STUDIES ON THE BIOLOGY AND CHEMICAL CONTROL OF *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794) TICKS ON THE ACCRA PLAINS OF GHANA

BY

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DEDICATION

To my beloved mother and my late father.

DECLARATION

I hereby declare that the work contained in this thesis is the result of my own original research and that this thesis has neither in whole nor in part been presented for any degree elsewhere.

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Lord take all the glory.

ABSTRACT

In a study of the life cycle of *Amblyomma variegatum* under laboratory conditions of 72%±2 RH and temprature of 28°C±2, the feeding periods of larvae and nymphs on rabbits were 6-9 days and 6-13 days respectively, while that of adults on sheep was 17-19 days. The mean unfed weights of larva, nymph and adult were 0.149 x 10^{-3}g, 0.906 x 10^{-3}g and 28.5 x 10^{-3}g respectively, while their mean fed weights were 2.14 x 10^{-3}g, 48.6 x 10^{-3}g and 2874 x 10^{-3}g respectively. The moulting periods for larvae and nymphs were 14-19 days and 17-19 days respectively. Oviposition took an average of 26 days,with a range of 24-28 days.

Saturated KCL and NaCL solutions with water as control were used to study the weight changes in and the laying pattern of ovipositing adult female *A. variegatum* ticks. Relative humidities of 83%±2, 72%±2 and 95%±2 were provided by saturated KCL and NaCL solutions and water respectively throughout the period of the experiment. Total number of eggs laid were 9,655 for KCL, 10,583 for NaCL and 5,074 for H_2O. Under these conditions, saturated NaCL solution appeared to provide the best favourable humidity

conditions for the ovipositing *Amblyomma variegatum* tick.

In a study to assess the sex of moulting nymphs, 55 fed nymphs of *Amblyomma variegatum* were randomly selected and weighed. Nymphs which weighed above 0.059g resulted in adult females, while nymphs which weighed below 0.031 g resulted in adult males, with 0.031 g - 0.059 g as area of overlap.

In an experiment to look at the efficacy of some acaricides, Supamix, Steladone, Lindane and Amitraz were assayed against fed and unfed larvae, nymphs and adults of *Amblyomma variegatum*. Based on their lethal concentrations (LC50), Supamix and Steladone placed first and second respectively in all the stages except at the unfed nymphal stage where Lindane led with LC50 of 0.001629, while Supamix and Steladone had LC50 of 0.001794 and 0.002258 respectively. Amitraz appeared not to be effective against all the stages since the percentage mortalities were less than 50% at the recommended concentration of 0.025, especially the larval stage where no mortality was recorded, even when the concentration was increased to 0.045 (almost twice the manufacturer's recommended concentration).

It however, had a quick knock down effect on larvae and nymphs with the ticks recovering after 3 hours . Lindane produced only 66% inhibition of oviposition of the laying females at the recommended concentration of 0.025, while Amitraz had no inhibition at the recommended concentration. Supamix and Steladone showed 100% inhibition of oviposition at their recommended concentrations.

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CHAPTER 1

1.0. GENERAL INTRODUCTION

Ticks are important ectoparasites which are a serious threat to both animals and human beings all over the world. Apart from mosquitoes, ticks are the most important in disease transmission (Balashov, 1984), and in tropical Africa, they are considered to surpass all other arthropod vectors (Agbede, 1981). Ticks belong to the order Acarina and superfamily Ixodoidea. Three families are known; the soft ticks (Argasidae), the hard ticks (Ixodidae), which are also referred to as the 'true ticks', and Nutalliellidae of which little is known (Hoogstraal and Aeschlimann, 1982 as cited by Andre et al, (1990).

There are about 150 species of soft ticks belonging to four genera; *Argas, Ornithodoros, Otobius and Antricola* (Service, 1980). These lack a hard dorsal scutum or shield, have a tough and leathery integument and have breathing pores or spiracles usually anterior to the bases of the hind legs. The capitulum or 'false head' is situated ventrally which

makes it not visible dorsally. Soft ticks have a more or less world-wide distribution, but are especially common in dry areas (Service, 1980).

The family Ixodidae, on the other hand, is made up of about 650 species belonging to 11 genera. Those of medical and veterinary importance are *Ixodes, Dermacentor, Amblyomma, Haemaphysalis, Hyalomma, Boophilus and Rhipicephalus*. These are characterised by a hard dorsal scutum or shield which covers almost the entire dorsal surface of the body in the male. In the adult female, nymph and larva, however, the scutum is restricted to the anterior part of the body, just behind the capitulum (Robinson, 1926). Mouthparts are terminal with the spiracles found posterior to the legs. Ixodid ticks have a world-wide distribution, but appear to be more prevalent in the tropics and subtropics.

Clear habitual distinctions exist between these two groups of ticks. Adult soft ticks feed rapidly for short periods, being measured in minutes and hours. Their eggs are laid in small batches of 10 - 100 eggs after each blood-meal. On the other hand, adult ixodid ticks feed slowly and lay single mass of eggs, ranging

from 1,000 to 20,000 (Neitz et al, 1971). The duration of the life cycle of argasids, from egg laying to adult depending on the species of ticks, favourable temperature and humidity and the availability of blood-meals, is often 6 - 12 months. Adult soft ticks are known to live up to 14 years in the laboratory and can survive long periods of starvation (Oyoun et al, 1990). Life cycle of ixodid ticks is similarly affected by temperature, humidity and availability of suitable host. Some may live up to seven years (Service, 1980).

The economic importance of ticks are quite numerous. Several important protozoal, rickettsial, bacterial, viral and nematodal diseases are transmitted to either man or his animals (Neitz, 1956; Hoogstraal, 1966; Agbede 1981; Balashov, 1984)). East Coast Fever (*Theileria parva* infection of cattle), transmitted by *Rhipicephalus appendiculatus* is known as a killer disease because of its high mortality (Uilenberg et al, 1976; Youdeowei and Service, 1983). In Nigeria, cowdriosis is increasingly becoming a common disease in cattle, sheep and goats (Leeflang,1976) . In South Africa, farmers experience losses of 1.3% and 3.8% due to heartwater in cattle and in sheep and goats

respectively (Plessis *et al*, 1994). Babesiosis and anaplasmosis are also frequently diagnosed in ruminants in Nigeria (Leeflang, 1976). Ticks are also known to cause physical damage to hides and skins, thus affecting the leather industries, and also discourage its consumption as food (Leeflang, 1976). An estimated milk loss in cows in Queensland (Australia) due to the presence of ticks is 180 litres per animal annually, also about 4 tons of beef in a herd of 100 cattle is lost to tick infestation annually (Shaw, 1973). *Amblyomma variegatum* in particular have received strong incrimination in the aggravation of dermatophilosis in cattle (Koney et al, 1994). Ten percent of the cattle population in Nigeria may be affected with a 30 to 50% loss of their normal market value (Agbede, 1981).

Acaricides appear to be the chief weapon in tick control. It started with arsenicals from the early years of this century (Youdeowei and Service, 1983). In recent times, many groups of acaricides such as organochlorines, organophosphates, carbamates, synthetic - pyrethroids, cyclic amidins and avermectins are continuously flooding the markets at alarming rates. Awumbila and Bokuma (1994), recently reported that there were 20 different brands of pesticides on

the Ghanaian market, with nine (45%) of them being organophosphates. There are, however, no strict regulation guiding the use of these acaricides.

Certainly, the use of acaricides has created a great number of serious problems. Tick resistance to acaricides have widely been observed (Luguru et al, 1987; Matthewson et al, 1980; Beugnet and Chardonnet, 1995). In Australia, at least 8 acaricide-resistant strains of *Boophilus microplus* have been reported (Shaw, 1973). The escalating costs of commercial acaricides have also been identified as a major constraint (Young et al, 1988). It is a major drain on the foreign exchange reserves of African countries, and annual importation costs have been estimated at US$ 6 - 10 million for Kenya (Chema, 1984), US$10 million for Zambia (Pegram *et al,* 1988) and US$150,000 for Burundi (Niyonzema and Klitz, 1986). Health and environmental hazards such as pollution of water sources also results from uncontrolled use and disposal of acaricides.

There are alternatives to the use of acaricides. Anti-tick vaccines have been developed against *Boophilus microplus* (Willadson et al, 1989) and *Rhipicephalus appendiculatus* (Dipeolu et al, 1990), but

not a single of such vaccines is as yet obtainable on the Ghanaian market. Pasture spelling which is another alternative (Wharton and Harley, 1962), seems not to be practicable by most our farmers and herdsmen who rely solely on free range grazing. Moreover, this controls mainly larvae of *Boophilus spp* which is one - host tick, as the technique demands that the free-living stage is short lived (Youdeowei and Service, 1983). The problems associated with the use of alternatives to acaricides have drastically limited their use, thus making the use of acaricide almost indispensable at least in the foreseable future.

Of late, attempts have been made to integrate the compatible control methods for more acceptable results. These methods includes biological control, vaccination, pasture spelling, resistant breeds of cattle, chemical control and other newer techniques yet to be perfected such as hormone antagonists and pheromone analogues. This approach appears to be promising especially when they are carefully combined to avoid negative interactions (Young et al, 1988). Such a method will however, depend on the establishment of economic thresholds, economic injury level and economic damage by the ticks on the various host species.

Since there is always a demand for the use of acaricides and at times the only method available to the farmer, it then appears prudent to continue to seek new chemicals as well as new systems for delivering the chemicals in effective, efficient, safe and less labour intensive manner (Miller, 1989). The introduction of insecticidal ear tags represents a major innovation in chemical control of livestock pests. It has been shown to be effective in controlling the Gulf Coast ear tick (*Amblyomma maculatum*, Koch)(Gladney, 1976; Ahrens and Cocke, 1978). In a recent study, neck and tail bands fabricated from plastic strips (ethylvinyl acetate) containing 12% amitraz have been shown to be effective in tick control (Miller, 1989). Ivermectin is a member of a potent new class of acaricides, and as subcutaneous injection, protects the animals for about 14 days (Drummond, 1985). Better still, is the intraruminal Ivermectin slow-release device, whose provision of 90 days protection against tick infestation has been demonstrated in Kenya (Tatchell, 1992). Apart from the use of synergists such as safrole, the combination of two acaricides especially from different groups such as organochlorines and organophosphates and rotational strategy based on the

alternation of several compounds, are being incorporated in the new delivery systems. This would prevent or eliminate the problems of resistance, health hazard and environmental unfriendliness and gradually would improve animal production.

However, any of these strategies adopted would be ineffective without adequate knowledge of the biology and the ecology of the ticks in question, as such information would be necessary pre-requisites for designing control strategies for attacking them when they are most vulnerable.

1.1. JUSTIFICATION

Recent survey of pesticides in Ghana have exposed new acaricides which are experiencing high patronage by farmers and herdsmen (Awumbila and Bokuma; 1994), but studies on the efficacy of acaricides (Bafi-Yeboah 1974; Kyei-Nkrumah,1980) were limited to the organophosphate group. The present study is designed to update our knowledge by evaluating the efficacy and potency of commonly used members of each of the following acaricide groups (organochlorine, organophosphate, carbamate and cyclic amidin) on *Amblyomma variegatum* which has proven to be the most

abundant on the Accra Plains (Koney et al, 1994). This study also looks into the life cycle of this tick at different relative humidities with the intention of providing data to explain its incidence and prevalence. It is also aimed at finding out the capability of the different salts in the creation of the required relative humidity for the maintenance of tick colonies.

