sites was an indication of a complex phenomenon, involving a struggle by the host to rid itself of a foreign invader and the will of the latter to remain attached and feed on the former. Ticks remained attached to hosts until final engorgement, despite host-mounted defense mechanisms. Tick attachment was aided by the secretion of cement material which ensured firmness at the tick-host interface. It is to the advantage of the parasites that more autolytic reactions by the host provides ample tissue fluids which it feeds on. The release of pharmacoactive substances such as histamine and heparin when leukocytic cells degranulate during tick feeding only help to cause vascular permeability and prevent blood clotting to the advantage of the tick (Willadsen, 1980). Thus, ticks which feed on animals that had earlier been exposed/sensitized to tick bites may actually be better disposed, in the absence of any detrimental effects, to those feeding on tick-naïve hosts.

Regeneration of new epithelial cells below the atrophied epithelium in Yankasa sheep between days 6 and 9, and the absence of the same in the Uda breed cannot be easily explained as no evidence of marked differences in the histochemical constitution of the skin or its thickness was studied in the present study. There is the possibility that Yankasa sheep have acquired certain genetical characteristics as a result of long exposure to a more tick -infested environment, and therefore have become better adapted to tick bites over prolonged periods compared to the sudan-savannah-inhabiting Uda, or that breed differences based on substances which

promote rapid healing in the former , which may be poorly present or even absent in the latter.

The hyperkeratosis of host tissues bordering the long mouth parts of the ticks prior to tick detachment at engorgement weakened the tick attachment to the host tissue. This, coupled with the enormous weight of engorged female ticks between days 8 and 9, and the contraction of muscles within the tick hypostome (Brown and Knapp, 1980) must have aided tick detachment from the two breeds of sheep, 7 to 9 days post infection.

The large-sized ulcers observed at sites where more than one tick attached could be due to the coalescence of bite injuries caused by the ticks' mechanical destruction of intervening tissues and/or secondary bacterial actions. This phenomenon has been commonly reported in infestations involving *Amblyomma spp.* in which more than one tick aggregate at the same feeding site, causing large ulcerated lesions to develop (Brown and Knapp, 1980; Mohammed and Agbede, 1980; Bourdeau,1982; Norval *et al*,1991). The latter was, however, not investigated, but the purulent exudates observed suggested it. The fact that this observation was more severe in the Uda breed than in the Yankasa indicated that the latter was probably more adapted to tick bites than the former.

From observations made in the present work, it is not easy to explain how disease causing organisms such as *Cowdria ruminantium* pass into host systems, successfully escaping the barrage of host defense elements mounted at the tick/host interface without getting destroyed or engulfed by leucocytic phagocytes and enzymatic reactions. Even if they are not destroyed by these cells and enzymes, it would be expected that fluids resulting from oedema and infiltrates from vascular tissues would flush them out of the gapping feeding lesions. Yet organisms are able

to penetrate this line of host defense, causing infections in susceptible hosts. Focus should therefore be centered on the elucidation of the mechanisms by which infections occur, despite host immunological defences. This should form the basis for further research work .

3.5 CONCLUSIONS.

To our knowledge, this is the first documentation of a study of the sequential tick feeding lesion by *Amblyomma variegatum* on local breed of sheep in Nigeria. The macroscopic and microscopic observations made from the tick bite sites of both breeds of sheep indicated an inititial inflammatory/hypersensitive reaction in which eosinophils were very prominent. As tick feeding continued, there was damage to capillary vessels which resulted in haemorrhage. The feeding lesion became enlarged due, partly, to further generation and secretions of basophils, neutrophils and lymphocytes by host defense system, and also mechanically by the presence of tick mouth parts embeded in the lesion.

The lesions in Uda breed of sheep became ulcerated and contained a higher population of neutrophils and lymphocytes, cells often associated with chronic and/or purulent ulcers. The healing process in Yankasa sheep was faster and apparently spontaneous, which perhaps was aided by the regeneration of epithelial tissues from day 6 onwards. This was not observed in the Uda , whose ulcers had to be treated chemotherapeutically.

The differences observed in the lesions between Yankasa and Uda sheep could be due to breed-specific acquired or genetic characteristics. This has not been ascertained in this study. Studies using crosses of the two breeds could shed more light on this observation.

The duration of tick attachment and feeding was the same in the two breeds of sheep. Tick detachment could have been aided by a weakened, hyperkeratinized epidermal tissue which, in addition to the vacuolation of the feeding lesion, held

losely to the tick hypostome, causing the enormously weighted engorged female tick to fall off. The importance of ticks and tick-borne diseases to the development of livestock in Nigeria is immense, considering the fact that majority of arthropod-borne disease agents are transmitted by ticks (Hoogstraal, 1956). The loss in revenue due to loss of condition and production in infested animals should be of paramount concern.

CHAPTER FOUR

THE RESPONSE OF YANKASA SHEEP TO TICK BITES FOLLOWING INOCULATION WITH EXTRACTS DERIVED FROM THE MID-GUT MEMBRANE OF 5-DAY FED FEMALE *Amblyomma variegatum.*

4.1 INTRODUCTION

The emergence of widespread resistance of ticks to many chemical acaricides and the increasing cost of treatments with these chemicals, in addition to the colossal amounts of money required to develop new chemical acaricides to replace obsolete ones prompted the development of alternative methods of tick control (Adamson *et al,* 1991). Alternative methods of non-chemical tick control such as pasture spelling, the use of parasitoids and predatory animals, use of herbal derivatives and replacement of animals with more tick-resistant breeds have been practiced by farmers in a number of countries (Wharton and Norris, 1980; Mwangi *et al,* 1991; Malonza *et al,* 1992; Gronvold *et al,* 1996). These measures can reduce tick burdens but only to a limited extent (Johnston *et al,* 1986). Tick resistance can be induced in animals such as cattle, sheep, goats, rabbits, guinea pigs etc by immunization with tick antigens (Wikel, 1981; Johnston *et al,* 1986; Kemp *et al,* 1986; Opdebeeck *et al,* 1988; Essuman *et al,* 1991; Dipeolu *et al* 1992; Kocan, 1995). Most of the work reported involved the cattle tick *Boophilus microplus,* a temperate climate tick infesting European breeds of cattle, and *Rhipicephalus appendiculatus,* infesting Boran and Zebu cattle in East Africa. A degree of success was recorded in the control of *Dermacentor andersoni,* a temperate climate tick species, using tick-derived antigens as inoculums (Allen and Humphreys, 1979). The only available report on the use of antigens from the three host tick *A. variegatum* in immunization

studies was that of Heller-Haupt *et al* (1987), in which guinea pigs were successfully immunized using homogenates derived from unfed larvae of the same species. There appears to be no documented report on the immunization of sheep with *A. variegatum*- derived antigens.

The advantages of employing non-chemical means of tick control such as vaccines over sustained chemical applications stem from the facts that , in principle, they offer sustained action ; they are mostly free of residues ; they are intrinsically specific with fewer chances of cross-species (non-target) action ; they may be cheaper than chemicals and tick resistance is less likely to develop than when chemicals are used, and even if this occurs, the effects may be circumvented in ways that are impossible with chemicals (Willadsen, 1997a).

Although the parasitized host in a host-parasite relationship is capable of mounting an effective immune response, eventhough with majority of ectoparasites this is not acheived no matter the level of exposure, vaccines are needed for the acceleration of the process if the proper antigens are used. Anti-tick vaccines may likely have a deleterious effect on disease agents that are transmitted by such vectors by causing a change in the normal physiology and morphology of the gut or by direct phagocytotic action of leucocytes that are mobilized to the bite site. These are hypothesis which may be ascertained only after thorough studies. Whatever effects vaccines will have on ticks fed on vaccinated hosts, the fact still remains that intergrating more than one form of tick control will be the most effective way of tick control.

The overall objective of this study is to evaluate the ability of extracts derived from the midgut membrane of female *A .variegatum* to protect sheep against challenge by nymphs and adult females of the same species.

4.2 MATERIALS AND METHODS

4.2.1. Experimental Animals:

Rabbits:

Six adult rabbits were purchased from the market in Samaru village, Zaria and kept in wire-mesh steel cages in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. They were fed on maize and wheat brans, leguminous plant residues and limited amounts of green vegetables. Water was provided *ad libitum*.

Sheep:

Two male and six female Yankasa sheep aged between 6 and 8 months were obtained from relatively tick-free sources in Sabon-gari and Samaru areas of Zaria. According to the previous owners, no ticks were ever detected on the animals. They were housed in an arthropod-proof pen and separated into two groups, which were kept in two separate cubicles. The animals were allowed to acclimatize for a period of 4 weeks within which time blood and faecal samples were collected and analysed for the presence of haemo- and helminthic parasites. They were subjected to acaricidal baths twice, at two-week intervals, within the period. Feed stuff included leguminous and grass hay, wheat and maize brans, and groundnut cake. Salt lick and clean drinking water were provided *ad libitum*.

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4.2.2 Antigen Preparation:

Larval *A.variegatum* ticks were obtained from the laboratory source as described in **3.2.2**. These were fed on the ears of rabbits with the aid of ear bags. Detached, fully engorged larvae were collected from the cage trays between days 4 to 7 pi. (Plates XVI, XVII and XVIII). They were incubated at 28°C and 85%

RH(Plate XIX) in order to obtain nymphs. About two hundred nymphs were placed again on the ears of rabbits to obtain young adult ticks 5 to 8 days later. These were collected and stored in an incubator at 25°C and 80%RH for use in the infestation of 8 Yankasa breed of sheep four weeks after.

One hundred 5-day fed female *A.variegatum* were collected, washed and rinsed twice in 25% ethanol and then with distilled water. In order to obtain the midguts, each tick was placed on partially melted candle wax contained in a petri-dish, so that the legs were properly secured and embeded in wax (Plate XX). Using a new sharp razor blade, the dorsal cuticular areas, including the scutum and parts of the dorsal idiosoma, were dissected out and the midgut was exposed. The midgut was then excised from the tick with a pair of hand forceps, and immediately immersed into a cold test tube on ice containing cold phosphate-buffered-saline (PBS) at 4°C. After all the 100 midguts had been excised, they were rinsed twice in cold PBS so as to substantially remove the host blood ingested by them.

To the washed midguts contained inside a cold porcelin crucible was added 5ml of cold PBS (4°C, pH 7.2) to which 1mM EDTA(wt/v), 0.5mM(wt/v) phenol and 1mM(wt/v) phenylmethylsulphonylfluoride (PMSF) as a protease inhibitor were added. These were disrupted and partially homogenized in a homogenizer after which 10ml of cold PBS solution was added. More vigorous blending for 5min was carried out to ensure greater homogeneity. The homogenized material was centrifuged at 10,000g for 15min in a refrigerated centrifuge (Beckman, Model J-21B) at 4°C, and the supernatant was collected in a plastic test tube. This was further subjected to centrifugation at 4°C, 30,000g for 30min. The supernatant was separated from the precipitate and both were stored at – 20°C in a deep freezer until

required. Prior to storage, about 1ml of each of the two portions were used to determine the protein concentration as described by Lowry *et al* (1951).

4.2.3. Inoculation Protocol:

As indicated earlier, the eight sheep were allocated into two separate cubicles, 3 females and 1 male in each. For the purpose of vaccination, the refrigerated homogenate were brought out and completely thawed at room temperature. The supernatant contained 2.70mg/ml of protein while the reconstituted precipitate, made up to about 12mls by adding 10ml of cold PBS, contained 2.55mg/ml of protein. Two inoculation procedures were carried out, the second coming at two-week interval after the first to serve as a booster. In the first inoculation, control sheep 201, 202, 203 and 204 were injected with divided doses of PBS (pH 7.2) containing 1mM(wt/v) of phenol and 1mM(wt/v) of PMSF intramuscularly (im) in the neck and thigh muscles, while the experimental group animals 205,206 207 and 208 were inoculated with, in each case, 3ml of the homogenate containing 2.70mg/ml of protein in the same manner as the control. The criteria for dose determination were based on an earlier work conducted by Essuman *et al.* (1991) in which homogenates from the midguts of *Rhipicephalus appendiculatus* were used in vaccinating cattle in East Africa. The second inoculations were similarly administered but with the 2.55m/ml protein homogenate in the experimental sheep, and the same solution of PBS (pH 7.2) in the control sheep. A period of 28 days was allowed after the second vaccination before infesting animals in both groups with starved nymphal and adult *A.variegatum* ticks.

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4.2.4. Tick Challenge:

In order to facilitate attachment of ticks to the vaccinated sheep, the skin of the scapulo-thoracic regions on the left flanks of each animal was cleanly shaved using a sharp razor blade, providing attachment areas measuring about 15sq cm on each animal. White cotton cloth (shettin) of equivalent size with the shaved portions were glued to the shaved areas on each host, leaving only the top open for the purpose of inserting ticks during challenge. Masking tape was used in sealing the open tops after ticks have been inserted. A total of 30 nymphs and 20 adult (16 females and 4 males) ticks were enclosed on each of the eight animals. The top and sides of the adhered cloth could be opened for the purpose of inspection and tick counting by removing the tapes. A 24-hour period was allowed after tick insertion before counts of detached ticks and collection commenced. This was to allow ticks gain firm attachment to feeding sites without hindrance. All detached ticks were collected separately and placed in properly labelled sample bottles. Detached ticks of the same stage were then collectively weighed using an electronic weighing balance and mean weights calculated. They were also inspected thoroughly for evidence of immunological damage or death by use of a dissection microscope. Immunologically damaged ticks , especially engorging larvae and nymphae, were seen to be bright red in colour while engorged ones were reddish-grey initially, but turned dark greyish/blackish after about two days. These normally died subsequently. Six weeks after the termination of the first tick challenge, the animals were re-challenged as in the first instance. The same parameters as in **Experiment 1** were determined.