1.2. OBJECTIVES

This study intends specifically to:

1. to compare the incubation performance of *Amblyomma variegatum* ticks under different humidity conditions created using different Saturated salt solutions.
2. to look at the relationship between nymphal fed weights and sex of emerging adults.
3. compare the efficacy of commonly used acaricides in Ghana.
4. ascertain the relative susceptibilities of the different instars of *Amblyomma variegatum* ticks to some acaricides.

CHAPTER 2

2.0.LITERATURE REVIEW

2.1. Distribution

The genus *Amblyomma* has a remarkable extensive distribution, comprising both the old and the new worlds. Its approximate latitudinal distribution is between 40°N and 40°S (Robinson, 1926). Of the eighty-six (86) definitely established species, about one-half are American, some eighteen species are found in Africa and Asiatic species are about seven. Australia has six while the rest are distributed among the Pacific Islands (Robinson, 1926). In Nigeria, five species have been reported, viz; *A. variegatum, A. pomposum, A. gemma, A. hebraeum and A. nuttalli* (Agbede, 1981). In Ghana, however, only two species, *A. variegatum* and *A. nuttalli* are known. *A. variegatum* is frequently encountered while *A. nuttali* was only seen on two occasions (Agyen-Frempong, 1967). In a recent survey of ticks on the Accra Plains of Ghana, *A. variegatum* and *Rhipicephalus senegalensis,* were found in high percentages (Koney et al, 1994). Other clearly identified genera in Ghana include *Aponomma, Argas, Boophilus, Haemaphysalis, Hyalomma* and *Ixodes* (Agyen-

Frempong, 1967).

A. variegatum (Fabricius, 1794) is also found in Senegal, Congo, Angola, Ivory Coast, Sierra Leone, Niger, Uganda, Kenya, Gambia and Guinea. This African tick was introduced to Guadeloupe (West Indies) about 150 years ago and has become established. Inspite of its slow sexual maturity but high individual fecundity, the species has a relatively limited potential to spread(Barre, 1989). Barre(1989) however, observed that *A.variegatum* has established itself widely in South Africa.

2.2 Host range

Although *Amblyomma variegatum* is essentially a tick of herbivores, it has been recovered from a broad spectrum of animals viz; horse, donkey, dog, cat, hartebeeste, camel, zebra, elephant, buffalo, water-buck, eland, congoni, rhinoceros, wart-hog, Jackson's hartebeeste, sable, antelope and bush-buck (Robinson, 1926). In Guadeloupe, birds particularly the cattle egrets are infested by the immatured stage (Barre 1989).

In Ghana, like in other places, *A. variegatum* is

predominantly found on cattle sheep and goats. Nonetheless, a single specimen was collected from turkey. *A. nuttalli*, on the other hand was collected from a tortoise and a snake (Agyen-Frempong, 1967).

2.3 Prevalence and seasonal incidence

A survey of cattle ticks from the Waterberg District of the Transvaal showed *Rhipicephalus appendiculatus* to constitute 59.4% of all the ticks recovered. *Rhipicephalus evertsi* accounted for 21.5%, *Amblyomma hebraeum* was 13.8%, while *Hyalomma* spp accounted for 4.6%. Seventeen *Ixodes cavipalpus* and one *Boophilus decoloratus* were also found out of the total 3011 ticks collected in a 14 month survey (Schroder 1980). In a broad sense, the seasonal fluctuation in tick numbers in this survey corresponded to those found in Natal (Baker and Ducasse, 1967) and Rhodesia (Jooste, 1966).

Koney et al (1994) found five species of ixodid ticks on cattle during a 26 month study on the Accra Plains of Ghana. They included *Rhipicephalus senegalensis*, *Boophilus annulatus*, *Boophilus decoloratus*, *Hyalomma marginatum rufipes*, with

Amblyomma variegatum being the most abundant species (55.6%). Adults of this dominant species peaked in the early part of the rainy season. Mohammed (1977) and Bayer and Maina (1984) found a similar tick fauna on cattle in Nigeria.

2.4 Morphological characteristics of *A. variegatum* (Fabricius,1794)

Considerable taxonomic difficulties still exist in separating closely allied species of *Amblyomma*. The distinguishing factor among *Amblyomma spp* is the shape and colour pattern of the scutum. The genus *Amblyomma* is specifically attractive on account of the beautiful ornamentation which many of its members exhibit. The ornamentation, being directly related to the underlying musculature, presents well-defined and constant features which are helpful in identification.

Amblyomma variegatum male is medium size, scutum ornate, black markings on pale coppery-red background; entirely black festoons with coarse punctations which are situated peripherally. Eyes are small, dark coloured, hemispherical and orbited. The body is 4-5mm in length and the width 3.7-4.5 mm. The capitulum is

rectangular, posterior margin slightly concave, lateral margin convex; palps long and fairly stout. Legs are stout, reddish-brown, articles with broad, pale annulations at their distal extremities (Arthur 1962).

The female is moderately a large tick, scutum triangular, and its posterior margin broadly rounded. The median field is more or less extensively pale coloured. The body is 5 mm by 4-5 mm (engorged ones may attain dimensions of 25 x 18 mm). Other features are similar to that of the males (Robinson 1926).

2.5 Reproduction, development and life history

It has been suggested that, mating of ticks on the host is essential for female ticks to complete full engorgement (FAO, 1984). Successful mating of females is, however, preceded by some period of feeding (Balashov, 1984). After continuous feeding of females for some days on the host, they emit one or more pheromones which attract the males (FAO, 1984). Li and Jiang (1992) have shown sex pheromones released via the foveae of the female *Hyalomma asiaticum* to facilitate copulation through the crawling of the male towards the female after perceiving the pheromone odour.

In a report of Animal Research Institute (ARI)

(1975) 95% RH and 30°C were suggested to be the optimum conditions for rearing *Amblyomma variegatum*. This had earlier been shown by Neitz et al, (1971, 1972) in other species of ticks. Ntiamoa-Baidu (1987) demonstrated the interacting influence of temperature and humidity on development and survival rates of *Rhipicephalus simpsoni*. The preoviposition period at 25°C and 95%RH was 5-8 days. Humidity had practically no influence on survival and development of engorged nymphs (Ntiamoa-Baidu, 1987). This finding is similar to that of Stampa and du Toit (1958) who established that engorged nymphs of *Ixodes rubicundus* survived almost everywhere and were less affected by adverse environmental conditions than were unfed nymphs and either the unfed or fully engorged larvae.

Oviposition in *A. variegatum* does not take place until the attainment of engorgement weight of 0.4gm, and the degree of daily weight loss was shown to be directly proportional to the intensity of egg output (Dipeolu and Ogunji, 1980). Recent study (Mattioli and Cassama, 1995) has revealed that the percentage of *A. variegatum* eggs hatched were lower in ticks collected on N'Dama than on Zebu cattle. Egg production (1500-

2000) in *Rhipicephalus theileri* (Neitz et al, 1972) 0appears to be quite low as compared to the more recent report of 2220 - 9874 by Ntiamoa-Baidu (1987).

Interestingly, Neitz et al (1971) observed larval hatchings to be good even when eggs were kept at relative humidity of 50-60% which was well below the critical hatchability level of 70% given by De Vries and Davey as cited by Stampa and du Toit, (1958).

High mortality rates of larvae and nymphs of most ticks during the prefeeding, feeding and premoulting stages have been reported (Neitz et al, 1972). Of late, however, Kiara et al (1994) have suggested ways of averting these mortalities through techniques called confined and separate methods. With *Amblyomma variegatum*, the technique simply enhances its attachment and also shortens the feeding duration.

2.6 Economic losses

The major losses caused by ticks is due to their ability to transmit protozoal, rickettsial, bacterial,viral and nematodal diseases to livestock which are of great economic importance world wide (Jongejan and Uilenberg, 1994; Balashov, 1984).

The organisms transmitted by ticks are quite numerous and may cause several diseases which may reduce performance or may be fatal to the animals.

2.6.1 Dermatophilosis

Dermatophilosis also known as streptothricosis causes severe economic loss through reduced rates of live weight gain, damage to hides and death. Circumstantial evidence has implicated ticks, particularly *A. variegatum* in the pathogenesis of bovine dermatophilosis in the tropics, although the lesions do not always coincide with tick attachment sites. Koney et al (1994) showed that the acaricide treated group of animals in an experimental herd remained completely free of dermatophilosis once the lesions that were present on them at the start of the study had cleared. Oppong (1976) had earlier reported the isolation of the organism from the mouthparts of ticks removed from dermatophilosis-infected skin of cattle. This association between infestation with *A. variegatum* and the occurrence of clinical dermatophilosis on cattle is based on their similar pattern of seasonal occurrence (Plowright, 1956),

geographical distribution (Burridge et al, 1984, Morrow and Compton, 1991) and the observed effect of tick control in reducing the prevalence of the disease (Plowright, 1956; Koney et al, 1994)).

Dermatophilosis is known to have frustrated many attempts at using exotic breeds of cattle to improve bovine meat and milk production in the humid and sub-humid tropics (Coleman, 1967). Steward (1930; 1942) observed in Ghana that the disease was particularly severe in "grade" stock, serious in zebu cattle, but rarely fatal to West African Shorthorns. In a systematic survey of skin conditions of 5,375 cattle on the Accra Plains of Ghana, the prevalence of dermatophilosis during the dry season was 4.8%, increasing to 12.85% in the rainy season (Oppong, 1973). Based on management practices, Koney and Morrow (1990) recorded 3.3% incidence on farms with good tick control and 24.7% on farms with traditional husbandry and management.

In Nigeria, where up to 10% of the cattle population may be affected, it is estimated that about 2% of diseased local animals die annually, while a further 4% are culled because of dermatophilosis with up to 30 to 50% loss of their normal market value

(Agbede, 1981). Moreover, losses due to the disease in Nigeria have been estimated at over 10 million US dollars per annum (Agbede, 1981). Its economic losses to the livestock industry in Ghana have not been fully investigated and are difficult to ascertain. However, blemishes created on the skin of cattle are unsightly and discourage its consumption as food (Wele). Moreover, damage to hides affects the production of high quality or durable leather goods.

2.6.2. Heartwater

Heartwater, an often fatal rickettsial disease of domestic ruminants transmitted by *A. variegatum*, ranks with dermatophilosis as a major constraint to the introduction of exotic breed and therefore the upgrading of livestock productivity in Ghana. Aning (1978) analysed post-mortem reports from regional veterinary laboratories in Ghana and showed that the disease incidence in sheep and goats was highest in the forest zone (26% of all diseases at Kumasi between 1976 and 1978), followed by the Accra Plains (8%) and Pong-Tamale (6%). He also observed that about 83% of cases of heartwater diagnosed in the Accra, Ho, Kumasi and Sunyani laboratories involved animals which had been

moved to new localities. In South Africa, it has been indicated that farmers experience losses of 1.3% and 3.8% due to heartwater in cattle and in sheep and goats respectively (Plessis et al, 1994).

It has been observed that breeds of animals such as Creole goats in Guadeloupe, and the West African Dwarf goats and sheep appear to have developed resistance to heartwater. Presumably, through generations of natural genetic selection, as they can survive in areas where high tick infestations occur.

2.6.3 Anaplasmosis and Babesiosis

The prevalance of *Anaplasma maginale, Babesia bigemina* and *Babesia argentina* was observed to be 62.93%, 23.77% and 12.44% respectively in the tropical and sub-tropical climatic regions of Colombia. Severe losses due to acute anaplasmosis and babesiosis occur when susceptible cattle are imported and when indigenous cattle are moved downward from the mountainous areas (Corrier et al, 1976). Outbreaks of tick fever in Queensland between 1966 and 1976 showed that over 70% of all the outbreaks occurred in the *Boophilus microplus* enzootic area of Queensland south

of the Tropic of Capricorn (Copeman *et al*, 1976).

Epidemiological studies on *Babesia equi* and *Babesia caballi* infection undertaken by Barbosa *et al* (1995) in Brazil revealed the prevalence of *B. equi* antibodies in horses to range from 90.6% to 100% and that of *B. caballi* antibodies from 59.4 to 65.5%. Mohammed (1976) in Northern Nigeria, discovered the prevalence of *B.argentina* to be markedly higher, while that of *Babesia bigemina* to be lower in the northern Guinea vegetation zone than in the Sudan vegetation zone. Recently, Plessis et al (1994) reported that South African losses due to redwater and anaplasmosis in cattle were 0.3 and 0.2% respectively.

Babesiosis is also known to affect man and Loutan (1995) reported more than 400 cases in the USA and 21 cases in Europe.

2.6.4. East Coast Fever

Theileria parva, the causative agent of East Coast Fever (ECF) affects cattle in East and Central Africa. Previously, the disease was present in South Africa and Zimbabwe, but was eradicated from these countries following the implementation of vigorous

control and slaughter measures (FAO, 1984). The geographical extent of ECF is determined by the distribution of the tick *Rhipicephalus appendiculatus*, the only vector capable of transmitting and maintaining infection under natural conditions. *Theileria parva* infection (East Coast Fever) deserves intensive tick control because highly productive and valuable exotic cattle are exposed, even local East African Zebu cattle once matured are fully susceptible and will die from *T. parva* administered by a single tick. Furthermore, calves have an unacceptable high mortality rate from this disease. Infact, no other tick-borne disease kills virtually 100 percent of susceptible stock (Youdeowei and Service, 1983).

2.6.5. Viral Diseases

Argas persicus is one of the arthropods which transmit hepatitis B-virus among between children in West Africa. In Gambia for instance, 7% of children whose beds were infested with ticks had hepatitis B-virus (Vall-Mayans *et al*, 1990). The arbovirus causing yellow fever have recently been isolated from naturally infected *Amblyomma variegatum* (Vall-Mayans *et al*,

1990). He has further shown this tick to experimentally transmit the virus to their progeny, which can then infect monkeys, indicating that the ticks may serve as an alternative reservoir host.

2.6.6. Growth rate and milk loss

It has been estimated by Shaw (1973) that in Australia, an average daily infestation of five or more engorging adult *Boophilus microplus* ticks cause a loss in growth rate of 0.75kg of bodyweight per tick (nearly four tons of beef in a herd of 100 animals). Shaw (1973) has also estimated milk loss in Queensland (Australia) from the presence of ticks to be 180 litres per animal or 5,300 tons of butter annually. Nevertheless, Meltzer et al (1995) believed that there was no significant difference in the weight of calves regularly treated with acaricide and the non-treated ones. Moreover, the total measured amount of milk suckled by untreated calves from their untreated dams was significantly more than treated calves.

2.7. Tick control

Tick control is predominantly carried out with the

use of acaricides. Other supportive measures include the use of resistant breed of cattle and pasture spelling. Of late, however, vaccination and biological control are being considered.

2.7.1 Vaccination

This is a promising area of tick management, except for a few setbacks which it now encounters. The Bm 86 antigen, a membrane-bound protein from the tick gut has been shown to confer immunity against *Boophilus microplus* in cattle. Field trials in Queensland indicated that the principal effect of the vaccine is the progressive control of tick numbers through a reduction in a reproductive capacity (Willadsen et al, 1991). McGowan (1981) and Thakur et al (1992) demonstrated a significant reduced feeding rate and poor reproductive performance of *Hyalomma anatolicum anatolicum* on immunized rabbits and *Amblyomma americanum* on immunized calves.

Ackerman et al (1980), nevertheless, showed that immunity was not produced when the host was immunized with the extract of whole ticks but rather extract from the mid-gut. Perhaps a more serious constraint is the

significant degree of dermal reaction observed in cattle and rabbits inoculated with whole tissue extracts of engorged females of *Boophilus microplus*. This was characterised by inflammation, pain and redness of the inoculated site (Lata et al, 1986). Not until some of these problems are resolved, full commercialisation of tick vaccine would still not be realised.

2.7.2. Biological control

A lot is yet to be exploited, considering its adoptability. Among those tried is the experimental infection of unfed *Amblyomma variegatum* nymphs with hymenopteran parasitoid, *Ixodiphagus hookeri*. This parasitoid was originally obtained from ticks collected from cattle in the Trans-Mara area of Kenya where it naturally infects *A. variegatum* nymphs. At the moment the problem is the possibility of mass rearing for use as a biocontrol agent (Mwangi et al, 1994). Bittencourt et al (1994) assessed two isolates of *Metarhizium anisophae* (Metschnikoff, 1879) in the laboratory as potential biological agents of *Boophilus microplus*. All ticks (group of 10 engorged females)

were infected at all the conidial concentrations used. Its field application is under consideration.

2.7.3. Resistant breed of cattle

Research in Gambia suggests that the N'dama cattle is more resistant to major ticks that affects livestock in the area than the Gobra zebu cattle. Mattioli et al (1995) saw significantly higher numbers of *A. variegatum* and *Hyalomma spp.* on Gobra zebu than on N'Dama cattle. In addition, liveweight gain was significantly higher in N'Dama cattle compared with Gobra cattle during the period of abundance of these major ticks. Nevertheless, exposed *Bos indicus* (zebu breeds) rapidly reach their individual level of resistance, with the frequency distribution of resistant individuals skewed in the opposite way, so that vast majority are resistant (Youdeowei and Service, 1983). In Northern Brazil, the value of Zebu and Zebu cross cattle in aiding tick control has been well appreciated for many years. In Australia, the relative tick resistance of individuals in a herd of Australian Illawarra Shorthorn (AIS) cows have been identified (Wharton and Utech, 1961). These cows were

mated with a single A.I.S. bull with a similar character. The tick counts from the resulting calves showed an encouraging degree of correlation with the parent counts.

2.7.4. Pasture spelling, modification of habitat and others

Pasture spelling has been practised and some degree of success has been achieved. Tick burden of Shorthorn and Hereford cattle was found to reduce considerably by spelling pasture on a 3,4 and 5-month regimes (Wharton and Harley, 1962). *Boophilus microplus* larvae in particular die rapidly in the summmer and removing cattle from grazing paddocks for 8-10 weeks greatly reduces tick numbers (Youdeowei and Service, 1983). It has, however, been observed that the method demands that the free-living stage is short-lived and that uncontrolled wild hosts are not present which could serve as reservoir hosts.

Modification of habitat has been used successfully to alter the microclimate needed for the development of free-living stages. Drainage and pasture improvement combined with heavy grazing were used to cause

disappearance of *Ixodes ricinus* in Europe. In North America, *Amblyomma americanum* has been well controlled by clearing bushes and trees which provide shelter for ovipositing females and developing larvae (Youdeowei and Service, 1983). Unfortunately, the costs of these operations may be high and uneconomical. Bobrovskikh and Schulman (1991) have also suggested that re-establishment of ticks is likely to occur in 5-6 years.

In two field experiments conducted in Zimbabwe, chickens were found to be effective predators of *Amblyomma hebraeum, Rhipicephalus evertsi, Hyalomma spp* and *Boophilus decoloratus.* No apparent preference was shown for any particular tick species (Kohn and Norval, 1994) and this is suggested to offer an economic means of tick control that could be exploited by communal land farmers.

Interestingly, pot trials revealed that two legumes *Stylosanthes scabra* and *Stylosanthes viscosa* produce sticky secretions which effectively immobilized and poisoned larvae of *Boophilus microplus.* This potential could be exploited in the tropical and subtropical areas in which *Stylosanthes* can be grown as pasture (Sutherst et al, 1982).

2.7.5. Chemical control

Acaricides have been the major method of tick control in Africa since their introduction into South Africa around 1890 (Dipeolu and Ndungu, 1991). This is perhaps the case in other parts of the world. Khan and Strivastava (1992) found cypermethrin to be more effective than permethrin and fenvalerate at both in vitro and in vivo applications. In their earlier work, Khan and Strivastava (1988) compared the residual effects of both cypermethrin and permethrin with Dursban (chlorpyrifos). The residual effect of the pyrethroids was 8 - 15 days while that of chlorpyrifos was 3 - 5 days based on artificial infestation of *B. microplus* on cattle. According to Henderson and Stevens (1987), cypermethrin gave 92% control of ticks on ewes when tried on 11 farms at the rate of 5 ml/kg body weight. Flumethrin (1%) and cyphalothrin (2%) on their part, protected against reinfestation for 21-45 days (Henniger, 1988) while in India, deltamethrin gave reinfestation period of 8-10 days and 100% effective against *Hyalomma anatolicum anatolicum* (Patil et al, 1992). Cantoray and Dik (1988) established 99.5% and

100% effectiveness of flumethrin against *Hyalomma anatolicum anatolicum* and *Boophilus microplus* respectively at the dose of 1mg/kg bodyweight. Bittencourt et al (1989) established that the unfed stages of ticks were more susceptible than the respective engorged stages. Similar result was observed by Khurana et al (1992).

Field assessment of two geographically and climatically different ranches in Zimbabwe have also indicated treatment with flumethrin to result in significant reductions of ticks at both sites over 4 months due to its prolonged residual action. This was opposed to weekly dipping with chlorfenvinphos and dioxathion which suggested short-lived protection against re-infestation (Hamell and Duncan, 1986). This probably made Mitchell et al (1986) to suggest that the use of pyrethroids as prophylactic treatment was a useful alternative to dipping for tick control.

Barnard et al (1981) assayed the biological activity of 14 acaricides against nymphs of *Amblyomma americanum*. Amitraz, permethrin, chlorpyrifos and Lindane in that order were most toxic based on LD90 values. The remaining compounds ranked in descending

order of efficacy were: carbaryl, stirofos, diazinon, coumaphos, dioxathion, phosmet, toxaphene, methoxychlor, ronnel and malathion. Barnard and Jones (1981) later carried out field efficacy trials of 7 of these acaricides based on their commercial availability in South-Eastern Oklahoma. Each gave a significant (P=0.05) control of *Amblyomma americanum* (L) at 24h post-treatment. Field efficacy of Amitraz (0.025%) (Jacquiet et al, 1994) which was found to be 95% effective against adult *Hyalomma dromedarii* appears to confirm the assay of Barnard et al (1981) and Maske et al (1994). Nonetheless, it had no effect on the rate of egg hatching and survival and oviposition of females that attached in the first few days after treatment. Maske et al (1994) found engorged female *Boophilus* treated with Amitraz at 0.05%, 0.03% and 0.01% not to lay eggs. Ahrens et al (1989) also showed that laboratory bioassay results of Amitraz compared favourably with those obtained with the dip vat treatment.

In Ghana, the superiority of chlorfenvinphos (Supona) over Asuntol (coumaphos) and Bacdip was demonstrated using *Amblyomma* spp and *Rhipicephalus* spp

(Bafi-Yeboah, 1974). Similar results have also been recorded (Mohammed, 1973 as cited by Bafi-Yeboah, 1974; Rawlins and Mansingh, 1981 and Khan and Strivastava, 1987). Also in Ghana, laboratory studies of Asuntol (coumaphos), bromo-phos-ethyl, fenitrothion (Sumithion) and pirimiphos-methyl on adult *Amblyomma variegatum* showed fenitrothion to be most effective and Asuntol the least effective (Kyei-Nkrumah, 1980).