The following criteria were used in assessing the effects on ticks which fed on either the control or vaccinated animals:-

a) the number of ticks detaching daily from each animal and the defects observed on the ticks.

b) mean engorgement weights of ticks which fed on each host at the time of detachment.

c) mean mass of eggs produced by female ticks which fed on each host

d) percentage of hatched eggs from that produced by detached female ticks

e) moulting success of nymphal ticks which fed on control and vaccinated hosts.

4.2.5. Statistical Analysis:

Differences between means were established by analysis of variance (ANOVA) and analysed by the students' t-test; and relationships between mean engorgement weights and egg mass, percentage hatchability and ecdyses were determined by the use of correlation matrix.



Plate XVI Feeding larvae of *A. variegatum* on the ears of rabbits. Note the attached ear bags.



Plate XVII Pinnae of rabbit laden with engorging larval ticks (arrow). Note the dropping , oedematous disposition of the ear.



Plate XVIII Photogragh showing cage tray containing rabbit faeces (Rf) and detached engorged larvae (LL) of *A. variegatum*. The pair of forcep is used in picking the larvae into the plastic bottle.



Plate XIX Incubating ticks contained inside cotton wool-stoppered plastic bottles in a Revco incubator at 28°C and 85%RH

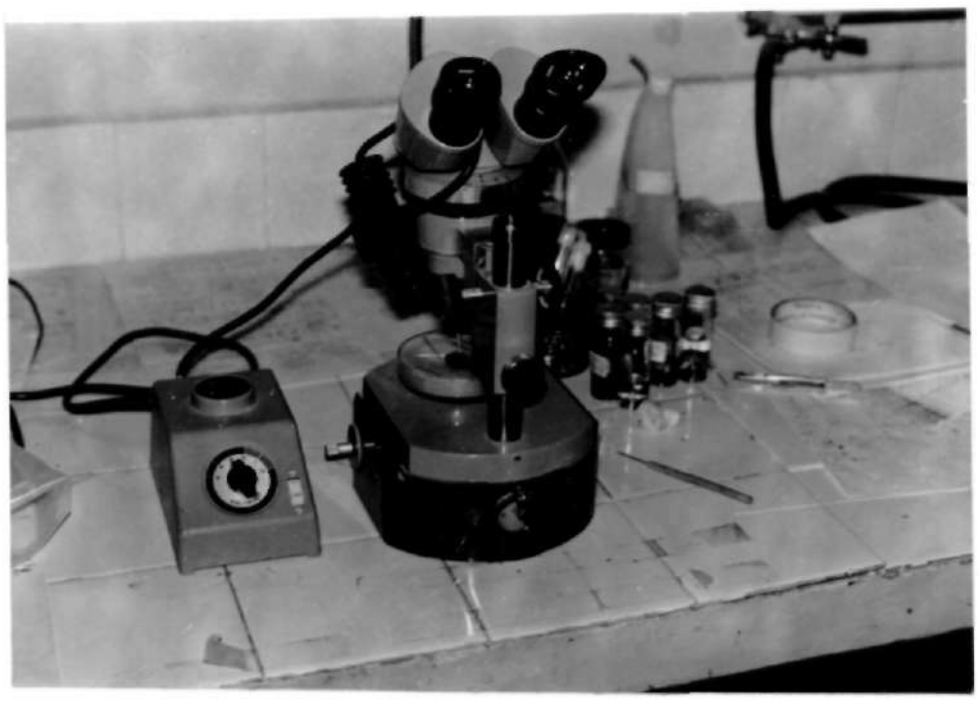


Plate XX Instruments used in the dissection of 5-day fed female *Amblyomma variegatum* ticks. A female tick can be seen embeded on wax in a petri dish mounted on the microscope.

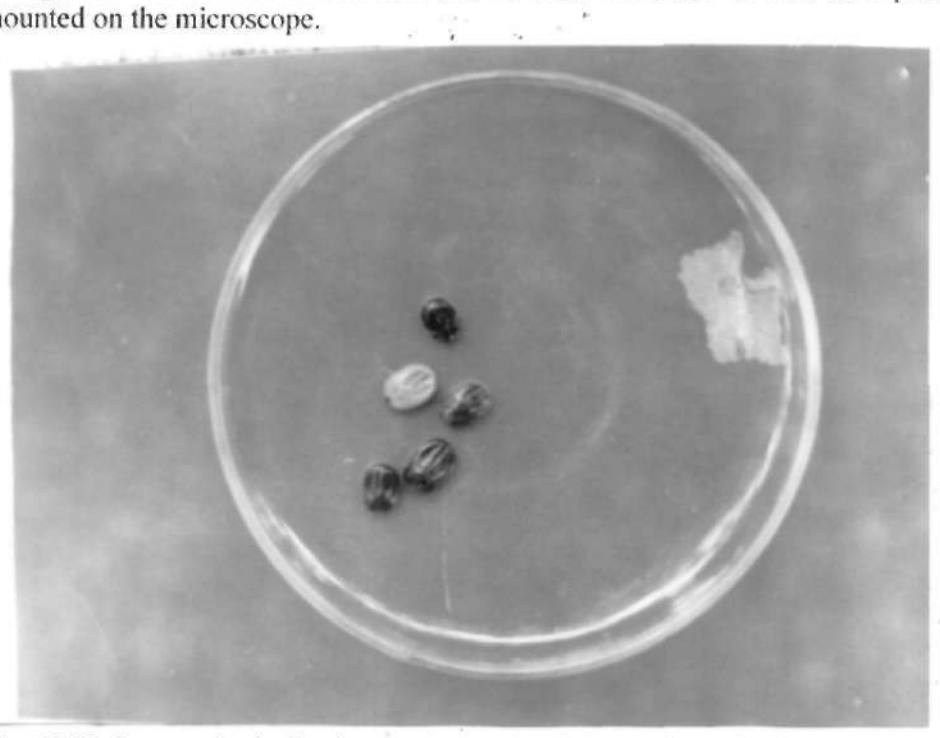


Plate XXI Immunologically damaged nymph (lighter coloured) of *A. variegatum* as compared with apparently normal nymphs (greyish/bluish coloured) fed on vaccinated and control sheep respectively.

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4.3 RESULTS

4.3.1. Daily counts of Detached ticks and their engorgement weights

Table 4.1 shows the result of infesting nymphal and female *A.variegatum* ticks on control tick-naïve Yankasa sheep during primary infestation. On host 201, 2 nymphs (NN) detached on day 2 post infestation (pi); and the same number on day 4. The highest detachment of 12 and 11 occurred on days 5 and 6 respectively, while only 3 were recorded on day 7 and none on day 8. Female ticks (FF) which fed on this host began to detach from day 5 until day 8 pi. The highest number of detached females was recorded on day 7 where 8 were counted. The total weight of nymphal and female ticks which fed on host 201 were 5.5gm and 5.5gm respectively, and the mean weights were 0.18gm (±13.0) and 0.34gm (±27.1). There were no immunologically damaged (DM) or dead (DD) ticks observed. Except for slight differences in the number of detaching ticks on a daily basis from the other sheep hosts in this experiment (hosts 202, 203 and 204), the general trend of tick detachment for both stages of ticks were similar to those reported for host 201. The differences in the total weights of nymphal ticks which fed on their respective hosts, and also the differences in their mean weights were not significantly different ($P>0.05$). The same trend was observed in the female ticks. There were no immunologically damaged or dead ticks observed except for two nymphs (6.7%) recorded on day 5 from host 203, which showed the typical bright-red colouration, indicating gut damage and infiltration of host blood into haemolymph of the ticks.

Generally, only two nymphs detached on day 2 from all the sheep, with no females. Neither nymphs nor females were counted on day 3, while another 8 nymphs and no females were counted on day 4. The highest number of detached nyumphs were counted on days 5 and 6 (54 and 46 respectively), while it was on day

7 for the females (36). The cummulative total weights of nymphs and females were 21.6gm and 23.0gm respectively, and the cumulative mean weights were 0.72gm and about 1.44gm for the nymphs and females respectively.

Table 4.1 Engorgement weight, number of immunologically damaged and dead , detached nymphs and females of *Amblyomma variegatum* fed on control Yankasa sheep during primary infestation.

Daily counts of detached ticks and their mean weights														
	Days	2	3	4	5	6	7	8						
Sheep	Tick	NUMBER							Tno	Twt (gm)	Mwt (gm)	SE (±)	DM %	DD %
201	NN	2	0	2	12	11	3	0	30	5.5	0.18	13.0	0	0
	FF	0	0	0	2	4	8	2	16	5.5	0.34	27.1	0	0
202	NN	0	0	0	17	12	1	0	30	5.2	0.17	13.9	0	0
	FF	0	0	0	2	3	10	1	16	5.7	0.35	33.5	0	0
203	NN	0	0	5	13	11	1	0	30	5.3	0.18	7.5	6.7	0
	FF	0	0	0	1	3	8	4	16	6.1	0.38	51.7	0	0
204	NN	0	0	1	12	12	5	0	30	5.6	0.19	10.4	0	0
	FF	0	0	0	2	2	10	2	16	5.7	0.36	38.1	0	0
Total (Mean)	NN	2	0	8	54	46	10	0	120	21.6	0.72		(1.6)	0
	FF	0	0	0	7	12	36	9	64	23.0	1.44			

Legend: NN - Nymphs; FF - Females , Tno - Total number; Twt - Total weight; Mwt - Mean weight; SE- Standard Error; DM%- Percentage of immunologically damaged ticks; DD%- Percentage of dead ticks.

Table 4.2 shows the number of detached nymphal and female ticks counted dailyfrom 4 tick-naïve Yankasa sheep vaccinated with the extracts derived from the midgut membranes of 5-day fed female *A. variegatum* during primary infestation.

Results show that nymphal ticks feeding on all 4 sheep hosts began to detach as from day 2 pi. There were no more ticks attached by day 8, and the highest number of detached nymphs were recorded between days 5 and 6 pi. The pattern of detachment of female ticks was not as clear-cut, although the highest number detached between days 4 and 6; none detached on day 2.

Although there was a lot of irritation caused by the attached ticks as evidenced by the several attempts made by hosts to groom themselves , there were not likely to be ticks that were detached by this activity.

Table 4.2 Engorgement weights, number of immunologically damaged and dead nymphs and females of *Amblyomma variegatum* ticks fed on vaccinated Yankasa sheep during primary infestation.

Daily counts of detached ticks and their engorgement weights															
	Days	2	3	4	5	6	7	8							
Sheep	Tick	NUMBER							Tno	Twt (gm)	Mwt (gm)	SE (±)	DM %	DD %
205	NN	3	5	3	6	13	0	0	30	2.9	0.10	15.3	19	22
	FF	0	2	5	3	3	2	1	16	4.5	0.28	64.4	25	19
206	NN	2	3	3	12	9	1	0	30	3.2	0.11	15.8	29	25
	FF	0	3	5	2	5	1	0	16	4.5	0.28	46.9	6	25
207	NN	3	5	2	13	7	0	0	30	3.1	0.11	19.0	11	19
	FF	0	2	5	5	2	2	0	16	4.4	0.27	76.8	6	19
208	NN	2	3	2	12	8	3	0	30	3.9	0.13	26.0	11	19
	FF	0	2	6	2	3	2	1	16	4.8	0.30	46.4	13	13
Total (Mean)	NN	10	16	10	43	37	4	0	120	13.1	0.45		(18)	(21)
	FF	0	9	21	12	13	7	2	64	18.3	1.14		(13)	(19)

Legend: NN- Nymphs; FF- females; Tno- Total number; Twt- Total weight; SE- Standard Error; DM%- % of immunologically damaged ticks; DD%- % of dead ticks

The total number of detached nymphs on day 2 from all four hosts was 10, and subsequently 16, 10, 43, 4 and 0 on the respective subsequent days. The highest number of attached nymphs were therefore recorded on days 5 and 6, while it was on day 4 that most female ticks detached. The difference in total weights of the different types of ticks which fed on the different hosts were not too aberrant from one host to the other, although ticks which fed on host 208 had relatively higher mean weights. The cumulaive total weights for nymphs and females which fed on all four hosts were 13.1gm and 18.3gm respectively, and the cumulative mean

weights of the same ticks (as above) were 0.44gm ±0.06 and 1.14gm ±0.03 respectively.

Immunological damaged and dead nymphal and female ticks were recorded from all four vaccinated sheep. The mean percentage of immunologically damaged ticks for nymphs and females counted from all the sheep were 17.5% and 12.5% respectively; the mean percentage of dead nymphs and females were 21.3% and 19.0% respectively.

Table 4.3 Engorgement weights, number of immunologically damaged and dead detached nymphs and females of *Amblyomma variegatum* fed on control Yankasa sheep during secondary infestation.