Many natural products such as plant extracts have been used in assay trials. "Kupetaba" which is a ground mixture of dried tobacco leaves (Family Solanaceae) and a mineral called "Magadisoda" has proved to be effective as an acaricide against all stages of *Rhipicephalus appendiculatus* and drastically reduced the hatchability of the eggs (Dipeolu and Ndungu, 1991). Research conducted in Minas Gerasis, Brazil on the essential oil of molasses grass (*Melinis minutriflora*) revealed its lethal effect on *Boophilus microplus* larvae within 10 minutes of exposure (Prates et al, 1993). Recently, *Stemona collinsae* extract was found to be effective against all stages of *Boophilus microplus* (Jansawan et al, 1993). Their field application would have to be fully investigated,

considering the influence that weather may have on their chemical constituents.

2.7.6. The chemistry of pesticides

Pesticides are grouped according to their chemical composition and perhaps their mode of action. Based on their chemical composition, the groups include organophosphates, organochlorines, carbamates, synthetic pyrethroids and cyclic amidins (Hamel and Duncan, 1986).

2.7.6.1. Organophosphates

During the second world war, German scientists under the direction of Gerhard Schrader were engaged on the task of developing highly toxic nerve gases for potential use in warfare. As a spin off, several organophosphorus compounds structurally related to the nerve gases, tabun and sarin were found to be effective insecticides (Kenneth, 1982). An important feature of the group is that different members possess very different physicochemical properties, in particular, they have different vapour pressures at room temperature and different solubilities in water. They

also vary considerably in their chemical stability and their toxicity to mammals (Eto, 1974).

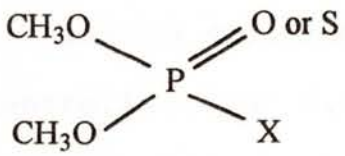


Fig.I General structure of organophosphorus insecticides.

The two R groups are usually methyl or ethyl and are the same in any one molecule, while x is frequently a rather complex aliphatic, homocyclic or heterocyclic group (Hollingworth, 1976).

Members of this group include chlorfenvinphos, dichlorvos, malathion, dioxathion and coumaphos. They are compounds of low chemical stability, soluble in water but more or less rapidly hydrolysed by it. They are used as low persistent contact insecticides (Kenneth, 1982).

2.7.6.1.1. Chlorfenvinphos (Estelladon 30^{R}, Supona^{R}, Birlane^{R}, Steladone^{R})

It is used againstseveral genera including *Amblyomma, Boophilus, Haemaphysalis* and *Hyalomma*, and as insecticide against horn flies, lice and mites. It is marketed as a 20% and 30% emulsion and is applied to the dip in concentrations of 0.01 - 0.1% active substance without producing any side effect; concentrations above 0.2% cause intoxication (Seifert, 1996).

2.7.6.1.2 Mode of action of organophosphates

The primary site of action of organophosphorus compound is the enzyme (acetyl) cholinesterase which is present in the nervous system (Kenneth, 1982). In the transmission of nerve impulses, acetylcholine a chemical transmitter, carries the signal across the synapse and must be destroyed immediately it has done its job or else the consequence would be the same as if the second nerve cell (or the effector cell) were to receive a continuous impulse (Romano and Greco, 1982).

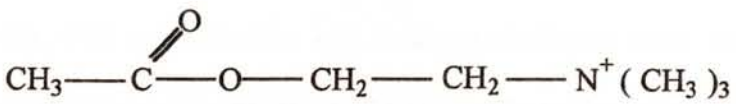


Fig.II Structure of acetylcholine

Acetylcholine binds to cholinesterase at two attachment sites. One of these, the esteric forming site, probably contains a serine residue in the protein chain, this unites with the carbonyl carbon of acetylcholine. The other, the negative or anionic site, may contain a glutamic acid residue. This stabilizes the positively charged choline nitrogen (N+) (Meyer, 1990).

The carbon atom of the carbonyl group of the substrate carries a slight positive charge and makes an electrophilic attack on the hydroxyl group of the serine. This results in acetylation of the enzyme and in the splitting and deactivation of the acetylcholine, the free choline readily leaves the enzyme surface (Holler, 1986).

The esteritic bond (EB) is weak and is rapidly hydrolysed during the recovery stage of the enzyme, the hydrolysis probably being facilitated by a basic histidine residue nearby (Eto, 1974). The surface of the enzyme is then free to accept another molecule of

acetylcholine. It has been estimated that about 300,000 molecules of acetylcholine are destroyed by one molecule of acetylcholinesterase per minute at 37°C (Kenneth 1982).

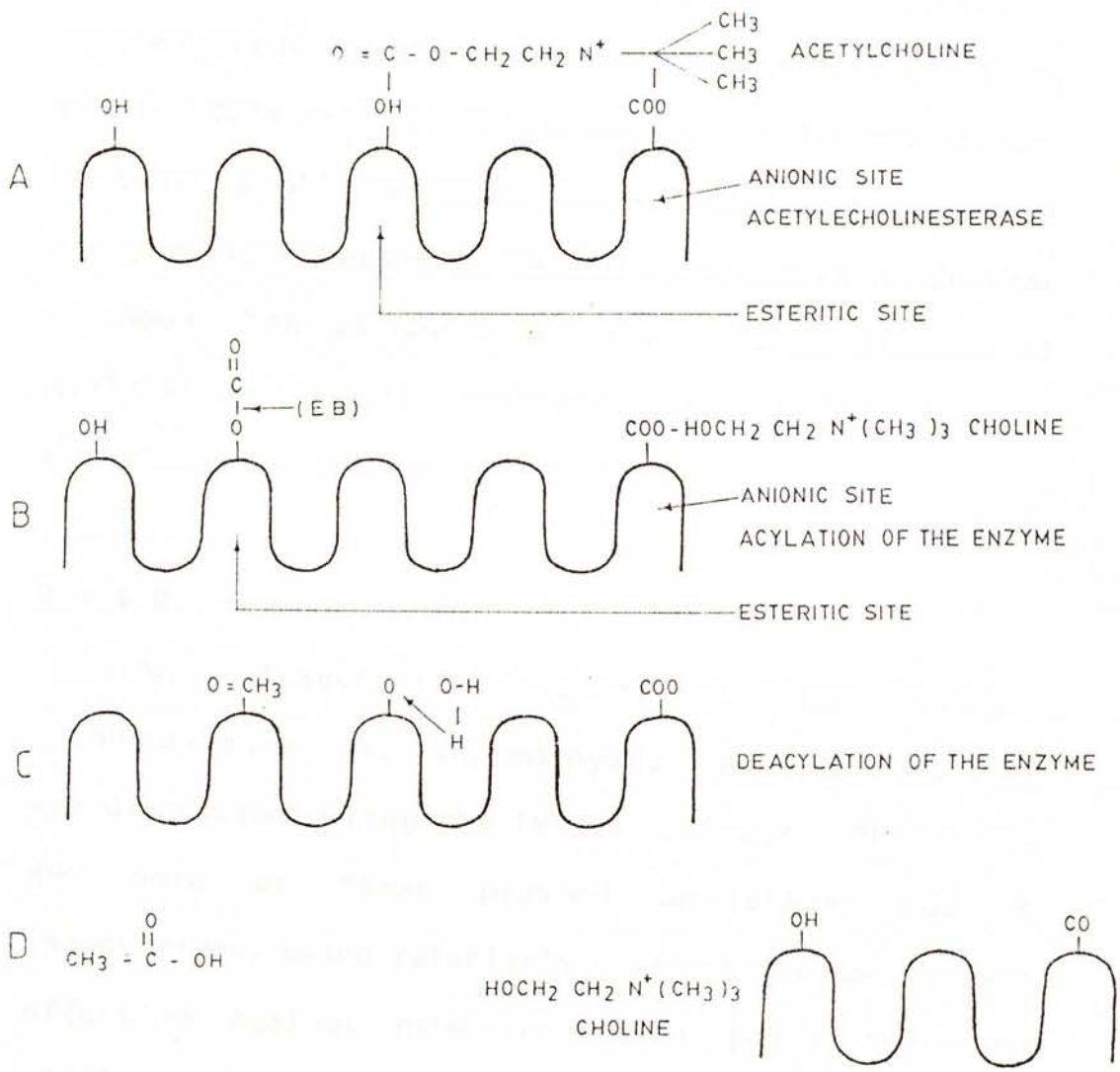


FIG. MODE OF ACTION OF ORGANOPHOSPHATES

Since organophosphorus compounds have structural similarity to the natural substrate, acetylcholine and because it has stronger affinity to pull electron more than acetylcholine, it then binds to the enzyme, thus acetylcholine is left free in the system. The enzyme is then phosphorylated instead of been acetylated (Eto, 1974). This bond is relatively stable to hydrolysis. It takes a million times longer for the molecule of acetylcholinesterase to become operational again than it does when it undergoes the natural process of acetylation which enhances the build up of acetylcholine (Ahrens et al, 1989).

2.7.6.2. Organochlorine

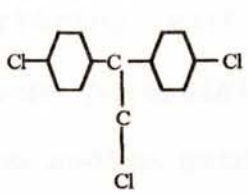
The insecticidal potential of certain organochlorine (chlorinated hydrocarbon) insecticides was discovered during the 1939 - 1945 war. DDT and r-BHC were at first praised as almost perfect insecticides, being relatively cheap to manufacture and effective against numerous insect pests (Kenneth, 1982). DDT is believed to have saved so many lives, because for a time, it had outstanding success in controlling the vectors of parasites responsible for such mortal and debilitating diseases as malaria, river

blindness and yellow fever (Hollingworth, 1976).

Unfortunately, organochlorines have received serious criticisms, as some members are notoriously persistent in the environment and so can be a real hazard to wildlife, and many believe a potential hazard to man. Carson (1963) specifically pointed out that their use has led to the indiscriminate killing of beneficial as well as harmful insects.

Structure

The chemical is divided into three families namely; DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane); BHC (1,2,3,4,5,6-hexachlorcyclohexane) and chlorinated cyclodiene . BHC family has become the most important of late.



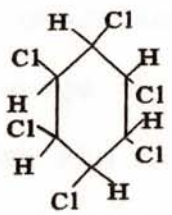


Fig.III DDT BHC

r-BHC (1,2,3,4,5,6 - hexachlorcyclohexane)

It was developed in 1942 and has been used since 1949, especially as r-isomer Lindane for mosquito and tsetse control. It has also been used as an acaricide for cattle dip (0.025-0.05%), as a wettable powder and as an emulsion. Lindane is absorbed through the skin as well as by the internal mucosa of the organism (Seifert, 1996). It is highly soluble in water and quickly catabolized by vertebrates. Because of its high lipid solubility, Lindane is deposited in fat, but less so in the liver, kidney and brain, and is excreted above all in milk.

2.7.6.2.1. Mode of action of organochlorines

DDT and other organochlorines are thought to interfere with ion channels. This they do by interfering with the steady state condition and membrane potential (Kenneth, 1982). A wedge is formed in the sodium gate, retarding its closure and allowing a high influx of sodium ions. When this open state created is transient, there would be repetitive firing, and when it is maintained, the membrane will be fully depolarised. The resultant symptoms include

hyperexcitibility, tremor, convulsion, paralysis and death (Kenneth, 1982).

2.7.6.3. Carbamates

Carbamates with insecticidal properties possess the general structure:

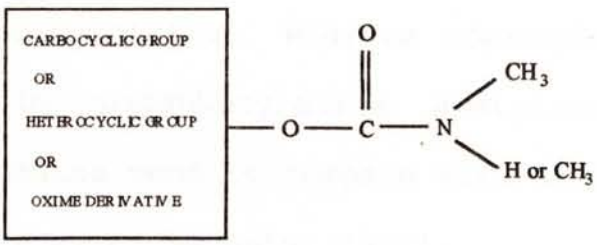


Fig.IV General structure of carbamates

Carbaryl is the most widely used carbamate insecticide. It has a moderate residual action.

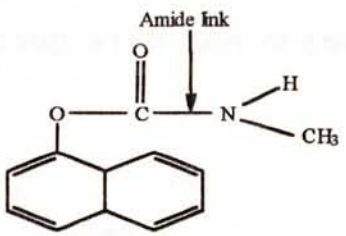


Fig.V carbaryl

Carbaryl (1-naphthyl-N-methylcarbamate) is sold as a

wettable powder and used in dips. Inspite of its low dermal absorption, residues appear in the milk after spray application of 0.05% solution to cattle (Seifert, 1996).

2.7.6.3.1. Mode of Action

Carbamates act in a similar way to organophosphates. Whereas organophosphates appear to act by phosphorylating acetylcholinesterase, the carbamates seem to compete with acetylcholine for its active site (Kenneth, 1982).

2.7.6.4. Pyrethroids

Pyrethroids are etheric oils and they are synthetic esters from chrysanthemum acid or its derivatives. The chemical structure is similar to that of natural pyrethrins which are substances contained in chrysanthemum spp. (Seifert, 1996). Pyrethroids act in the same way as DDT by interfering with ion channels.

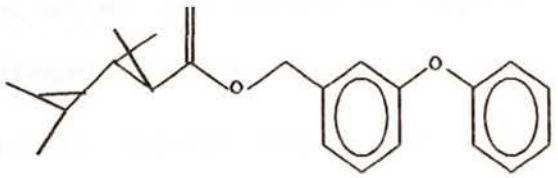


Fig.VI Pyrethrin

Their low toxity to higher animals, limited persistence and quick knock down effect and ease of application makes them preferable (Barlow and Hadaway, 1975). The effect of pyrethroids on nervous system of the arthropods depends on the dose and is irreversible. Therefore, once paralysed, arthropod do not recover.Even with a low dose,a tick's oviposition is inhibited (Stendel and Hamel,1990).

2.7.6.4.1. Flumethrin (alpha-cyano-(fluoro-3-phenoxy)-benzyl-3-2-chloro-2-4-chlorophenyl)-ethyl-2,2-dimethyl-cyclopropane-carboxylate),Bayticol)

As a pour-on application,(1 mg/kg b.w.),ticks and tsetse flies are controlled for almost four weeks (Meyer,1990). Due to its broad therapeutic range, the spot-on formulation may be used even by people with little experience in animal health practice

(Duncan,1991). It spreads over the entire body of a treated cattle after 2-4 hours of application (Hamel and Van Amelsfoort, 1986). Infestation is reduced drastically after 24-48 hours of application and oviposition of ticks is also inhibited (Hamel and Duncan, 1986). With a 2.5 times the normal strength, no residues were found in the milk (Hamel and Duncan, 1986; Stendel and Hamel,1990).

2.7.6.5. Cyclic amidins (Formamidins)

Cyclic amidins belong to a heterogeneous group of compounds. These compounds are also known as detaching agents. Although their mode of action appears not to be clearly defined, it is believed that the group interfere with the metabolism of the ticks, reduce the glycogen and glucose level and block the development of ova. They also interfere with the respiratory enzyme system of the arthropod by blocking the NADH-fumarate-reductase system and also cause a neuromuscular blockage (Seifert, 1996).

The most widely used member of the group, amitraz (n-methylbis(2,4 xylyliminomethyl)-amine, Taktic^{R}, Triatix^{R}) is used for the control of one, two and three-host ticks as well as midges and lice as a 0.025%

dip or spray. It has a good residual effect and a fast action. Ninety percent of tick drop within 8 hours of application (Ahrens, et al, 1989). Being unstable in the dip over a longer period of time, the application as a spray is preferable. The dip solution however, can be stabilized by adding lime adjusting to a pH of 12 thus making the compound very efficient. It is believed in Australia that there are fewer farms with resistance to amitraz than to pyrethroids (Seifert, 1996).

2.7.6.6 Avermectins

The avermectins are a family of closely related macrocyclic lactones produced by an actinomycete, *Streptomyces avermitilis*, which was isolated at the Kitasato Institute in Japan 1975, from a soil sample (Seifert, 1996). They are divided into the components A1, A2, B1 and B2 because of their structural differences (Drummond, 1985). The compound is active against different genera of ticks, intestinal parasites, lungworms, warble flies, lice and midges (Ahrens and Cocke, 1978).

2.7.6.6.1. Mode of action

Avermectins seem to stimulate the pre-synaptic release of Gama Amino Butyric Acid (GABA) and enhance its binding to the post-synaptic receptors. The function of the inhibitory neurotransmitter, GABA, is to open the chloride channels on the post-synaptic side, allowing Cl- ions to flow in and to induce the "vesting" condition. In the presence of avermectins, the chloride channels are kept opened when they should be shut. Cl- ions flow in even when Na+ ions alone should be entering. The motor neuron remains negatively charged and so both inhibitory and excitatory signals are not registered by the recipient muscle cell. Thus the muscle cell does not function and results in paralysis.

2.7.6.6.2 Ivermectin (Ivomec^{R})

It is a derivative of avermectin B1, contains 80% dihydroavermectin B1a and a maximum of 20% dihydroavermectin B1b (Seifert, 1996). The residual effect of a subcutaneous injection of ivermectin lasts for about 14 days, while the intraruminal ivermectin slow-release device provides a 90 day protection

against tick infestation, as demonstrated in Kenya (Tatchell, 1992). A withdrawal time of 42 days for beef and 28 days prior to milking is indicated. The compound is deposited for a short time in the liver, fat and only in small amounts in muscle and kidney.

2.8.0 Acaricide resistance

This is a developed ability of ticks to tolerate doses of the acaricide which will prove lethal to the majority of individuals in a population of the same species (Hollingworth, 1976). It could result from genetic changes which occur over several generations. Explanation for such changes are decreased sensitivity of target sites to acaricides and improved capacity to metabolically detoxify insecticides (Kenneth, 1982). It was postulated that resistance to organochlorines is due to some physiological or structural modification of the site of action in the nervous system (Winteringham, 1962; Bridges and Cox, 1959 as cited by Kenneth, 1982). Another example to insensitivity to target site is knock down resistance (KDR) which is associated with recessive gene which confers resistance to pyrethroid (Seifert, 1996).

There has been widespread acaricide resistance over the years. This of course is suggesting the discouragement of the use of certain acaricides. Beugnet and Chardonnet (1995) recently reported the resistance of *Boophilus microplus* to deltamethrin in New Caledonia, with the resistance factors from 8.3 to 97.7. A survey carried out in three important cattle areas of Zambia revealed resistance to acaricides in *Rhipicephalus appendiculatus* (Neumann), *Amblyomma variegatum* (Fabricius), *Boophilus decoloratus* (Koch) and *B. microplus* (Canestrini). A relatively high resistance factor was recorded for *R. appendiculatus* against dimethoate and dioxathion. However, most samples of *A. variegatum* and *B. microplus* were susceptible to the acaricides (Luguru et al, 1987). Matthewson et al (1980) had earlier recorded high level resistance to dioxathion in strains of *Boophilus decoloratus* from farms around Lusaka, Zambia. In Western Ethiopia, *Boophilus decoloratus* collected from 18 dairy farms and 6 veterinary clinics proved resistant to toxaphene (Camphechlor) and dieldrin, but there was no resistance with *A. cohaerens, A. variegatum, Rhipicephalus bergeoni and R. praetextatus*

collected from the same place (Regassa and Castro, 1993). In Okpara State Farms of Benin, however, *A. variegatum* and *Hyalomma marginatum* were slightly resistant to coumaphos, dioxathion and diazinon (Pangui et al, 1993).

2.8.1 Management of resistance

Several authors have proposed ways for the management of acaricide resistance. One of such ways is the use of synergists such as safrole, and piperonyl butoxide through their abduct formation. They can help to delay resistance formation or to overcome the established resistance (Hollingworth, 1976). Suggestion for the combination of two acaricides especially from different groups has been made. In Argentina, Romano and Greco (1989) demonstrated the supression of resistance of ticks to coumaphos by combining it with flumethrin, that is coumaphos 16%, flumethrin 1.2%. The majority support is for a rotation strategy based on the alternation of several compounds. This prevents the resistance from setting in, however, the cost of purchasing several acaricides at the same time is perhaps a limiting factor (Roush

and Tabashnik, 1990). Integrated pest management (IPM) could be another way of generally reducing total reliance on chemical thus preventing or slowing down the development of resistance (Young et al, 1988).

CHAPTER 3

3.0 LABORATORY REARING OF A. *VARIEGATUM* (FABRICIUS 1794) TICKS UNDER DIFFERENT HUMIDITY CONDITIONS.

3.1 INTRODUCTION

A. variegatum species in terms of abundance and their role in disease transmission have been well reported (Mohammed, 1974; Dipeolu, 1975). However, only a few field and laboratory studies have been conducted on various aspects of the biology of this tick species.

Survival of female ticks after engorgement and during oviposition appears to significantly affect the number of instars available during the subsequent stages. For instance, poor laying due to the adverse environmental conditions would drastically reduce the number of ticks available for the immediate and subsequent generations.

Relative humidity (RH) and temperature are perhaps the most important requirements for survival during the free-living and pre-oviposition phase (Neitz et al,

1971). Without the appropriate or suitable RH conditions, ticks succumb to desiccation and die. Since the survival of ticks depends on the appropriate relative humidity, the maintenance of required relative humidity in the laboratory culturing of ticks by the use of saturated solution of certain salts have therefore become important. This has been used in the setting up of tick colonies. Solomon (1951) and Winston and Bates (1960) showed that KCl gives a constant RH of 83%±2 at 28°C±2; NaCl gives RH of 72%±2 at 28°C±2 and distilled water gives RH of 95%±2 at 28°C±2.

The segregation of the fed nymph of *Amblyomma variegatum* ticks into different sexes before adult life is not yet documented. However, Knight *et al* (1978) observed that nymphs of *Hyalomma marginatum* which developed into females fed longer than those which developed into males. Krinsky (1979) has also reported that pre-eclosion period was longer in *Ixodes dammini* nymphs that became females than those which became males. This suggests that there are at least some differences between nymphs emerging as males or females which could be exploited. The need to sort out the

sexes before incubation is very important in tick research where there is the need to study the effects of the different sexes on the hosts. With this demand, it should therefore be possible in continuous tick culture to produce the required numbers of the different sexes at any point in time. If that could be done,it would help to save time and allow maximum utilization of resources by avoiding the breeding of ticks of unrequired sex. With cues from Krinsky's (1979) observations it was decided that the relationship between feeding performance and weight of the fed nymph would be assessed to find whether differences existed between the fed nymph emerging as either female or male.

3.2 MATERIALS AND METHODS

3.2.1 Preparation of saturated salt solutions

The preparation of saturated salt solutions, for the creation of required RH conditions was according to the methods of Solomon (1951) and Winston and Bates (1960). Three (3) desiccators each of which contained a different salt viz; saturated KCl which was observed to maintain humidity of 83%±2 at 28°C±2; saturated NaCl

with a relative humidity of 72%±2 at 28°C±2 and distilled H_2O with a relative humidity of 95%±2 at 28°C±2 were provided.

3.2.2 Collection and maintenance of ticks

Forty-five engorged adult females of *A. variegatum* ticks weighing between 1.5-3.6g were collected from cattle at Katamanso, Oshiye, Kotoku and Kasoa all in the Greater Accra Region. They were carefully removed from cattle with the aid of thumb forceps to avoid mutilation of their mouthparts, kept in small cylindrical plastic tubes and brought to the laboratory.