Daily counts of detached ticks and their engorgement weights															
Days		2	3	4	5	6	7	8							
Sheep	Tick	NUMBER							Tno	Twt (gm)	Mwt (gm)	SE (±)	DM %	DD %
201	NN	0	0	5	10	8	6	1	30	5.0	0.17	37.9	23	37
	FF	0	0	2	5	3	3	3	16	4.7	0.29	55.8	6	19
202	NN	0	0	2	6	10	10	2	30	5.2	0.17	10.9	23	17
	FF	0	0	3	3	5	4	1	16	5.0	0.31	13.8	13	13
203	NN	0	0	3	7	10	7	3	30	5.1	0.17	8.0	27	27
	FF	0	0	1	4	3	6	2	16	4.5	0.28	15.4	25	0
204	NN	0	0	2	8	9	8	3	30	5.1	0.17	5.8	24	24
	FF	0	0	0	3	5	6	2	16	5.1	0.32	13.0	31	13
Total/	NN	0	0	12	31	37	31	9	120	20.4	0.68		(24)	(26)
(Mean)	FF	0	0	6	15	16	19	8	64	19.3	1.20		(19)	(15)

Legend: NN- Nymphs; FF- Females ; Tno- Total number; Twt- Total weight; Mwt- Mean weight; SE- Standard Error ; DM%- Percentage of immunologicaly damaged ticks; DD% - Percentage of dead ticks.

Table 4.3 shows results of tick infestation on control sheep previously exposed (primary infestation) to tick bites. The pattern of tick detachment generally showed that no ticks detached on day 2 and 3, and that the majority of nymphs detached between days 5 and 7. The total daily counts of detached nymphs from all

the sheep showed a range between 9 to 37. It was between 6 and 19 in the female ticks.

The total weight of nymphs which fed on each host did not differ significantly ($P>0.05$) from each other. A similar observation was made in the case of female ticks. Cumulative total weights of both nymphs and females were 20.4gm and 19.3gm respectively. There were no significant differences between the mean weights of ticks which fed on one host or the other ($P>0.05$) with respect to their stages. The cumulative mean weights of nymphs and females were 0.68gm and 1.20gm respectively. The mean percentages of immunologicaly damaged and dead nymphal ticks were 24.3% and 26.3% respectively; it was 18.8% and 15.0% in female ticks. The difference in percentages of morbidity and mortality between nymphs and females was significant ($P<0.05$).

Table 4.4 Engorgement weights, number of immunologically damaged and dead detached nymphs and females of *Amblyomma variegatum* fed on vaccinated Yankasa sheep during secondary infestation.

Daily counts of detached ticks and their engorgement weights														
	Days	2	3	4	5	6	7	8						
Sheep	Tick	NUMBER							Tno	Twt (gm)	Mwt (gm)	SE (±)	DM %	DD %
205	NN	0	3	6	7	7	7	0	30	3.7	0.12	23.0	40	50
	FF	0	2	4	7	3	0	0	16	3.1	0.19	29.2	25	31
206	NN	0	0	3	11	10	6	0	30	3.5	0.12	11.5	27	37
	FF	0	3	4	5	4	0	0	16	2.8	0.18	19.9	31	44
207	NN	0	2	5	7	12	3	1	30	2.7	0.09	12.0	27	33
	FF	0	3	5	8	0	0	0	16	2.8	0.17	4.6	19	17
208	NN	0	5	2	13	9	1	0	30	2.5	0.08	7.6	27	30
	FF	2	2	5	5	4	0	0	16	2.7	0.17	5.8	13	31
Total/ (Mean)	NN	0	10	16	38	38	17	1	120	12.4	0.41		(30)	(38)
	FF	2	10	18	25	11	0	0	64	11.4	0.72		(22)	(31)

Legend: NN- Nymphs; FF- Females; Tno- Total number; Twt- Total weight; Mwt-Mean weight; SE- Standard Error; DM%- Percentage of immunologically damaged ticks; DD%- Percentage of dead ticks

The results shown on Table 4.4 are of nymphal and female *A.variegatum* which were fed on previously vaccinated sheep during primary infestation. The only detached ticks recorded on day 2 were two females from host 208. The pattern of tick detachments in both nymphs and females appeared to be generalized, although the former mostly detached between days 5 and 6 while the latter mostly detached on days 4 and 5. The range in number of detached nymphs from all the four hosts was between 1 and 38; it was between 2 and 25 for the female ticks.

The difference in total engorgement weights of nymphs which fed on hosts 205 and 206 (3.7gm and 3.5gm respectively) was not significant ($P>0.05$); this was similar in ticks which fed on hosts 207 and 208 (2.7gm and 2.5gm respectively). There were, however, significant differences ($P<0.05$) between the engorgement weights of these two groups of ticks. On the contrary, there were no significant differences ($P>0.05$) between the engorgement weights of female ticks which fed on the different sheep hosts. The cumulative total weights of all the nymphs and females which fed on all four sheep were 12.4gm and 11.4gm respectively; the cumulative mean weights were 0.41gm and 0.72gm respectively. The mean percentages of immunologically damaged and dead nymphs were 30.3% and 37.5% respectively; it was 22.0% and 30.8% in female ticks respectively. The difference in morbidity and mortality of the two stages of ticks were only slightly significant ($P>0.05$).

As regards the pattern of tick detachment, there was no glaring difference in the detachment pattern of nymphs and females which fed on control sheep during the primary and secondary infestation periods (Tables 4.1 and 4.3). However, while there were no detached female ticks recorded by day 4 during the primary infestation, 6 were counted on the same day during the secondary infestation.

Similarly, no nymphal tick fed up to day 8 during primary infestation; but 9 were recorded on this day during the secondary infestation. Also, majority of nymphs detached between days 5 and 6 during primary infestation, but this was more widespread during secondary infestation to include day 7. In the female ticks, slightly more than half spread during the secondary infestation to include days 5 to 7.

The difference in total engorgement weights of nymphs and female ticks during the two periods of infestation was not very obvious but the percentages of morbidity and mortality of both tick types were clearly different ($P<0.05$). There was only a 1.7% morbidity in nymphs during primary infestation with no mortality in control sheep while it was 24.3% and 26.3% in nymphs and 18.8% and 15.0% in females respectively during secondary infestation.

In the vaccinated sheep, there were 10 detached nymphal ticks recorded on day 2 during primary infestation and none during the secondary infestation; similarly, no female tick was counted on day 2 during primary infestation while 2 were recorded during the secondary infestation. These observations were recorded in reverse order for both tick types on day 8 during the two periods of infestations. In addition, while most nymphs detached on days 5 and 6 during primary and secondary infestation periods, most female ticks detached between days 4 and 6 respectively. The difference in total weights between nymphs in the two infestation periods was not significant ($P>0.05$). There was a significant reduction in the total weight of female ticks during secondary infestation. The percentages of immunologically damaged and dead ticks were significantly higher ($P<0.01$) during secondary infestation for both tick types.

A comparison of ticks fed on control sheep and those fed on the vaccinated during the primary infestation showed obvious dissimilarities. In contrast to the 2 detached nymphs on day 2 in control sheep, there were 10 in the vaccinated sheep; there was no nymphal tick recorded on day 3 in the former while 16 were counted in the latter. The highest number of tick detachment in nymphs were, however, between days 5 and 6 in both cases. No female ticks were recorded between days 2 and 4 in control sheep, while tick counts were made from day 2 in vaccinated sheep. The highest daily count of detached female ticks in the control sheep was 36 on day 7, and the rest was spread in days 5, 6 and 8 (Table 4.1). Female tick counts in vaccinated sheep were more fairly distributed between days 3 to 8, with relatively higher numbers counted on days 4 to 6.

There was a slight reduction in the total engorgement weights of detached nymphs and females from all the vaccinated sheep.when compared with ticks which fed on control animals. The mean percentages of ticks which suffered morbidity and/or mortality were significantly higher ($P<0.05$) in ticks which fed on the vaccinated sheep when compared with ticks fed on control sheep.

Similarly, there was a significant difference ($P<0.05$) in the engorgement weights and percentage morbidity and mortality of ticks which fed on control and vaccinated sheep during the secondary infestation period. All the ticks fed on vaccinated sheep during the primary and secondary infestations did not attain the critical engorgement weight (CEW) of about 0.17gm for nymphs and 0.35gm for females (Dipeolu, 1991) of *A. variegatum*. In ticks fed on control sheep, all nymphs (100%) and 70.3% of females attained CEW during primary challenge. Similarly, 64% of nymphs and none of the females attained CEW during secondary challenge.

4.3.2. Mean engorgement weight, Egg mass, and Percentage of hatched eggs

Tables 4.5 and 4.6 show the relationship between mean engorgement weights of female ticks and the mean egg mass produced; and also the relationship between these two parameters and the percentage of larvae successfully hatched from eggs of ticks fed on control and vaccinated sheep.

Table 4.5 Mean engorgement weight, Egg mass and Percentage of hatched eggs produced by female *Abmblyomma variegatum* fed on control and vaccinated Yankasa sheep during primary challenge

Host (Contrl).	No. of ticks	MWt (gm)	MMs (gm)	% Hatched
201	16	0.34	0.33	80
202	16	0.35	0.34	80
203	15	0.38	0.35	80
204	16	0.36	0.34	80
Mean	15.8	0.36	0.34	80
Host (Vaccn).	No. of ticks	MWt (gm)	MMs (gm)	% Hatched
205	16	0.28	0.23	65
206	12	0.29	0.27	77
207	12	0.27	0.27	75
208	13	0.30	0.24	67
Mean	13.3	0.28	0.25	71

Legend: Contrl. - Control hosts, Vaccn.- Vaccinated hosts; MWt = Mean engorgement weight; MMs = Mean egg mass

Female ticks fed on control sheep during primary challenge had a mean engorgement weight of 0.36gm, and a mean egg mass of 0.34gm. Eggs incubated from these ticks produced about 80% of successfully hatched larvae. On the other hand, mean engorgement weight of ticks fed on vaccinated hosts was 0.28gm and the mean egg mass was 0.25gm, which were 0.08gm (21%) and 0.9gm (26%) less in weight than ticks fed on control sheep respectively. The percentage of successfully hatched larvae was about 71% (Fig 4.1).

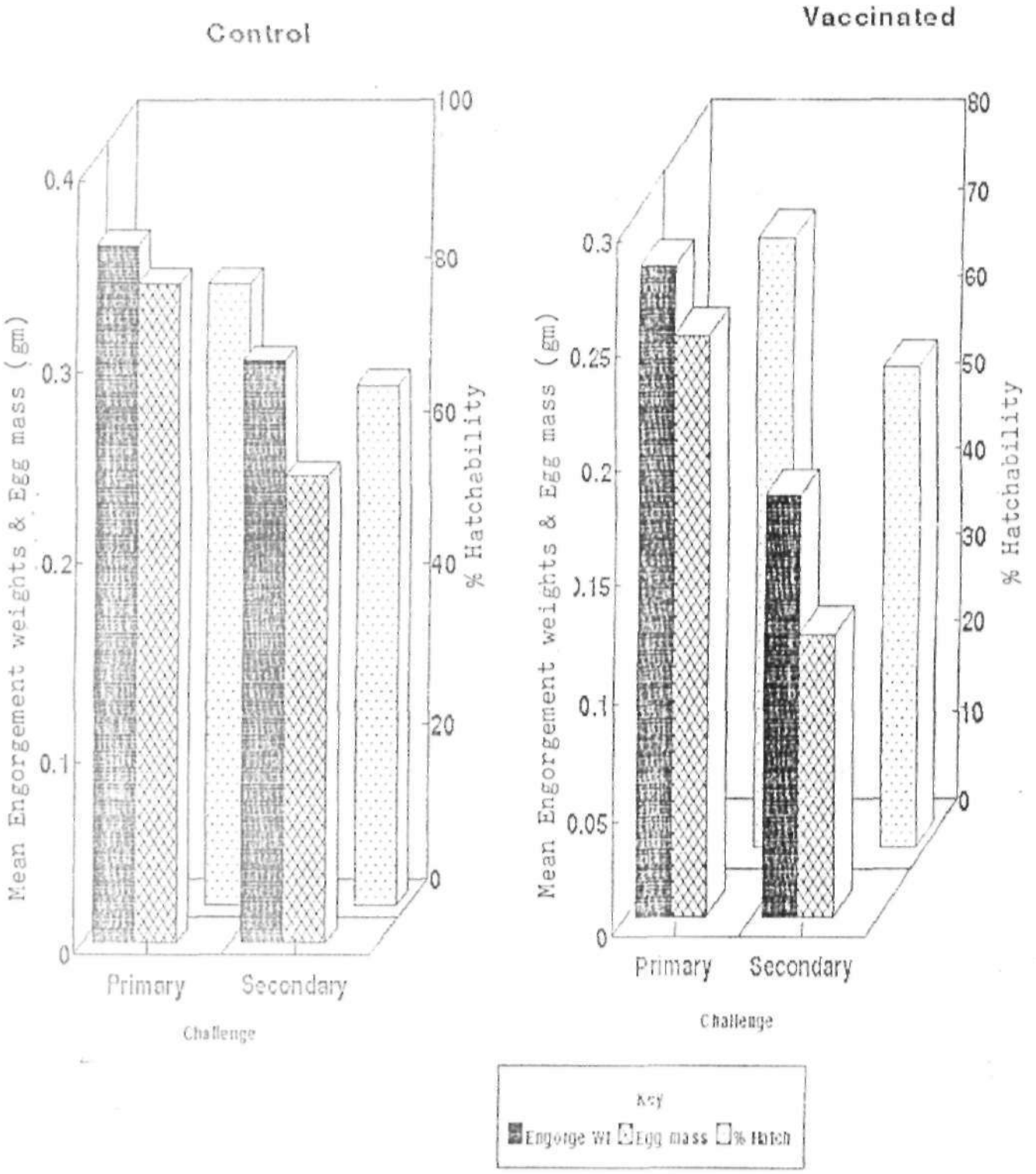


Figure 4.1 Mean engorgement weight, egg mass and percentage hatchability in female *Amblyomma variegatum* fed on control and vaccinated sheep during primary and secondary challenge.