Each tick was cleaned and weighed and then put in a sterile plain plastic tube (9cm by 2.4cm) with the mouth covered with muslin cloth held in place with rubber band. Ticks were then randomised into three groups with each group consisting of 15 ticks and having a similar mean group weight.

Each of the groups was then put in a different desiccator containing saturated NaCl solution, saturated KCl solution and distilled water. All ticks were examined daily and those found with fungal infections (mould) were cleaned.

3.2.3 Egg collection, weighing and counting

Eggs laid by each tick were collected at every other day during oviposition with the aid of fine hair brush. Particular care was taken to disturb the ovipositing ticks as little as possible. Eggs collected from each tube were counted with the aid of an Olympus SZ30 dissecting microscope, this was rather difficult and time consuming, as a result of this, some number were carefully counted from each batch and weighed. The number in every batch was then calculated according to the method developed by Dipeolu and Ogunji(1980). Laying females were weighed at the same time. The eggs were then allowed to hatch into larvae.

3.2.4 Hosts

Male Blue Vienna, New Zealand White and Flemish Giant rabbits weighing between 1.5 - 2.1kg at 12 weeks old were purchased from the Animal Breeding Station, Nungua, Accra, and used as hosts for the laboratory-reared larvae and nymphs. The rabbits were allowed three weeks of adaptation before being used for tick feeding. They were fed ad libitum on pelleted feed (Gafco Tema) and *Cajanus cajan* (Pigeon Pea) leaves.

For the feeding of the adult ticks, four Djallonke male sheep from the Animal Research Institute sub-Station, Pokuase were used. The rabbits, before being used, had the fur between the fore and the hind limbs clipped very close to the skin and a corset fitted around the torso (Alani, 1984). The corset was made from calico with cuffs of soft elastic band at the ends to grip the border areas between the clipped and unclipped fur. The open ends were brought together with safety pins which facilitated daily inspection. Some of the rabbits were used a few times while others were used several times for larval and nymphal feeding.

With the sheep, one of the flanks was clipped of hair very close to the skin and a sleeve made to fit the clipped area attached with the aid of a glue (Evostik, Stafford). The open end was tied. This formed a pocket which permitted inspection of ticks before and after engorgement and detachment.

3.2.5 Handling and feeding of larvae

Larvae were used 10 days after hatching for the hardening of the cuticle to occur. They were applied on rabbits in batches of about 500-1000. The rabbits and larvae were examined daily and the detached

engorged larvae were collected and some were picked randomly and weighed. They were kept in plain plastic tubes, covered with muslin cloth, held in place with rubber band and then kept in desiccators with RH of 72%±2 at 28°C±2 until they moulted into nymphs. Time taken for this phase was recorded.

3.2.6 Handling and feeding of nymphs

Nymphs were also allowed 10 days for hardening of cuticle before being applied to the rabbits. They were attached in batches of about 200-300. The rabbits were examined daily, and engorged and detached nymphs removed. They were kept in plastic tubes (20 per tube) then covered with muslin cloth held in place with rubber band and then kept in desiccators with RH of 72%±2 at 28°C±2 until they moulted into adult.

55 fed nymphs were randomly selected, weighed, and put in individual tubes. The tubes were properly labelled and nymphs allowed to moult into adults. After moulting, their sexes and adult weights were also recorded.

3.2.7 Handling and feeding of adults

Five (5) males were applied before twenty (20) females were added two days later to feed on a sheep. The tied end was opened daily for inspection. Engorged females which detached were removed.

3.2.8 Statistical Analysis

Data were analysed using Student-t-test, analysis of variance and correlation analysis were applied.

Plate 1: Rabbit with the corset for the feeding of the Larval and the nymphal stages of *A. variegatum*

Plate 2: Fed nymphs of *A. variegatum* tick

Plate 3: Djallonke male sheep with the corset on the flank for the feeding of adult *A. variegatm*

Plate 4: Unfed adult *A. variegatum* ticks

Plate 5: Fed adult *A. variegatum* ticks

3.3 RESULTS

3.3.1 Preoviposition and oviposition periods

The preoviposition periods were 9-15 days, 10-12 days and 9-12 days in KCl, NaCl and H_20 respectively; and they were not significantly ($P<0.05$) different. The number of eggs laid in KCl was 9,655 in NaCl, 10,583 and 5,074 in H_20, as shown in Table 1, (Appendix I-VI). Eggs laid per day was highest in NaCl (441), 345 for KCl and 211 for H_20, corresponding to one egg in 3.3min., 4.2min and 6.8min respectively (Table 1).

The highest peak of laying corresponded to the highest peak of weight loss which was observed on day 6 in both NaCl solution and H_20. In saturated KCl, the highest peak was from the 4th day to the 6th day, with the highest weight loss seen on day 4 (Table 1). In saturated KCl solution with a relative humidity of 83%±2 at 28°C±2, there was a gradual fall in egg laying, declining from 1,300 eggs on day 8 to 953 eggs on day 12, after the initial peak. The second fall was seen from day 14-16 where the laying was between 520-693 (Figure 1). From day 22, there was laying of 87 eggs regularly for 4 days, with the laying terminating

Table 1: Preoviposition and oviposition periods of *A. variegatum*.

Solution	Total eggs	Egg/ day	Rate of egg laying (mins. /one egg)	Pre-ovipositio n period (days)	Total number of peak of egg laying	Highest peak day of laying (days)	Peak of weight loss	Total number of days of laying
KCL	9,655	345	4.2 min	11 (9-15)	2	4-6	4	28
NaCL	10,583	441	3.3 min .	10 (10-12)	4	6	6	24
H_2O	5,074	211	6.8 min	10 (9-12)	3	6	6	2

Table 2: Sex ratio of 55 fed nymphs as determined by their weight

	Number from the total	Percent-age	Fed nymphal weight (g)	Mean fed nymphal weight	Unfed adult weight (g)	Mean unfed adult weight (g)	Correl-ation (r)
Fednymphs which yielded to males	20	36.36	0.0180-0.0590	0.0377	0.0020-0.0290	0.0143	0.690
Fednymphs which yielded to females	31	56.36	0.0310-0.0740	0.0555	0.0060-0.0390	0.0211	0.450

Four (7.27%) nymphs did not moult

Table 3: Feeding period, moulting period and average weight of larva, nymph and adult at fed and unfed states

	Larva	Nymph	Adult
Mean fed weight (g)	0.00214 (13.36 times the unfed weight)	0.0486 (52.64 times the unfed weight)	2.874 (99.84 times the unfed weight
Mean unfed weight (g)	0.000149	0.000906	0.0285
Difference of fed and unfed weight (g)	0.00199	0.0477	2.8455
Feeding period	6-9 days	6-13 days	7-17 days
Moulting period	14-19 days	17-19 days	–

with 60 eggs on day 28. The laying female's weight showed three (3) distinct dips in the pattern of weight loss on days 2-10, 10-16 and 16-28. The first dip being the steepest, had the mean weight reduced from 1.56g to 0.84g (0.72g loss), the second dip declined from 0.84g to 0.54g (0.30g loss) and the last dip being the least steep had the mean weight reduced from 0.54g to 0.41g (0.13g loss).

In saturated NaCl solution with a relative humidity of 72%±2 at 28°C±2, the number of eggs laid increased from 867 eggs on day 4 to 2,426 eggs on day 6, followed by a rapid decline to exactly 50% (1,213 eggs) on the 8^{th} day. Thereafter, there were fluctuations in the number of eggs laid, until egg laying stopped with 91 eggs on day 24 (Figure 2). In saturated NaCl solution the laying ticks had an initial sharp mean weight loss of 0.75g from day 6 to day 18 and the last period which was day 18 to day 24 had a mean weight loss of 0.04g.

The pattern of egg-laying by the ticks and the weight loss in H_2O were similar with those in saturated NaCl solution except for fewer peaks. After the peak of 1,127 eggs on the 6th day, a drastic fall of 48% (520 eggs) on day 10 followed, with as low as 40 eggs being laid on day 24. The

mean weight loss of 0.98g occurred from day 2 to day 10, a 0.43 gm loss from day 10 to day 20 and the last period from day 20 to day 24 had a mean weight loss of 0.12g. There was positive correlation ($P<0.05$) between the loss of weight in and number of eggs laid by ticks in the three solutions (KCl, $r = 0.533$, NaCl, $r = 0.425$ and H_2O, $r = 0.786$).

3.3.2 Feeding periods of larvae, nymphs and adults

The feeding periods for larvae, nymphs and adults were 6-9 days, 6-15 days and 7-12 days respectively in saturated NaCl (Table 3). On two hosts, however, (host 2 and 4) nymphs were collected up to the 20th day (Appendix VIII). Average fed weight for larvae, nymphs and adults were 0.00214g, 0.0486g and 2.874g respectively. Fed larva was 13.36 times the weight of the unfed larva, for nymphs and adults, it was 52.64 times and 99.84 times respectively (Table 3). It took larvae 14-17 days into moult to nymphs, while from the nymph to the adult took 17-19 days.

The yield of fed larvae obtained from rabbits was lower during the second infestation than the primary infestation, however, rabbits which received

third infestation gave better yield than the first (Appendix VIII). Fewer yields were usually recovered on the first and the last days. When naive rabbits were used for nymphal infestation, 100% yield was obtained over rabbits which have had a primary larval infestation (Appendix VIII).

3.3.3 Sex ratio of adults from moulting nymphs

Thirty-one female and twenty male ticks were recovered from 55 randomly selected fed nymphs. Mean weight for the male nymphs was 0.037g, while that of females was 0.055g (Table 2). There was a significant ($P<0.05$) difference between the nymphal mean weights.

Adult males had a weight range of 0.020-0.029g, while that of females ranged between 0.006-0.039. There was a stronger positive correlation in male nymphs with adults ($r=0.69$) than in female nymphs with adults ($r=0.45$).

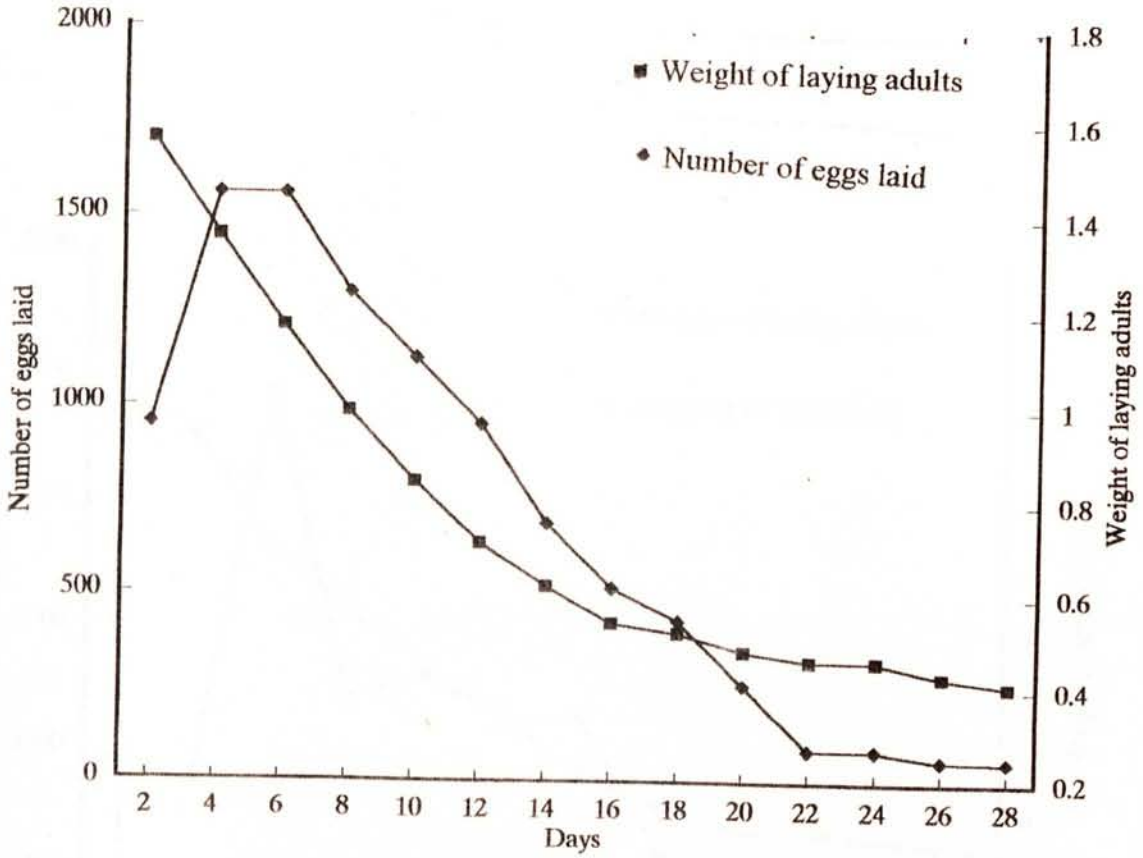


Fig 1: Showing the laying pattern and weight (g) changes in engorged adult females of *Amblyomma variegatum* in saturated KCL solution.