Table 4.6 Mean engorgement weight, Egg mass and Percentage of hatched eggs produced by female *Amblyomma variegatum* fed on control and vaccinated Yankasa sheep during secondary challenge.

Host (Contrl)	No.of ticks	MWt (gm)	MMs (gm)	% Hatched
201	13	0.29	0.28	76
202	14	0.31	0.23	66
203	16	0.28	0.22	64
204	14	0.32	0.25	67
Mean	14.3	0.30	0.24	68
Host (Vaccn)	No. of ticks	MWt (gm)	MMs (gm)	% Hatched
205	11	0.19	0.10	50
206	9	0.17	0.12	54
207	8	0.17	0.13	55
208	11	0.17	0.12	50
Mean	9.8	0.18	0.12	52

Legend: Contrl.- Control hosts; Vaccn.- Vaccinated hosts; MWt- Mean engorgement weight; MMs- Mean egg mass;

During the secondary challenge, the mean engorgement weight of female ticks fed on control sheep was 0.30gm, and the mean egg mass was 0.24gm . These represent a reduction in weight of 0.06gm (16%) and 0.10gm (29%) in ticks which fed on control sheep during primary challenge respectively. The percentage of successfully hatched eggs was about 68%, similar to that obtained for ticks fed on vaccinated sheep during primary challenge. The mean engorgement weight of ticks fed on vaccinated sheep and their eggs during this time were 0.18gm and 0.12gm respectively. These weights/mass were less than those recorded in ticks fed on vaccinated sheep during the primary challenge by 0.11gm (37%) and 0.14gm (54%) respectively. It also showed that the mean weight of these ticks and their eggs were 0.12gm (41%) and 0.13gm (52%) less than in ticks fed on control sheep in the secondary infestation period. These results show significant differences ($P<0.05$) in the engorgement weights and egg mass of ticks which fed on control sheep and those

fed on the vaccinated hosts (Fig 4.1). There was very strong correlation (r=0.90) between mean engorgement weight and mean egg mass (Fig. 4.2 ; see Appendix A).

4.3.3_ Mean engorgement weight and percentage of successfully ecdysed nymphs.

Tables 4.7 and 4.8 show the mean engorgement weights of nymphal ticks and the mean percentages of those which successfully ecdysed into young adults following incubation. The mean engorgement weight of nymphs fed on control sheep during primary challenge was 0.18gm, and the mean percentage of successfully ecdysed ticks was 86.5%. In ticks fed on vaccinated sheep, the mean engorgement weight was 0.12gm, while the mean percentage of successfully ecdysed ticks was 66.5% (Fig 4.3).

Table 4.7 Mean engorgement weight of nymphs of *Amblyomma variegatum* and percentage of successfully ecdysed adults during primary challenge.

Host (Contrl)	No. of ticks	MWt (gm)	Ecdy.	%
201	30	0.20	28	93
202	30	0.17	25	83
203	30	0.18	27	90
204	30	0.19	26	80
Mean	30	0.18	26.5	86.5
Host (Vaccn)	No. of ticks	MWt (gm)	Ecdy	%
205	24	0.10	17	71
206	23	0.11	19	83
207	25	0.11	15	60
208	25	0.14	13	52
Mean	24.3	0.12	16	66.5

Legend:__Contrl.- Control hosts; Vaccn.- Vaccinated hosts; MWt= mean engorgement weight; Ecdy= Number successfully ecdysed

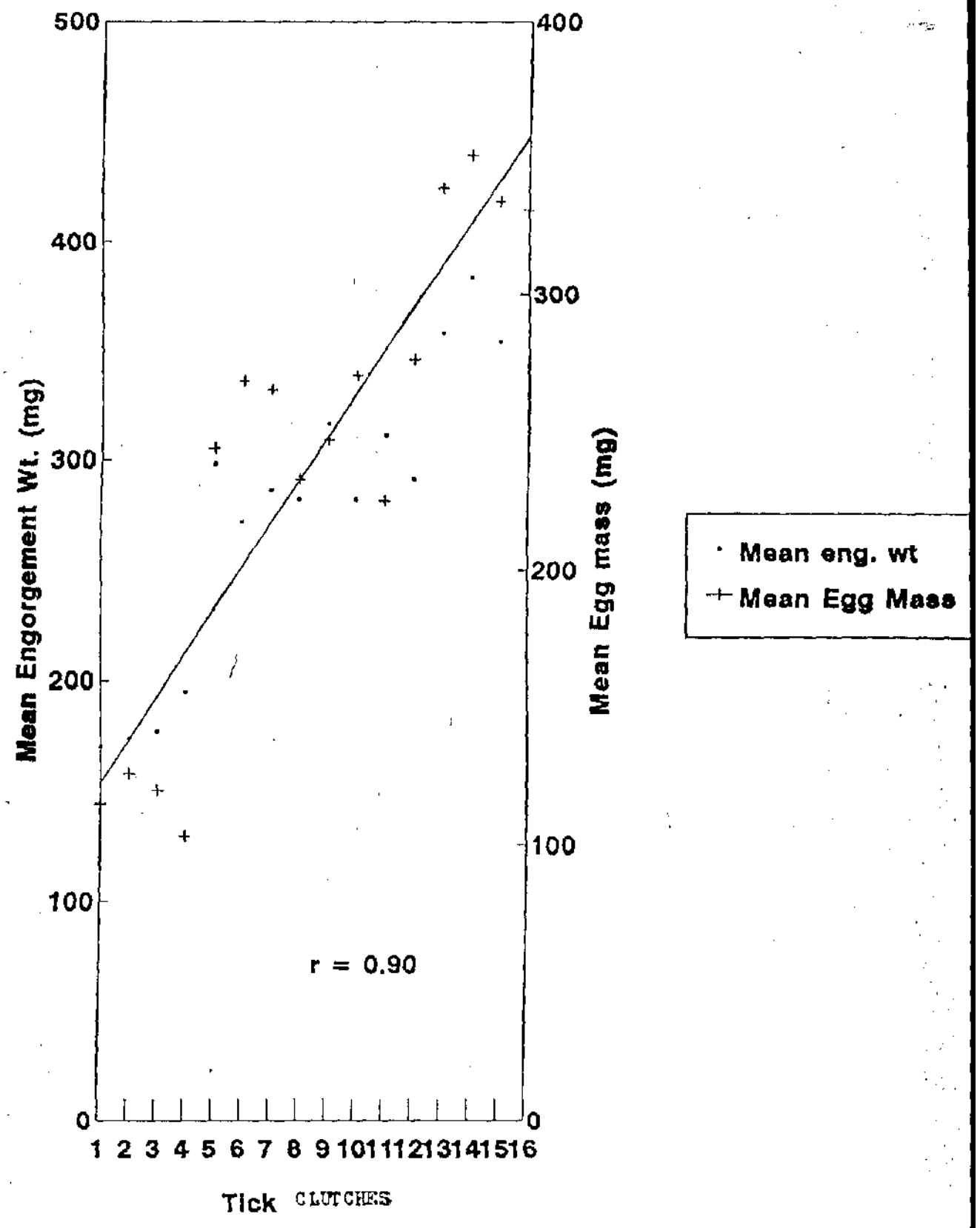


Figure 4.2:Correlation between engorgement weight of female A. variegatum and mean egg mass

Table 4.8 Mean engorgement weight of nymphs of *Amblyomma variegatum* and percentage of successfully ecdysed adults during secondary challenge.

Host (Contrl)	No. of ticks	MWt (gm)	Ecdy	%
201	19	0.17	17	89.5
202	25	0.17	18	72.0
203	22	0.17	13	59.1
204	23	0.17	15	65.5
Mean	22.3	0.17	15.8	71.5
Host (Vaccn)	No. of ticks	MWt (gm)	Ecdy	%
205	15	0.12	7	46.7
206	19	0.12	11	57.9
207	20	0.09	8	40.0
208	21	0.08	8	38.1
Mean	18.8	0.10	8.5	45.7

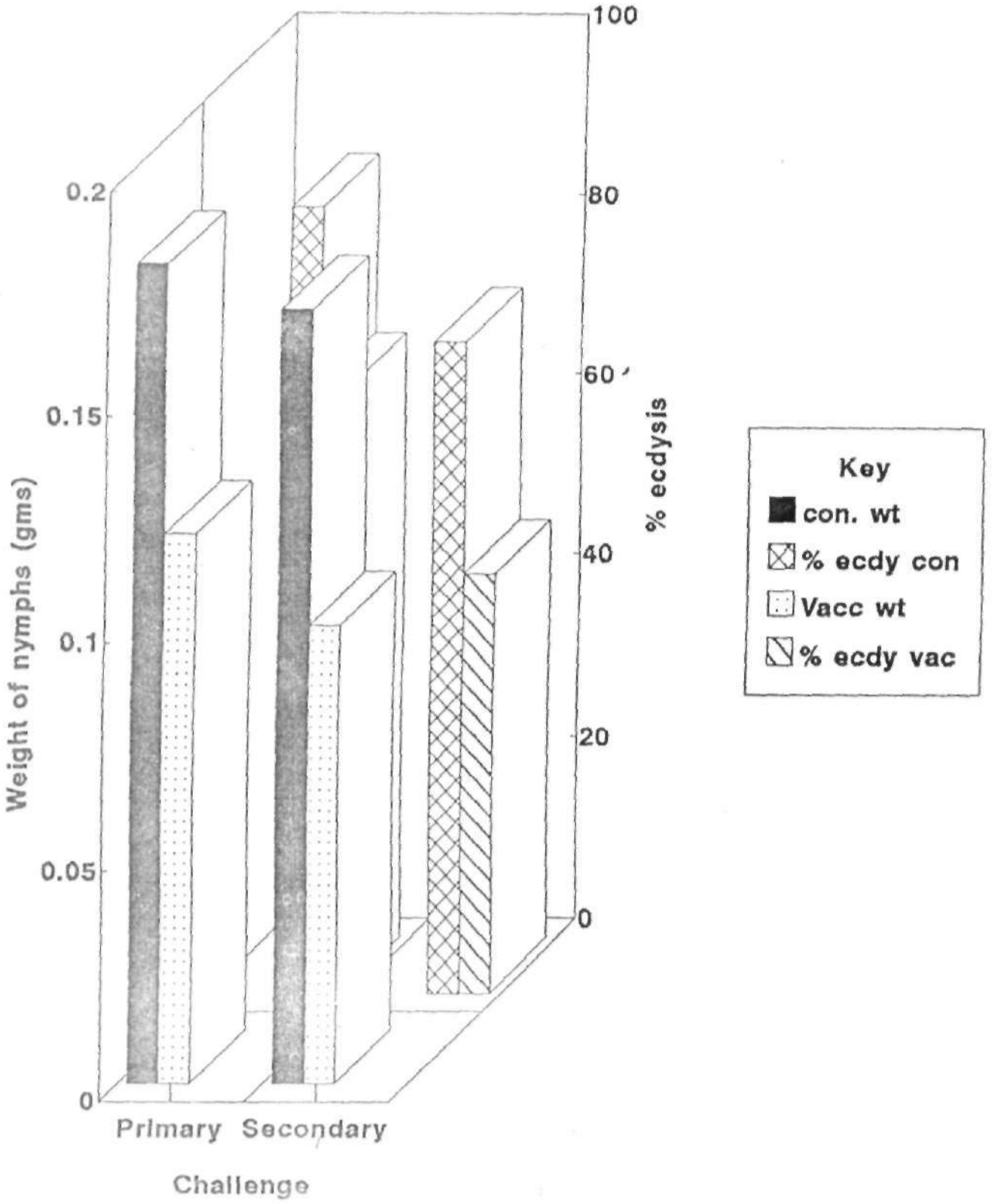
Legend: Contrl.- Control ; Vaccn.- Vaccinated ; MWt- Mean weight ; Ecdy- number successfully ecdysed

Nymphs fed on control sheep during the secondary challenge period had a mean engorgement weight of 0.17gm, and the number / percentage of successfully ecdysedticks was 15.8 (71.5%) ; it was 0.10gm and 8.5(45.7%) respectively in tick fed on vaccinated sheep during the same period.

Results obtained showed no significant difference in the mean engorgement weights of ticks fed on control sheep in the two periods of tick challenge ($P>0.05$), and also in ticks fed on vaccinated sheep during the two periods. There was, however, significant differences in the mean engorgement weights of ticks fed on vaccinated sheep compared with those fed on control hosts ($P<0.05$) in the two challenge periods. This difference was more marked between ticks fed on control sheep during the primary challenge and those fed on vaccinated sheep during the secondary challenge period.

The difference in the mean number/percentage of successfully ecdysed nymphs which fed on control and vaccinated sheep in the primary and secondary challenge periods was significant ($P<0.05$), especially in the latter period. There was strong correlation between engorgement weight of nymphs and the number/

percentage of successfully ecdysed young adult ticks (r= 0.71) (Fig. 4.4 see Appendix A)



ure 4.3: Mean engorgement weight and percentage of successfully ecdysed A. variegatum nymphs during primary and secondary challenges in vaccinated and control sheep.

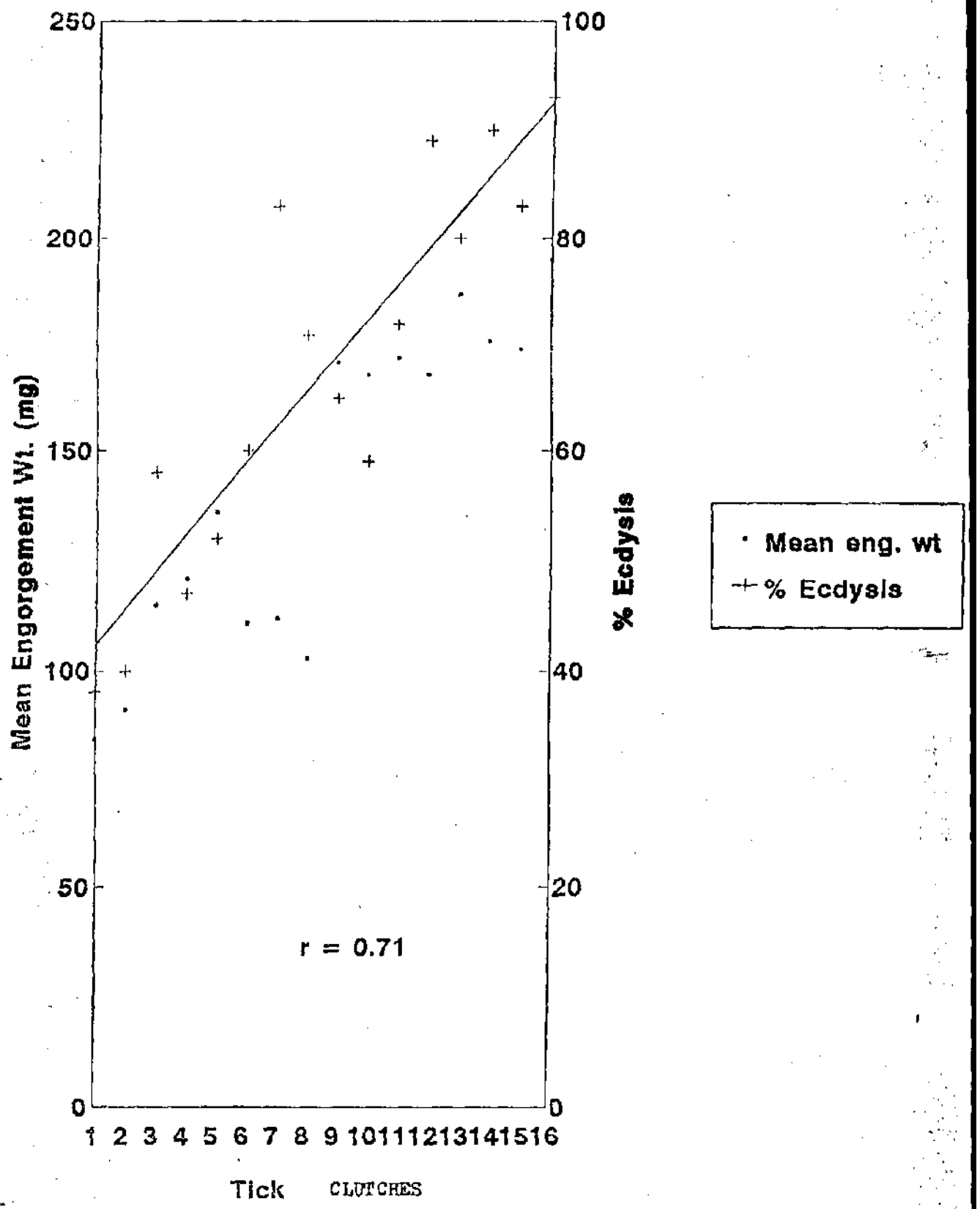


Figure 4.4:Correlation between engorgement weight of female A. variegatum and percentage ecdysis.

4.4 DISCUSSION

From the results obtained in the present work in which immunologically damaged and dead ticks were reported, it could be asserted that certain immunologic changes (Kemp *et al*, 1986) especially in sheep that were vaccinated with antigenic material derived from the midgut membranes of 5-day fed female *A.variegatum* had taken place. This must have interfered with the normal feeding process of adult female and nymphal ticks. These changes were manifested in the greater number of ticks detaching from their hosts without attaining the critical engorgement weight (CEW), reduction in the mean mass of eggs in ticks which fed on vaccinated hosts as compared with eggs of ticks fed on control sheep during secondary infestation, the presence of apparently gut-damaged and dead ticks, the reduction in viability of eggs produced by female ticks fed on vaccinated hosts, interference with the success of ecdyses in nymphal ticks and the reduction in activity of larval ticks hatched from eggs derived from females which fed on vaccinated hosts in comparison with those fed on control sheep. These changes were more evident during the secondary infestation which were manifested by the greater number of immunologically damaged and dead ticks. These observations are consistent with earlier reports by Agbede and Kemp (1986), who studied the histopathology of *Boophilus microplus* fed on immunized cattle that were vaccinated with extracts derived from the midgut of female *Boophilus microplus* ticks; Allen and Humphreys (1979), who immunized guinea pigs and cattle against *B.microplus* using extracts of larval and adult ticks; Dhadialla *et al* (1990) who immunized rabbits against *Rhipicephalus appendiculatus* using solubilized tick tissue proteins; Essuman *et al* (1991) who immunized cattle using semi-purified fractions of solubilized tick gut membrane antigens against *R.*

appendiculatus; Heller-Haupt *et al* (1987) who immunized laboratory animals (guinea pigs) against *A.variegatum* using homogenates from unfed larval ticks; Johnston *et al* (1986) who immunized cattle against *B. microplus* using extracts derived from adult female ticks and Kemp *et al* (1986) who immunized cattle against *B.microplus* using extracts derived from adult female ticks. Opdebeeck *et al* (1988) also immunized Hereford cattle against *B .microplus* with extracts derived from female ticks, and observed similar effects on ticks fed on the vaccinated animals.

Detachment of ticks before the attainment of CEW, especially in vaccinated sheep, may be the result of immunological rejection mechanisms mounted by host immune systems against the ticks. This rejection mechanism is elicited by foreign antigenic proteins introduced into the host via inoculation. Agbede and Kemp (1986) and Kemp *et al* (1986) observed the formation of perforations along the gut lining of immunologically damaged ticks which fed on vaccinated cattle. Engorged host blood in such ticks leaked out into the haemolymph, which made the ticks look reddish in colour (Plate V). Kemp *et al* (1986) however reported that some of such female ticks survived long enough to lay eggs, even though the viability of the eggs were very low. The affected females and nymphs observed in the present study did not, generally, survive longer than 3 to 7 days in the incubator before dying. A few of such female ticks did lay eggs which, after incubating same for a period of about 14 days, produced few larvae which were obviously not as agile as those produced from normal female tick eggs. The result of the present study therefore showed that eggs of most female ticks fed on vaccinated sheep were not as viable as eggs of those fed on control sheep. The implication of these observations on the propagation of tick populations is that affected females and other developmental tick stages, even if they survived, may not be as viable as their unaffected counterparts. This will, in

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effect, reduce tick populations in the field and on host animals with time, as long as the practice of vaccinating ticks continued. Since the engorgement weights of female and nymphal ticks are strongly tied to the mass of eggs produced and the percentage of success during ecdyses respectively, it could be inferred that all ticks which could not attain CEW are bound to perform badly in the field as parasites. However, identification and successful purification of the target antigen in the crude midgut homogenate is necessary for validation of this inference. The ultimate goal is to produce an anti-tick vaccine against *A.variegatum* in Africa, as has been done in Australia and Cuba where TickGARD and GAVAC vaccines have been produced respectively and commercialised against *B.microplus* (Willadsen *et al*, 1995; 1996; Willadsen, 1997). An attempt, in line with the present work, towards semi-purification of the crude antigen derived from ticks in East Africa has not been as successful (Essuman *et al*,1991). A lot more needs to be done.

Tick detachment from infested hosts may also be enhanced by the grooming activity of sensitized/vaccinated animals (Essuman *et al*, 1991). Although attempts at grooming were observed in all the animals, especially in vaccinated hosts as a result of intense irritation during the secondary challenge period, ticks were well protected by the cotton cloth used in demarcating the attachment sites. Intense irritation is created by the attachment and feeding of ticks due to hypersensitivity reactions brought about by secretions from the salivary glands of ticks during feeding (Willadsen, 1980; Opdebeeck and Daley, 1990). Detachment of ticks in the present study may not, therefore, have been aided by host grooming activity but rather by the degree to which ticks were affected by host immune mechanisms. This appeared to be dependent on the intensity of exposure to ticks and the antigens they pass into hosts during feeding, and also tick antigenic materials injected into the

hosts. This is because more gut-damaged and dead ticks were recorded during secondary challenge, and mostly in ticks fed on vaccinated sheep. This cumulative build-up of antibodies and other immune factors such as complement ensures more vigorous reaction against target antigens increasingly with time and exposure (Dipeolu *et al*, 1992). This not withstanding, there appears to be superiority in the quality and intensity of reaction seen in vaccinated sheep where more ticks with mean engorgement weights below CEW, and a higher number of immunlogically damaged and dead ticks were recorded. This is consistent with observations made by Johnston *et al*, (1986) in cattle challenged with *B. microplus* after vaccination with tick derived antigens.

Differences observed with regards to the skin reaction and number of immunologically damaged and dead ticks between the two breeds of sheep could best be explained from the sphere of immunogenic differences, which in turn could be due to genetic differences , however small. Perhaps a relationship similar to that between the Ndama and the White Fulani zebu cattle exists in this case This aspect has not been ascertained in this study but should form an area for further research work.

Deductions made from results obtained in the present study indicate the possibility of producing an anti-tick vaccine against *A.variegatum* using membrane-bound proteins from the midguts of female ticks. The number of detached ticks which could not attain CEW, especially in vaccinated sheep, and also the percentage of immunologically damaged and dead ticks from vaccinated as compared to control sheep serve as pointers.

Vaccinated sheep appeared to have mounted a superior, more intense rejection reaction than control sheep which were only exposed to tick challenge. This became obvious when the number of immunologically affected ticks, viability of eggs and percentage of successfully ecdysed ticks became more during the secondary challenge period .

This study represents a preliminary attempt at determining the protective effect of *A.variegatum* derived antigens against infestation by the tick species. It is recommended that further studies be conducted to identify and characterize the antigens for eventual use in the development of an anti-tick vaccine. The potential for cross-protection against other tick species should also be investigated, considering the enormous diversity of tick species of veterinary importance in Africa.

CHAPTER FIVE

INNÖCULATION OF RABBITS USING FRACTIONS OF A SEMI-PURIFIED MEMBRANE – BOUND ANTIGEN PREPARATION DERIVED FROM MIDGUTS OF 5-DAY FED FEMALE *Amblyomma variegatum.*

5.1 INTRODUCTION

Laboratory animals, including rabbits have been used in several experiments involving parasitic organisms like protozoans, helminths and arthropods. Much work has been carried out using insects on laboratory animals (Jones, 1970), but far less so with ticks. Brown and Knapp (1980), studied the sequential histopathology of the feeding sites of *A .americanum* using guinea pigs. Similarly, Shapiro *et al* (1986) studied the anitigens of *Rhipicephalus appendiculatus* that were recognized by serum of guinea pigs; while Heller-Haupt *et al* (1987) carried out immunization studies using guinea pigs as hosts to *A .variegatum.* Mongi and Aganyo (1990) used rabbits in studying immunity and immunological responses to antigens derived from the tick species *R .appendiculatus.* Even domestic chickens have been used (Mwangi *et al* 1991) as laboratory hosts to larval ticks of *A .hebraeum* (and similar tick species). Willadsen (1980), reported that laboratory animals could acquire resistance to ticks after several and long periods of exposure. Newson *et al* (1980) observed that ticks which fed on hosts that had acquired resistance to ticks dramatically reduced daily in population when compared with those feeding on tick naïve animals. Likewise, antigens derived from whole ticks , tick salivary glands and midguts have been used, with varying degree of success in inducing resistance to tick infestation (Johnston *et al*, 1986; Kemp *et al*, 1986; Agbede and Kemp, 1986;

Opdebeeck *et al,* 1988). Besides reducing the chances of tick- borne diseases, acquired immunity can directly interfere with the development of parasites in the tick vector (Newson and de-Castro, 1983). Acquired tick resistance is an immune phenomenon involving cutaneous hypersensitivity to some antigens secreted by feeding ticks (Allen *et al,* 1977; Askehase, 1980). The use of concealed antigens , such as the midgut antigens, which are not normally exposed to the host by feeding ticks have been shown, in immunization studies, to be superior over natural acquisition of resistance (Shapiro *et al,* 1986; Opdebeeck *et al,* 1988; Kocan, 1995). Although some advances have been recorded in the use of crude tick antigen in immunization studies, there is need for a more efficient method of artificial immunization by obtaining purified antigens that could be used with different adjuvants in order to attain better and lasting immunity (Shapiro *et al,* 1986).

The aim of this study is to evaluate the degree of protection against nymphal *A. variegatum* challenge conferred on rabbits immunized with partially purified extracts obtained by ion-exchange chromatography of the crude midgut homogenate.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Animals;

Rabbits; Eight, six to ten month-old rabbits weighing approximately 3 to 5 kilograms were acquired from a livestock farmer in Tudun-Jukum, near Tudun-Wada in Zaria. The rabbit flock was adjudged to be free of tick infestation, based on visual examination of animals. The rabbits were housed in steel-wire cages in the arthropod-proof pen of the Department of veterinary Parasitology and Entomology , and were fed on maize bran (dussa) , carrots and carrot leaves, leguminous groundnut hay (harawa) and other green vegetables such as spinach and potato leaves. Clean drinking water was provided in the cages *ad libitum* (Plate XVI). They were allowed to acclimatize for a period of two weeks prior to the commencement of the experiment.