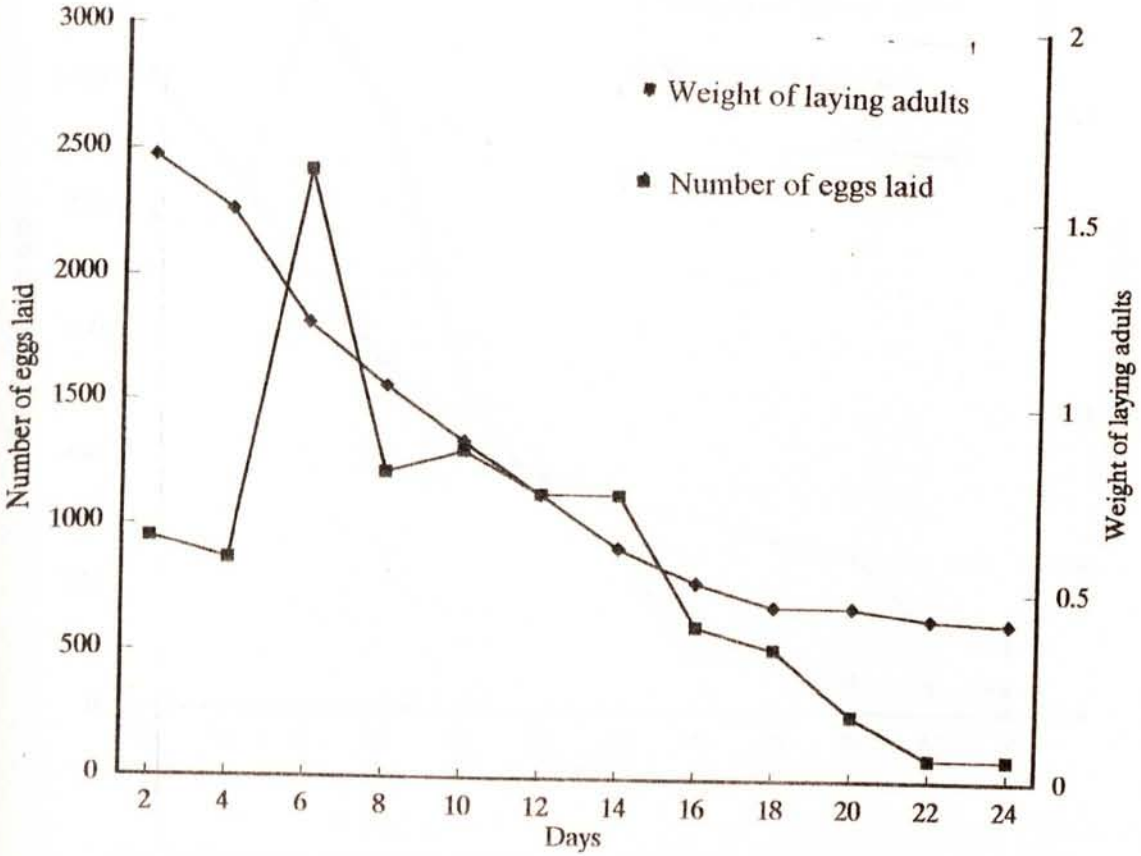


Fig 2: Showing the laying pattern and weight (g) changes in engorged adult females of *Amblyomma variegatum* in saturated NaCL solution.

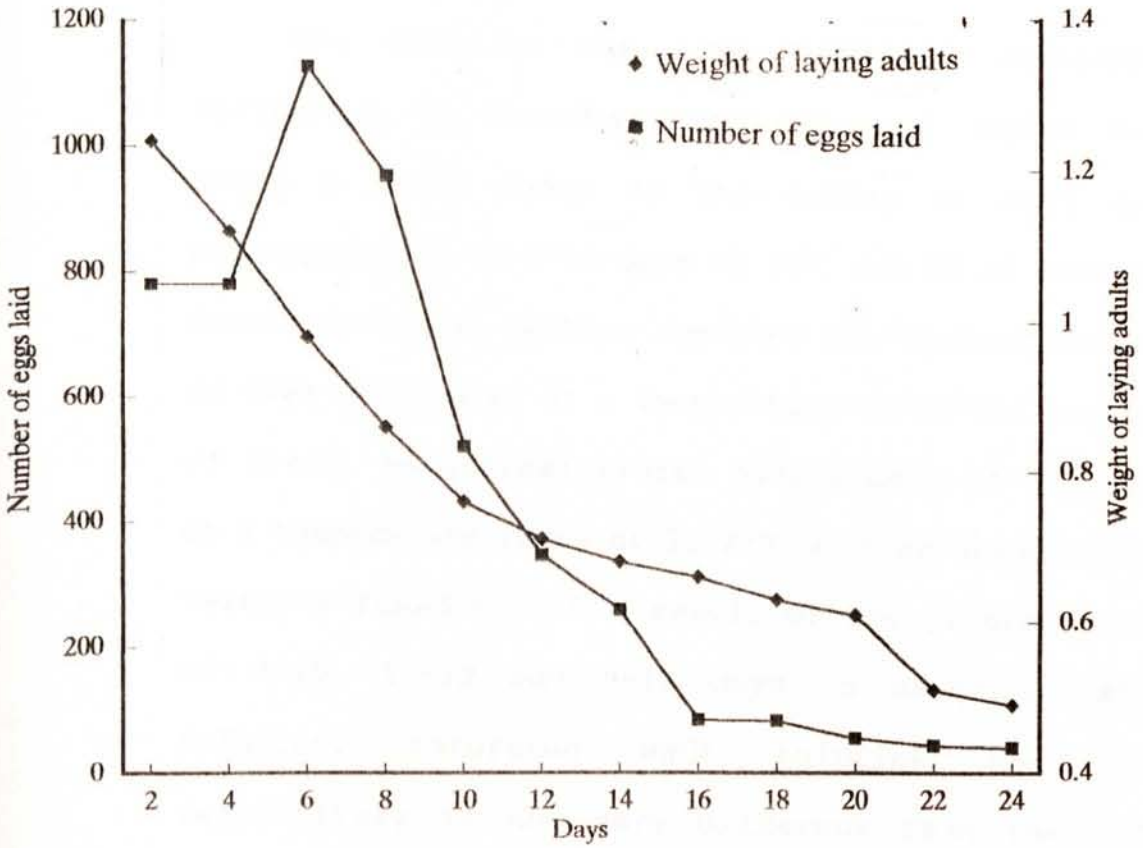


Fig 3: Showing the laying pattern and weight (g) changes in engorged adult females of *Amblyomma variegatum* in distilled H2O.

3.4 DISCUSSION

The work on the life cycle of *Amblyomma variegatum* by Ilemobade and Mohammed (1976) has shown a large range in the number of days for preoviposition of 5-14 days at 28°C and RH of 80-95%, Andreasen (1974) perhaps reported the highest number of days of 35 days at a temperature of 27°C±1 and RH of 85%±1. Hoogstraal (1956) gave a mean of 12 days at a temperature range of 25-27°C with an unspecified relative humidity. The result of the present work of 9-15, 10-12 and 9-12 days in saturated KCl solution, saturated NaCl solution and H_2O respectively is not very different from that of Ilemobade and Mohammed (1976).

While the discrepancies in the preovipositional periods recorded by the investigators (Andreasen, 1974; Hoogstraal, 1956 and Ilemobade and Mohammed, 1976) could be attributed to the different temperatures and relative humidities employed, and the use of different hosts, it is important to also consider the influence of engorgement weights of the ticks. It was observed that the engorgement weights of the ticks collected from the animals in the field varied greatly, and their influence on the number of eggs laid and hatchability have been noted by

Dipeolu and Ogunji(1980).

Dipeolu and Ogunji(1980) observed seven (7) peaks of egg laying in *Amblyomma* ticks that weighed between 1.4-2.0g, with the second peak being the highest. This weight range corresponded to the average weight of ticks used in this experiment. However, ticks in the saturated solution of KCl, NaCl and H_20 produced two, four and three peaks respectively (figures 1, 2 and 3). It is possible that the high number of eggs produced at the first peaks (1,560, for KCl, 2426 for NaCl and 1127 for H_20) in this experiment compensated the fewer number of peaks observed. Davey et al (1980) observed the first peak of 489 eggs on day 4 in *Boophilus annulatus,* while Agyei (1985) observed a peak of 570 eggs on day 3 with *Dermacentor reticulatus.* The total number of eggs laid in the three different conditions created by the three different solutions fell within the expected range of the standard conditions as established by Dipeolu and Ogunji(1980).

In figures 1, 2 and 3, the direct relationship between the number of eggs laid and the loss of weight is supported by the positive correlation in all ticks in the two saturated salt solutions and

water (r=0.533 in KCl, r=0.425 in NaCl, and in H_20, r=0.786). The relationship was strongest in H_20 than the two saturated salt solutions, suggesting that the bulk of the weight loss in it was a result of the egg laying.

Saturated NaCl solution produced significant ($P<0.05$) higher number of eggs than KCl solution though the laying females in the different solutions had no significant weight difference. The laying pattern in saturated NaCl solution appears to be closer to that of the standard conditions as stated by Dipeolu and Ogunji (1980).

The yields of *A. variegatum* larvae and nymphs on rabbits were quite similar in the sense that, the first batch of fed ones were first collected on the 6th day of application,and this collection continued for about four consecutive days,however no recoveries were made during the feeding period. The dispersion of these recoveries gives a normal curve or dum-shape, in which few were collected on the first and the last days and the majority collected in the middle. The first group perhaps have a natural fast feeding habit or might have been able to attach earlier, while those with prolonged feeding periods could be attributed to inability to

attach early. Some of the larvae and nymphs were found wandering on the second day after application.