Tick Source: Ticks were obtained from the existing laboratory colony as described earlier on in 3.2.2. About 35 female *A .variegatum* were fed on sheep for 5 days. Partially engorged by day 5, they were harvested and dissected following which their midguts were removed and stored in 5ml of PBS (pH 7.2) at – 20°C until needed.

5.2.2. Antigen Preparation, Purification and Separation:

The same procedures of antigen preparation as in **4.2.2**. above were followed. The crude antigen homogenate was further subjected to semi-purification by use of the ion-exchange chromatographical method of Peterson and Sober (1962) in which

diethylaminoethyl (DEAE) cellulose adsorbent was used. This method is most commonly used in protein separation and purification.

Briefly, a 2.2 X 18cm chromatographic column was obtained and thoroughly rinsed with distilled water and then with the starting buffer (0.01M PBS, pH 7.2) which was freshly prepared. It was mounted on a clamp stand in such a way that a 250ml flat-bottomed flask was fitted below. The column was then packed with about 8 gm of DEAE-cellulose which had earlier been weighed and washed in three rinses of distilled water and the equilibrating buffer solution. About 10 ml of the homogenate was then applied to the top of the DEAE-cellulose column and covered with several mililiters of the starting buffer. The flow rate was adjusted to about 20 ml/hr and, subsequently, two volumes of 250 mls of eluents, using 500 ml of starting buffer were collected. About 250 ml of the final buffer, 0.3M PBS (pH 8.0) was continually added until all 250 ml was collected. The three 250 ml flasks collected containing fractions of the eluents were sent , in ice-packed flasks, to NVRI, Vom, Plateau State, where they were freeze-dried. The optical densities of the three eluents were measured at 280nm in order to determine the protein content in each of the eluents. These were later reconstituted by adding 10ml of normal saline solution in each case before inoculating the rabbits , as in Johnston *et al,* (1986).

5.2.3 Inoculation Protocol and tick Challenge: The eight rabbits were randomly assigned into four groups of two rabbits each, and designated as follows: a) Control; b) group 1; c) group 2; d) group 3. The rabbits were allowed a two-week period to acclimatize after which inoculations were conducted. Control rabbits were injected intramuscularly with 2 ml of 0.01M PBS (pH 7.2) in the thigh muscles. Rabbits in the other three groups were inoculated with 2 ml of 0.7 mg/ml ,

1.8 mg/ml and 1.2 mg/ml of eluents respectively. All injections were administered i.m. in the thigh muscles. Booster injections were administered in the same manner two weeks later. All rabbits were then challenged, in each case, with 100 nymphs on the ears using ear bags to aid tick attachment. Tick challenge was carried out one week after the injections.

5.2.4. Daily counts of Detached ticks: In order to allow for tick attachment, ear bags were left secured on the rabbits undisturbed for 24 hours. Subsequently, count of detached ticks were done at about 9.00 am of each day, commencing from day 2 post-infestation, until day 7 when all ticks were expected to have fully engorged. Detached ticks normally fell to the bottom of the vertically oriented ear bags, so that removing and shaking out the bags yielded all detached ticks. The bags were then replaced over the feeding ticks still attached to the pinnae. The flat cage tray was also inspected in order to collect detached ticks which were able to crawl out of the ear bags (Plate XVII). The number, weight and other physical attributes of the recovered ticks were recorded before placing them inside properly labelled bottles and incubating them at 28°C and 85% RH for development to adulthood.

The same rabbits were again re-challenged four weeks later with the same number of nymphs belonging to the same tick species. This was meant to test for the development of immune reaction against tick infestation after the initial inoculation and tick challenge. The tiny size of larval ticks and the enormous size attained by adult (female) *A.variegatum* made them unsuitable for use in this experiment. Nymphs were used because of their moderate sizes.

5.3. RESULTS

5.3.1 Daily Counts of Detached ticks:

Tables 5.1 and 5.2 show results obtained when detached nymphs of *A.variegatum* were collected daily from the infested control and vaccinated rabbits during primary and secondary challenges. In control rabbits during the primary challenge, most of the ticks detached on day 5 post infestation (pi) and the rest were mostly spread between days 6 and 7. A similar observation was made during the secondary infestation where fewer number of ticks detached on days 4, 6 and 7. For the group 1 rabbits which were inoculated with eluent containing 0.7mg/ml of protein, most ticks detached on day 5 during the primary and secondary infestation periods (67% to 80% respectively), followed subsequently by the number of ticks which detached on day 6. Similar results were generally obtained from rabbits of groups 2 and 3 which were inoculated with eluents containing 1.8mg/ml and 1.2mg/ml of protein respectively. There was, however, a slight difference in detachment patterns of ticks fed on rabbits in group 2 where 2 nymphs detached on day 2 from host 05 during secondary challenge. The difference in number of detached ticks between control and the group 2 rabbits in both periods was very significant ($P < 0.01$).

The mean percentage of detached ticks, on a daily basis, were higher during the secondary challenge period (except on day 5 in the primary challenge). This showed that more ticks detached between days 2 and 4 during secondary challenge than during the primary. A similar pattern was also observed between days 6 and 7 (Fig. 5.2).

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Table 5.1 Engorgement weight, number of immunologically damaged and dead detached nymphs of *Amblyomma variegatum* fed on control and vaccinated rabbits during primary challenge

Daily counts of detached ticks and their engorgement weights															
Rabbit groups	Days	2	3	4	5	6	7	8	Tno	Twt (gm)	Mwt (gm)	SE (±)	DM %	DD %	EC %
Contrl.	01	0	0	0	94	4	2	0	100	7.8	0.08	41.6	0	0	100
	02	0	0	0	89	6	5	0	100	7.9	0.08	22.9	0	0	100
Grp 1	03	0	4	0	76	20	0	0	100	6.8	0.07	10.6	0	0	100
	04	0	0	5	83	12	0	0	100	6.8	0.07	8.6	0	0	100
Grp 2	05	0	0	15	82	3	0	0	100	6.7	0.07	10.5	0	0	92
	06	0	0	21	75	4	0	0	100	6.9	0.07	14.6	0	0	89
Grp 3	07	0	0	4	88	8	0	0	100	7.7	0.07	41.5	0	0	95
	08	0	0	8	84	8	0	0	100	7.4	0.07	16.7	0	0	97
Mean		0	0.5	6.6	84	8.1	0.9	0	100	7.3	0.07		0	0	96.6

Legend: Contrl.=control group; Grp= Group; Twt= total engorgement weight; Mwt= Mean weight; SE= Standard error; DM%= percentage damaged; DD%= percentage dead; EC%= percentage successfully ecdysed.

Table 5.2 Engorgement weight, number of immunologically damaged and dead detached nymphs of *Amblyomma variegatum* fed on control and vaccinated rabbits during secondary challenge.

Daily counts of detached ticks and their engorgement weights															
Rabbit Group	Days	2	3	4	5	6	7	8	Tno	Twt (gm	Mwt (gm)	SE (±)	DM %	DD %	EC %
Contrl	01	0	0	10	80	5	5	0	100	6.2	0.06	24.5	1	0	88
	02	0	0	6	83	9	2	0	100	6.3	0.06	41.6	0	0	89
Grp 1	03	0	0	17	63	18	2	0	100	6.1	0.06	34.6	3	5	82
	04	0	0	22	70	8	0	0	100	6.2	0.06	7.9	2	10	76
Grp 2	05	2	30	45	22	3	0	0	100	5.9	0.06	12.3	13	10	[illegible]
	06	0	0	21	33	46	0	0	100	6.1	0.06	9.6	10	8	75
Grp 3	07	0	0	31	66	3	0	0	100	5.9	0.06	14.6	12	5	77
	08	0	0	34	61	5	0	0	100	5.9	0.06	20.7	15	8	71
Mean		.25	3.8	23.3	59.8	12	1.1	0	100	6.1	0.06		7.0	5.6	78

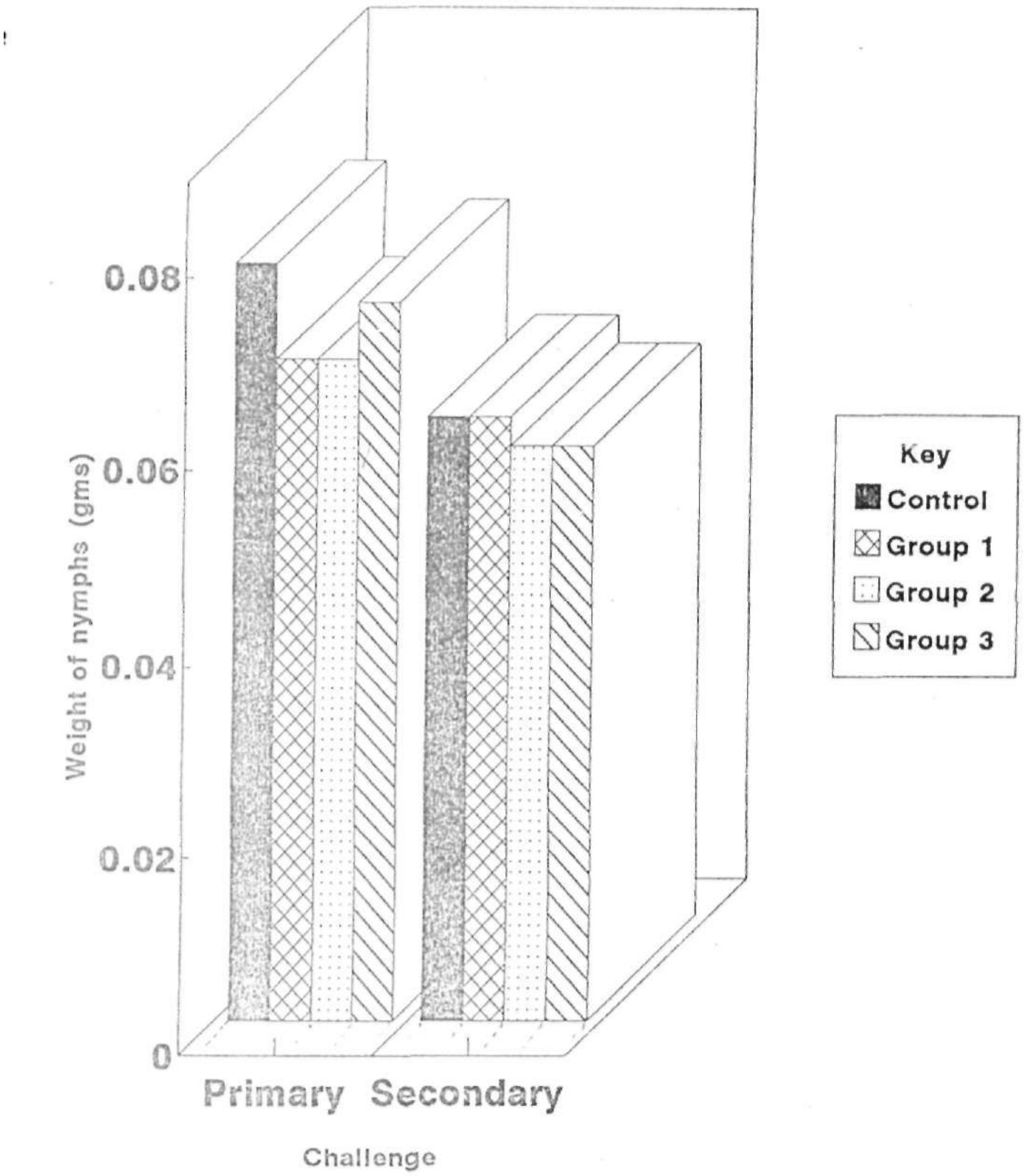
Legend: Contrl.- Control; Twt- Total engorgrmrnt weight; Mwt- Mean weight; SE- Standard error; DM%- percentage immunologically damaged; DD%- percentage dead ; EC%- Percentage successfully ecdysed.

5.3.2 Daily mean engorgement weights of detached *A. variegatum* nymphs during primary and secondary chellenges.

Generally, there was progressive increase in mean engorgement weight of ticks which fed on rabbits in all the experimental groups during the primary and secondary challenge periods, although ticks fed on control animals attained comparatively higher mean engorgement weights at detachment on days 6 and 7. Except in the control group and in the group 1 rabbits (during secondary challenge), all detachments of ticks were completed by day 6. Ticks had lower mean engorgement weights in all cases, during the secondary challenge period when compared with those which fed during the primary challenge period (Fig 5.1). The distribution of daily mean engorgement weights of nymphs fed on all groups of rabbits is as represented in Fig. 5.2.

5.3.3 Percentage of immunologically damaged and dead ticks:

There were no immunologically damaged or dead nymphs seen during the primary infestation (Table 5.1). Affected ticks were observed only during secondary challenge, especially in vaccinated rabbits of groups 2 and 3. The highest percentage of immunologically damaged ticks were recorded from host 08 in group 3 (15%), followed by host 05 (13%) in group 2. The highest percentage of dead ticks (10%) were recorded from hosts 04 and 05 in groups 1 and 2 (Fig 5.2). There were only 1% of immunologically damaged ticks in the control rabbits and no dead ticks were recorded. The differences in the percentages of damaged and dead ticks between ticks fed on control and those fed on vaccinated rabbits, especially those from groups 2 and 3 was significant ($P< 0.01$). This was also seen in ticks which infested rabbits during the primary challenge period compared with those in the secondary challenge period.



igure 5.1 Mean enngorgement weights of detached nymphs of A. variegatum fedon control and vaccinated rabbits during primary and secondary challenges

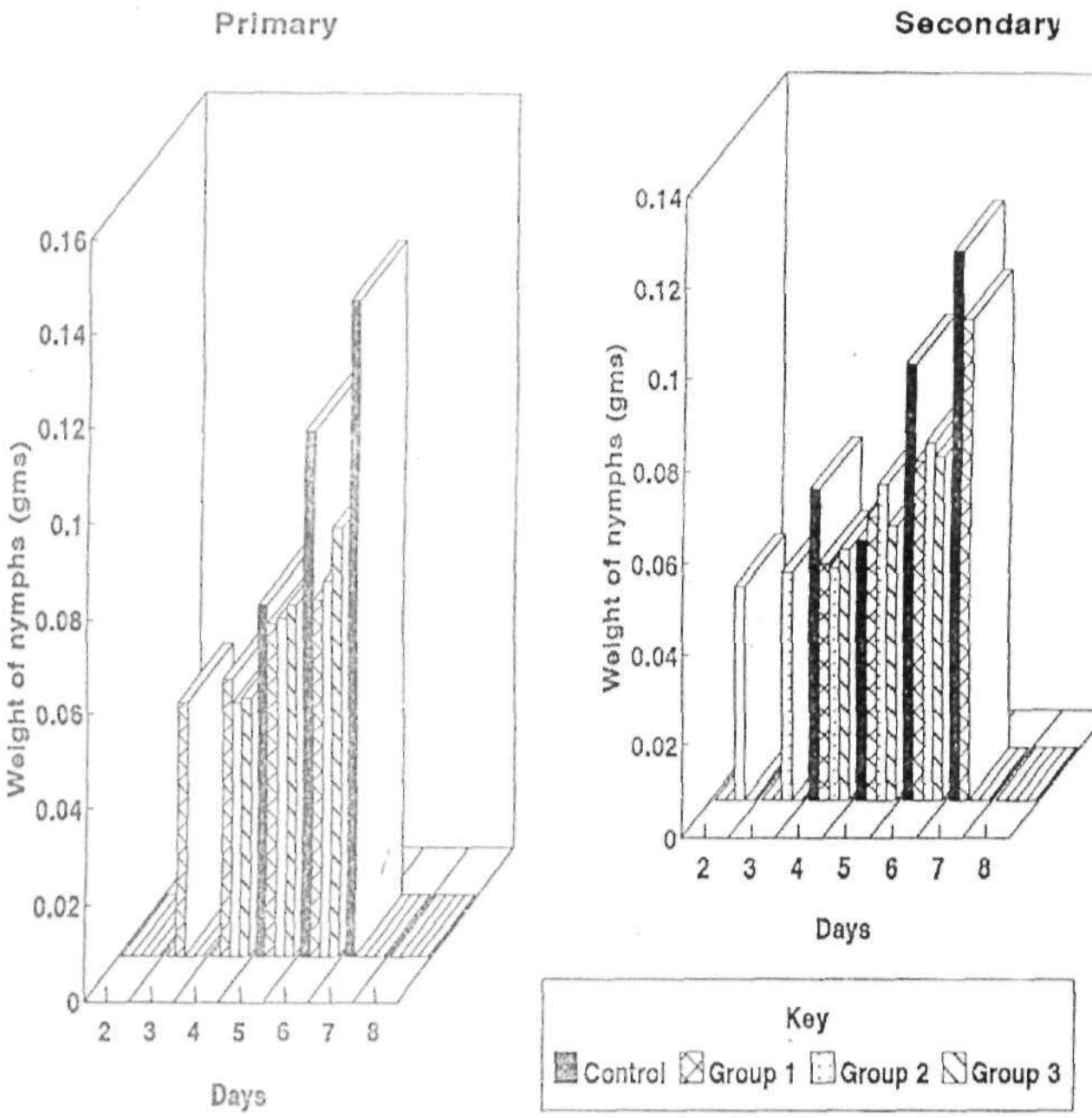


Figure 5.2: Distribution of daily mean engorgement weights of nymphal ticks fed on control and vaccinated groups of rabbits during primary and secondary challenge

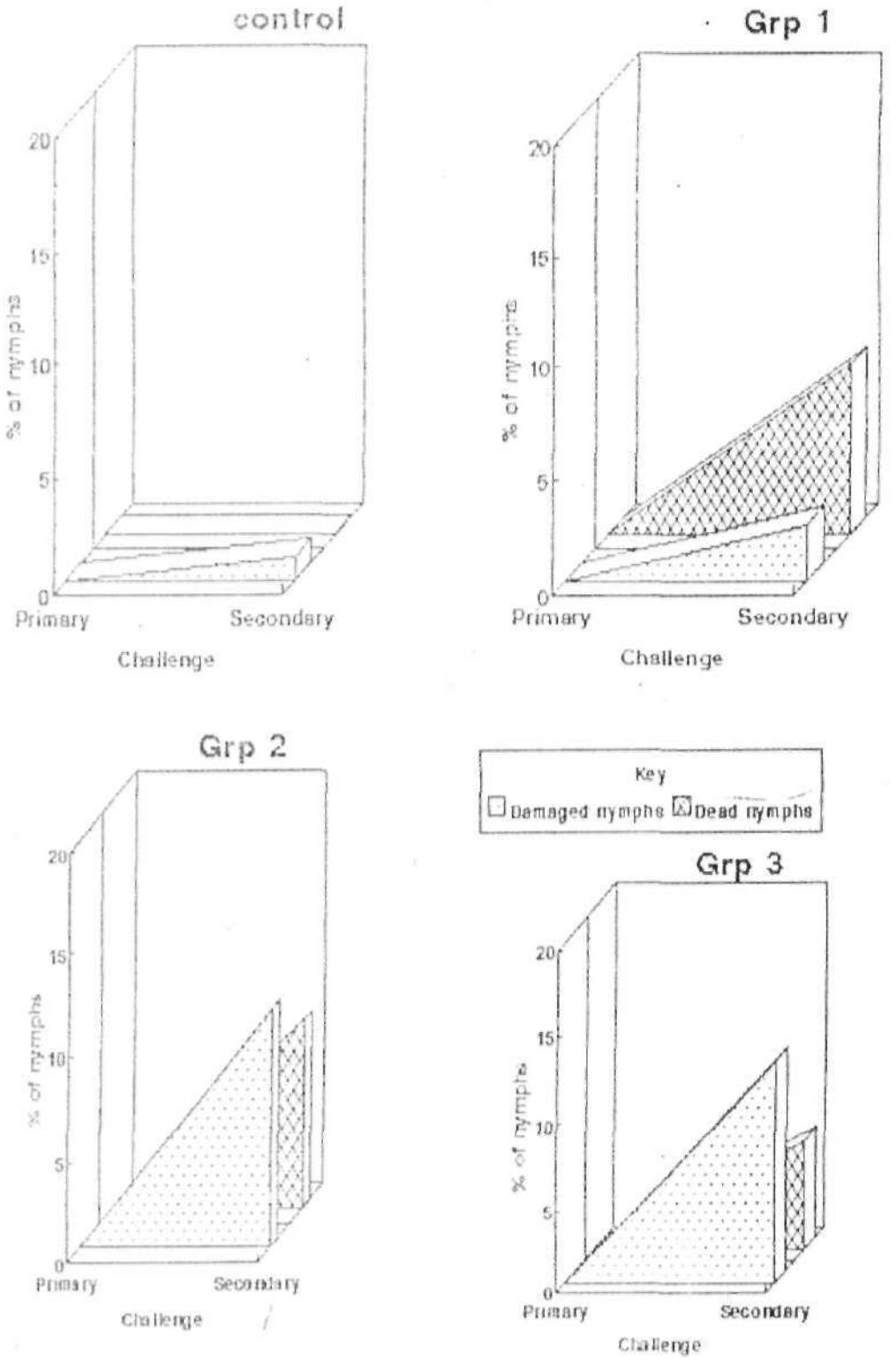


Figure 5.3: Percentage of immunologically damaged and dead nymphs fed on control and vaccinated groups of rabbits during primary and secondary challenge periods

5.3.4 Percentage of successfully ecdysed nymphs following incubation:

Tables 5.1 and 5.2 show the percentages of nymphal ticks which *successfully ecdysed into young adults after recovery from infested hosts during* primary and secondary challenges. All recovered ticks from the control hosts and the group 1 rabbits successfully ecdysed (100%) during the primary challenge period. About 89 to 97% of ticks which fed on rabbit hosts in groups 2 and 3 during the same period successfully ecdysed. The mean percentage of successfully ecdysed nymphs from all the four groups was 97%. During the secondary challenge period, between 71 and 89% of ticks recovered from the four experimental rabbit groups successfully ecdysed. A mean percentage of about 79% success was achieved

5.4 DISCUSSION

The detachment of ticks from their hosts is normally the result of attainment of full engorgement, or could be due to uncomplimentary host reactions geared towards rejection of ticks. Persistent grooming activity by the host due to intense irritation caused by the presence of the parasite (Dipeolu *et al,* 1992), and the mounting of an immunological defense mechanism, especially in previously sensitized hosts (Kemp *et al,* 1986) discourage continued tick attachment to hosts.

The lesser number of detached ticks counted on day 5 (mean 59.8) during the secondary challenge , when compared with the number which detached within the same period of time during the primary challenge (mean 84) may indicate that rabbits had developed a greater degree of resistance to the feeding ticks. This is because immunologically affected ticks, especially larvae and nymphae, take longer to engorge on resistant hosts (Ackerman *et al,*1980) and may even detach from hosts earlier due to gut damage (Willadsen, 1988). This may have resulted in early tick detachments, especially in hosts 05 and 06 during primary challenge, and in all hosts after secondary challenge but particularly those vaccinated. The detachment of most nymphs on day 5 in each of the two challenges was within the expected feeding time of this tick species (Hoogstraal, 1956). The larger number of nymphs which detached from the control rabbits on this day, compared with those from the vaccinated hosts suggests some differences in the environment of the feeding lesion which may have been brought about by effects of the inoculation. The differences in the number of detached ticks from control and vaccinated rabbits was, however, not significant ($P > 0.05$) at day 5 (except in the group 2 rabbits) . There was marked diference in the number of nymphs which detached from rabbits in group 2,

prior to day 5, when compared to rabbits in the other groups during both challenge periods. This also resulted in the ticks failing to attain CEW before detaching from the animals. The lower engorgement weights recorded in ticks fed on control rabbits during secondary challenge might be the result of their inability to feed normally due to host mounted anti-tick defense mechanisms. This must have been built up from the primary challenge period and then enhanced by the presence of feeding ticks during the secondary challenge period.

Results obtained in this study did not agree with a similar report by Heller-Haupt *et al* (1987) who fed *A. variegatum* larvae on guinea pigs that were vaccinated with homogenates derived from unfed, ground-up larvae of the same species. They reported significantly lower mean engorgement weights ($P< 0.01$) and a 35% rejection rate of larvae. These observed differences may have been brought about by factors such as the concentration of protein used in inoculums (they used 3mg/ml of protein); the stage of ticks used in the challenge experiments; methods of preparation of homogenates and the form in which it was used (they used crude homogenates without purification or separation into sub-units) and even the availability of relevant facility for carrying out the experiments may have affected outcome of the results. In addition, it could be that certain proteins or enzymes critical in the elicitation of specific immune responses in challenged hosts were destroyed or even lost during the chromatographical separation of proteins in the present study. Mongi and Aganyo (1990) demonstrated the presence of anti-tick antibodies in rabbits vaccinated with antigenic material derived from the midgut of *R. appendiculatus* female ticks, thus demonstrating that rabbits also have the ability to mount an immunological defense mechanism against ticks. The low level of

reactivity against ticks in vaccinated rabbits in the present study may not therefore be due to inability to react adequately if properly challenged.

The presence of immunologically damaged and dead ticks during the secondary challenge, though in comparatively low percentages, may be due to the activity of a rejection mechanism in sensitized hosts. This kind of reaction had been reported by Agbede and Kemp (1986) in which *Boophilus microplus* ticks were fed on Hereford (*Bos taurus)* cattle previously vaccinated with midgut–derived antigen from female ticks of the same species. Kemp *et al* (1986) and Opdebeeck *et al* (1988) also made similar reports. The similar but greater effects in terms of damage and death of ticks in the groups 2 and 3 rabbits when compared with the group 1 rabbits in the present study may be an indication of different immunological responses due to differences in the constituents of preparations used, however small these differences are.

The responses against feeding ticks in the present study indicates the need to derive a vaccine with known concentration of antigens that will be effective in the control of infesting hard ticks on hosts. A properly determined route of vaccine administration must also be worked out after several trials.

For the purpose of further research work on this subject, it is recommended that efforts should be geared towards isolation and purification of target proteins with potentials as good vaccine candidates that could be mass-produced and used in vaccine production. The semi-purified protein units used in vaccinating the groups 2 and 3 rabbits in the present work appear promising.

5.5. CONCLUSION:

In general, there was little difference in the detachment pattern of ticks fed on control rabbits and those fed on the vaccinated hosts (except in the group 2 rabbits) during the two challenge periods. This observation could be attributed to a number of factors, some of which include comparatively less protein concentrations, which perhaps also affected the immunogenecity in the inoculum preparations used; probable loss of vital proteins from tick homogenates during the process of semi-purification or even the use of a not too suitable route of vaccine administartion.

The mean engorgement weights of ticks fed on control and vaccinated rabbits within the same challenge period did not significantly differ from each other. However, there was a recognizable difference , which was not highly significant, in the mean engorgement weights of ticks which fed on the control and vaccinated rabbits after the secondary challenge.

The absence of immunologically damaged and dead ticks after the primary challenge, and their presence in the vaccinated hosts after the secondary challenge suggests the elicitation of a host- mounted defense mechanism which might have been in direct relationship with the dosage of protein administered in the animals.

Further work on the identification, isolation and purification of potentially beneficial antigenic proteins from the midgut of female ticks (*A. variegatum*) is suggested. This could lead to the production of the first anti-tick vaccine against African hard ticks, similar to the TickGARD produced against the temperate hard tick *B. microplus* in Australia (Willadsen, 1997a,b).

CHAPTER SIX

6.1. GENERAL DISCUSSION

An important component of livestock improvement in the developing world include good nutrition, proper management and the control of animal diseases, and the availability of good market for the sale of livestock products. The problem of livestock diseases is particularly serious in Africa, Asia, The Caribbean and Latin America (de-Castro, 1997). Ticks and tick-borne diseases (TTBD) constitute the most important limiting factors in the improvement of the livestock industry in the tropics (Callow, 1978). The diverse biological and ecological setting in the tropical and sub-tropical regions of the world provide very suitable environments for the development and propagation of ticks and the diseases they transmit (Young *et al.* 1988). The large number of tick species (Ixodidae) in Africa, with a wide variety of animal hosts in diverse ecological regions make the control of ticks virtually impossible on a large scale. This is in direct contrast to the situation in temperate climate countries where single or a few species of ticks infest animals. It is therefore easier to control or even eradicate ticks in temperate rather than tropical and sub-tropical countries (Bram and Gray, 1979).

In Nigeria, five genera of ticks of veterinary importance , and several species and sub-species parasitize livestock and wild animals (Strickland, 1961; Mohammed, 1974; George, 1987). *A.variegatum* stands out as one of the most economically important species, causing serious economic losses to the livestock industries which utilize by-products from livestock, such as the leather industry (Mohammed and Agbede, 1980). In addition to these, there is loss of production due to the detrimental activities of ticks and the parasites they transmit . Other effects in parasitized animals include worry through irritation and bites, loss of

blood by exsanguination , introduction of toxins during feeding which may result in paralysis in a number of organs , and damage to hides and skin . Callow (1978) and Young *et al* (1988) considered ticks and tick-borne diseases as the greatest problem facing the livestock industry in Africa. Economic losses globally due to TTBD has been estimated at about seven billion US dollars annually (McCosker, 1979).

The destruction of host tissues by ticks during attachment range in severity from pycnosis of the nuclei of adjacent cells to large scale damage of host skin. This often degenarates to into ulcers and cavity formation as was observed in the present study. The existence of such tissue damage has been used as evidence for the presence of cytolytic enzymes in tick saliva which aid the destruction and denaturation of host tissues, making it suitable for ingestion by the tick. Allen *et al* (1977) were of the view that pycnosis of cell nuclei and the dilation of blood capillaries were effected by salivary enzymes such as hyaluronidase, phospholipase, proteinase, lipase, esterases, aminopeptidases etc; but also that certain pharmacological agents and chemicals contained in salivary fluids which could cause mast cell degranulation may also be responsible (Schlegar *et al*.1976).

Prostaglandin E2 (PGE 2), a vasoactive agent was identified in the salivary secretions of female *B. microplus* ticks (Tatchell and Binnington, 1973) and was suggested as an agent which promoted cutaneous blood flow during tick feeding. Its highest activity was recorded at the stage of tick final rapid engorgement , suggesting involvement in the tick feeding process. The massive infiltration of host blood components into tick feeding lesions, and the erosion of host tissues, especially between days 6 and 8 in the Yankasa and Uda sheep may therefore be the result of enzymatic and pharmacological agents in the tick saliva, in addition to host autolytic activity. The more extensive damage observed in the skin of Uda sheep

may be due to the enhanced activity of all these factors for reasons that are not clear presently.

The role of the salivary gland in the development and transmission of tick-borne parasites, though not studied in this work, is not clear. Certain piroplasmic organisms such as *Babesia* and *Theileria* spp have been observed to prefer certain cell types in the haemolymph cells of ticks during their development and translocation (Binnington and Kemp, 1980). Certain parasites may even be using components of tick saliva in their development as evidenced in the depletion of cellular granules in infected cells when compared with normal cells (Kemp *et al*,1982; Agbede and Kemp, 1986; George, 1987). It is possible, therefore, that the relationship between ticks and the parasites they transmit is more complex than may be assumed. An insight into this relationship may aid control of TTBD.

The development of resistance by some species of ticks to a number of commonly used acaricides (Crampton and Gichanga, 1979) , and the high cost of purchasing these chemicals has made control of ticks by use of chemicals a less attractive method. On the other hand, a number of non-chemical tick control methods tried have not yielded appreciable success (Thompson *et al*,1978; Zimmerman *et al*,1984; Mwangi *et al*,1991; Gronvold *et al*, 1996). Drastic modification of the ecological setting involving the destruction of flora and fauna had no lasting effects on the population of ticks (Bram and Gray, 1979; Meyer *et al*,1982; de-Castro, 1997).

Species of *Amblyomma* ticks have been shown to respond to natural and synthetic aggregation pheromones applied on cattle. They attach to areas where the chemical was applied. This led to the development of a system of combining pheromones with acaricide and applying same on cattle. Ticks which fed from such

areas were killed (Norval *et al*,1996; de-Castro ,1997). This system is more selective in the species and stage of ticks killed since not all tick species respond to this pheromone, just as more adults of the attracted tick species respond and are killed. The development of non-chemical acaricides will reduce dependence on the use of chemicals in tick control.

One of the attractions in controlling tick infestations on livestock by immunization is that infected ticks could be impeded from further feeding which, in a way, reduces the chances of disease transmission. The reduced mass of eggs produced, coupled with the lower viability of same as examplified by the fewer larvae produced by ticks fed on immunized sheep and rabbits in this study, attests to this fact. It is therefore envisaged that the use of anti-tick vaccines in tick control will not only reduce the level of infestation, but also reduce the spread of disease causing organisms transmitted by ticks (Kocan, 1995).

The ability of host animals to produce antibodies to protect themselves partially or totally against ticks following sensitization with antigens was first reported by Galun (1975). Subsequent work in the area of research for anti-tick vaccine (Wikel, 1982; Willadsen, 1987;1988) using proteins derived from the ticks themselves culminated in the production of the first commercially available vaccine, the TickGARD (Willadsen, 1997a). This vaccine has further been improved in terms of potency by enhancing the antigenic capability of the antigens, and is presently sold as TickGARD Plus (Willadsen, 1997b). This forms one of the most important developments in the area of tick control in recent times. A similar vaccine, the GAVAC was produced in Cuba and is presently about to be commercialised (Willadsen, 1997b). Efforts in producing anti-tick vaccines against ticks of African origin are yet to yield success (Kocan, 1995).

In order to better address the problem of TTBD in Africa, Meltzer *et al* (1996), suggested the use of an Integrated Tick Management (ITM) approach , like the Integrated Pest Management (IPM) (de-Castro (1997). Tatchell (1992) had suggested approaching the perennial issue of tick-borne diseases (TBD) in consonance with IPM , in which control of the parasites transmitted by ticks will be carried out in a multi-faceted manner involving animal hosts , ticks on and off hosts and ecological modifications. The ultimate goal of such an approach was the attainment of endemic stability in an ecological setting involving the hosts, ticks and the parasite populations. He defined endemic stability as "a stable endemic situation with high prevalence of infection but little or no clinical disease in the target population, caused by a high transmission rate of the parasites between tick vector and vertebrate hosts". He advocated minimal or no tick control measures over a very long period of time , in addition to early exposure of offsprings to tick infestation. Clearly, a programme like this can only succeed in places with less diversity of flora and fauna , like in some temperate regions of the world. It may not appeal to livestock farmers in the tropics where the multiplicity and diversity of disease causing organisms and variety of animal species is overwhelming.

The relevance of the present study towards developing tick-derived anti-tick vaccines to control *Amblyomma variegatum* , and possibly other tick species which may cross react with antigens of this tick species cannot be overemphasized when consideration is given to the enormous losses encurred by livestock farmers worldwide. This is in addition to the fact that a good number of tick species had developed resistance to many chemical acaricides. The integration of more than one method of tick control towards effectively controlling ticks (and tick-borne

diseases) , as in the Integrated Pest Management system (IPM), stands out as the most practicable way of achieving an endemic stability in the livestock industry.

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6.2. CONCLUSION AND RECOMMENDATIONS:

The histopathological reactions observed at the tick-bite sites in the skins of both Yankasa and Uda sheep were sequentially similar . However, the adverse reactions observed in the skins of the Uda , especially between days 5 and 8 where ulcerative, necrotizing / liquefied tissue were formed , and the formation of large, fluid-filled vacuolated spaces by the feeding ticks differed from reactions observed in the Yankasa. These observations may be attributed to differences in biological and/or environmental peculiarities between the two breeds such as thickness of skin or other factors. These were not investigated during the present study.

Failure of ticks to attain CEW at the time of detachment, especially in those fed on vaccinated animals during this study, may be attributed to their inability to remain on the host long enough due to detrimental host reactions. The number of immunologically damaged and dead ticks, and the percentage of viable eggs produced by female ticks , in addition to the percentage of successfully ecdysed nymphs are also indicators of the effects of host reactions to the feeding ticks. Ticks which fed on vaccinated hosts, especially during secondary challenge, were most affected. The low number of immunologically damaged ticks which had fed on rabbits vaccinated with the different units of the semi-purified preparation was, perhaps, due to the low concentrations/immunogenecity of proteins in the inoculums . It is also likely that certain vital , immunogenic proteins could have been lost during processing and separation of the crude extracts. The fact that a number of ticks , especially those fed on vaccinated rabbits (compared with the control) showed some defects , similar to observations made in

experiment II could be evidence that the vaccinations did have some positive effects against the ticks.

In view of the results recorded during these studies, it is recommended that further work be carried out on the pathology of tick bite sites in the skins of livestock species in Nigeria so that the means by which pathogenic tick-borne parasites enter into host animals during tick feeding can be understood. Furthermore, work on the elucidation of potentially useful proteins towards the production of an anti-tick vaccine should be made priority if control of vectors and parasites causing diseases in livestock is the target. The aspect of cross-protection of *A. variegatum* derived antigens against other tick species should be the logical step following this work, and should therefore be the focus of another study.

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APPENDIX A

Correlation coefficient"r" value for the relationships:-

a) Mean engorgement weight X Mean egg mass

b) Mean engorgement weight X Percentage ecdysis

Using the formula

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left[\sum x^2 - \frac{(\sum x)^2}{n}\right]\left[\sum y^2 - \frac{(\sum y)^2}{n}\right]}}$$

a)

Tick Batch	Mean Engmt. Wt.(mg)	Mean Egg mass (mg)	$\sum x^2$	$\sum y^2$	$\sum xy$
1	170	115	28900	13225	19550
2	174	126	30276	15876	21924
3	177	120	31329	14400	21240
4	195	103	38025	10609	20085
5	298	244	88804	59536	72712
6	272	268	73984	71824	72896
7	286	265	81796	70225	75790
8	282	233	79524	54289	65706
9	316	247	99856	61009	78052
10	282	270	79524	48400	62040
11	311	225	96721	50625	69975
12	291	276	84681	76176	80316
13	357	340	127449	115600	121380
14	383	352	146689	123904	134816
15	353	335	124609	112225	118255
16	342	332	116964	110224	113544
$\sum$	4489	3851	1329131	1008147	1148281

$$r = \frac{1148281 - \frac{(4489)(3851)}{16}}{\sqrt{\left|1329131 - \frac{20151121}{16}\right| \left|1008147 - \frac{14830201}{16}\right|}}$$

$$r = \frac{67835}{75240}$$

$$r = \underline{0.90}$$

b)

Tick Batch (Nymphs)	Mean Engmt. Wt.(mg)	Percentage Ecdysis	$\sum x^2$	$\sum y^2$	$\sum xy$
1	84	38	7056	1444	3192
2	91	40	8281	1600	3640
3	115	58	13225	3364	6670
4	121	47	14641	2209	5687
5	136	52	18496	2704	7072
6	111	60	12321	3600	6660
7	112	83	12544	6889	9296
8	103	71	10609	5041	7313
9	171	65	29241	4225	11115
10	168	59	28224	3481	9912
11	172	72	29584	5184	12384
12	168	89	28224	7921	14952
13	187	80	34969	6400	14960
14	176	90	30976	8100	15840
15	174	83	30276	6889	14442
16	195	93	38025	8649	18135
$\sum$	2284	1080	346662	77700	161270

$$r = \frac{161270 - \frac{(2284)(1680)}{16}}{\sqrt{\left[346662 - \frac{5216656}{16}\right]\left[77700 - \frac{1166400}{16}\right]}}$$

$$r = \frac{7100}{9952}$$

475788

$$r = \underline{0.71}$$