Earlier suggestion of development of immunity to ticks (Trager, 1939; Agyei, 1985) appear to have occurred in this study. Examinations of the data (Appendix VIII) showed that hosts which had a primary larval infestation had poor yield of nymphs on subsequent infestations, as compared to yields from naive rabbits having their primary nymphal infestation. Dineen (1963) have suggested that there might be a threshold in the host-parasite relationship whereby a host is tolerant to a certain antigenic load of parasites, but responds when that threshold is exceeded and the threshold varies from one animal to the other. It could be through such a mechanism that the rabbit is able to regulate parasite population and their biotic potential. Bennet (1969) and Doube and Kemp (1975) have also suggested a physical response through increased irritability which culminates in the animal grooming itself to remove the ticks. This was observed in this study on a rabbit (Appendix VIII) which consistently pulled off its corset to enable it effectively groom itself, resulting in loss of ticks

and therefore low harvest. Health status of the host also plays a role in the level of infestation, as demonstrated in louse infestation (Gregson, 1964) and *Boophilus* ticks on Shorthorn steers (Riek, 1957) although this was not observed in this study. However, Norval (1978) did not see the effect of immunity in his study on *Amblyomma hebraeum* on rabbit. It was observed that all undeformed larvae and nymphs after feeding were able to moult, with the exception of a batch of five (5) fed nymphs collected from a particular rabbit on the same day. This good moulting was earlier reported in nymphs by Daniel et al (1980) and Agyei (1985) in *Dermacentor reticulatus*, which was suggested to reinforce the suitability of rabbit for larval and nymphal feeding (Feldman-Muhsam, 1964).

The possibility of determining the sex of *Amblyomma variegatum* (Table 2) at the nymphal stage, especially with the significant difference ($P<0.05$) between the mean weight of males (0.0377g, sd of 0.0979) and the mean weight of females (0.0555g sd of 0.0125) was observed in this study. However, for a more reliable sex determination at the nymphal stage, the area of overlap (0.031-0.059g) should be avoided, that is, nymphs with weights above 0.059g

are more likely to be females, while nymphs with weights below 0.031g are more likely to be males. This could be supported by the feeding periods which Knight et al (1978) observed to be longer in *Hyalomma marginatum* nymphs resulting in females and the pre-eclosion period which Krinsky (1979) has also reported to be longer in *Ixodes dammini nymphs* that became females.

Further investigation into the biology of *Amblyomma variegatum* could increase the probability of determining their sex even at stages earlier than the nymphal stage. This would facilitate the establishment of tick colonies of required sex at any point in time thus, saving time and cost.

CHAPTER 4

4.0 STUDIES ON THE EFFICACY OF SOME ACARICIDES AGAINST *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794) TICKS

4.1 INTRODUCTION

Studies on the efficacy of various acaricides have been undertaken by several workers (Drummond, 1981; Henniger, 1988; Duncan, 1991 and Jacquiet *et al*, 1994). Barnard *et al* (1981) have extensively studied the susceptibility of Lone Star tick *(Ambylomma americanum* (L) to not less than 14 commercially available acaricides in the USA. However, information on the efficacy of the various acaricides available on the Ghanaian market is limited.

Efficacy studies are carried out mainly to ascertain the potency of the chemicals and also the range of parasites affected. This is a necessary pre-requisite from the manufacturer's point of view as far as the uses of the chemicals are concerned. However, parasite control authorities also need to regularly assess these characteristics of the drugs. The regular screening of acaricides does not

only help to determine the efficacy of acaricides being used but would help to find out the development of resistance to any of the acaricides through the comparison of the present and the past results.

The fact that fake manufacturers also parade themselves on the market (Matthewson *et al*, 1980; Luguru *et al*, 1987 and Beugnet and Chardonnet, 1995) tend to aggravate the acaricide resistance problem and has resulted in the continuous reports of resistance globally. The acaricides apart from having different target organs in their hosts and different routes of administration or application, act mainly on the nervous system of the ticks either by inhibiting acetylcholinesterase (Eto, 1974) as in the case of organophosphates and carbamates or by interfering with the ion channels (Winteringham, 1962) as seen in the case of organochlorines and pyrethroids. These differential activity of the various acaricides means the different target hosts could be affected differently, particularly with the ability of the parasite to alter its physiological responses to the acaricide through resistance mechanisms (Eto,1974).

The developing countries including Ghana stand

to suffer more from the effects of acaricide resistance, because they are compelled to use the scarce foreign exchange to import these chemicals. The drain on the foreign exchange reserves of most African countries as a result of the annual importation of acaricides have been very alarming. It has been estimated that Kenya spends between US$6-10 million (Chema, 1984) US$10 million for Zambia (Pegram *et al*, 1988) and US$150,000 for Burundi (Niyonzema and Klitz, 1986).

The need for regular sreening studies therefore cannot be overemphasised. The studies conducted in this experiment were aimed at finding out the current status of those acaricides on the market in the country against all the stages of the most abundant tick *Amblyomma variegatum* (Fabricius, 1794), the results which could be extended and interpreted to other tick species present in Ghana. The results should be of applied significance, since the chemical control of ticks is directed at different developmental stages which usually co-exist on a host.

4.2 MATERIALS AND METHODS

4.2.1 Source of ticks

All stages of ticks (fed and unfed; larva, nymph and adult *Amblyomma variegatum*) used in this experiment were obtained from a colony maintained at the Parasitology Laboratory, ARI, Achimota. Larvae were allowed about three weeks for their cuticle to harden before use according to Roberts *et al* (1980). Unfed larvae were applied to rabbits and after engorgement and detachment were collected and used immediately to prevent the commencement of moulting. Some batches were subsequently allowed to moult into nymphs as they were placed in glass tubes covered with muslin and kept in the dessicator maintained at 72%±2RH and 28°C±2 until moulting.

Unfed nymphs were used at about three weeks of age (Roberts *et al*, 1980). Batches of unfed nymps were also applied to naive rabbits for feeding and after engorgment and detachment were immediately used for the engorged nymphal trials. Those allowed to moult into adults were used about three weeks after their emergence for the cuticle to get hardened. Unfed adults were applied to sheep using calico sleeve attached to the flank of the animals

after the fur was cut very close to the skin. Males were attached two days before the females. Engorged females which detached were collected and used immediately.

4.2.2 Types of acaricides used

The acaricides used were chosen based on their commercial availability and patronage by farmers (Awumbila and Bokuma, 1994),except for Amitraz which was recently introduced into the Ghanaian market. They included; Supamix which contains chlorfenvinphos 55.0% M.V and dioxathion 55.0% M/V (both organophosphates) with the recommended concentration of 0.025 and produced by (Cooper, Zimbabwe Ltd.); Steladone an organophosphate contains 300g chlorfenvinphos per litre (0, 0-diethyl 0-(1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate). Its recommended concentration is 0.050 and produced by CIBA-GEIGY Limited, Basel, Switzerland; Lindane, an organochlorine contains gamma benzenehexachloride of 200g/L. It has the recommended concentration of 0.025 and produced by Tema Chemicals Ltd Tema Ghana; Triatix an amidine contains 125g/litre amitraz (n-methylbis (2,4 xylyliminomethyl)-amine, with the recommended

concentration of 0.025 and produced by Cooper Zimbabwe Ltd.Harare,Zimbabwe.

4.2.3 Preparation of acaricide solutions

From the original stock, various concentrations were prepared for each acaricide and the ranges were as follows: Lindane 0.0009-0.025, Steladone 0.0009-0.050, Supamix 0.0009-0.025 and Amitraz 0.025 - 0.045. The recommended concentrations were included in each case (0.025 for Lindane, Supamix and Amitraz, and 0.050 for Steladone).

For every concentration of each acaricide, 1000 ml was chosen as the final volume and this was to allow enough quantity of acaricide to be taken from the initial stock, especially when very low concentration of about 0.0009 was to be prepared. The volume taken from the stock solution to make the final volume of 1000ml was calculated with the use of the equation $V_1 \times C_1 = V_2 \times C_2$.

V_1 = volume of acaricide to be taken from the original stock.

C_1 = concentration in the original stock.

V_2 = volume of the new concentration been prepared.

C_2 = new concentration required.

For instance, to calculate the volume that would be taken from the original stock if concentration of

0.0009 Steladone is to be prepared, given Steladone at 300 emulsifiable concentrate(EC) to make a final volume of 1000 ml.

V_1 = ?

C_1 = 30

V_2 = 1000 ml

C_2 = 0.0009

$V_1 \times C_1 = V_2 \times C_2$

$V_1 \times 30 = 1000 \times 0.0009$

V_1 = 0.03 ml or 30 ul.

Therefore, 30 ul of Steladone would be taken from original stock to prepare concentration of 0.0009.

With the use of micropipette (Finnpipette) the calculated volume was carefully taken from the original stock each time and dropped in a one litre round bottom volumetric flask. About 100 ml of distilled water was then carefully added and then gently shaken to ensure proper mixing of the solution, more distilled water was then added to make the final volume. This procedure was used in the preparation of all the concentrations of each of the acaricides used. The concentrations used in the various acaricides are as follows:

Steladone - 0.060, 0.050. 0.030, 0.020, 0.010,
0.0030, 0.0040, 0.0010, 0.0009, 0.0008,

0.0007, 0.0005

Supamix - 0.030, 0.025, 0.015, 0.010, 0.0050, 0.0030, 0.0010, 0.0009, 0.0008, 0.0007, 0.0005, 0.0001

Lindane - 0.030, 0.025, 0.015, 0.010, 0.0050, 0.0030, 0.0010, 0.0009, 0.0008, 0.0007, 0.0005

Amitraz - 0.020, 0.025, 0.030, 0.035, 0.038, 0.040, 0.045

4.2.4 Bioassay technique

Fifty each of unfed larvae and nymphs, 20 each of engorged larvae and nymphs and unfed adults and five fed adult female ticks were used for each concentration or test. Each test included the manufacturers' recommended dose. The bioassay technique used was the filter paper dip method as described by Shaw (1966). Ten (10) ml of each concentration of each of the acaricides was placed onto ticks held between two 11-cm grade 1 filter paper discs in the bottom half of a plastic Petri dish (10 by 100 cm); after 10 minutes, ticks were transferred to an untreated 9-cm, grade-1 filter paper disc for air drying, after which they were transferred to glass tubes and covered with muslin.

For the engorged adults, each was put in a separate glass tube. They were then transferred and kept in the desiccator for 24 h at 27°C±2 and 72%±2 RH; mortality was assessed after 24 hrs. A control group (with distilled water) was used for each test.

4.2.5 Observations on behavioural responses (state of activity)

The observations made on the responses of the ticks to the acaricides included change in colouration and shape of the ticks, type of mobility, time of death and the time before oviposition started in the fed adult females. Ticks were considered alive if, when breathed upon or agitated, they exhibited paddling of the legs or were mobile. Those unable to maintain the usual upright posture or that made uncoordinated leg movements when breathed upon or prodded were considered dead after 24hrs. For the fed adult females, ticks were considered dead if oviposition was inhibited. The same procedures were applied in all the concentrations of Supamix, Steladone, Lindane and Amitraz and were replicated three times.

4.2.6 Statistical analysis

Data were subjected to Abbott's formula and probit analysis according to Finney (1964).

4.3 RESULTS

4.3.1 Behavioural responses of ticks

4.3.1.1 Supamix

4.3.1.1.1 Unfed adults

The unfed adults *A. variegatum* were generally found to be immobile with occasional paddling of the limbs, while others staggered in their attempt to move 24h after applying the test.

4.3.1.1.2 Engorged adult females

The engorged adults had the natural pale coppery-red colour of the anterio-lateral part of the dorsum turning yellowish as early as the second day. This gradually darkened and spread to the entire dorsum. The dorsum appeared rough and wrinkled laterally. The shape of the fed tick was noticed to change from the initial robust form to an arc shape, with a curve in the ventral side. The cuticle appeared dried. Vigorous and continuous paddling of the limbs was noticed especially in those with concentrations of 0.003 and 0.004 in the first two days and later changed to occasional

movements. Colourless liquid (not analysed) was seen all over the cuticle when examined after 24 hours. Death of the tick occured between 8 and 60 days. For those that laid, laying started between 9-12 days. Laying took place in 0.0001, 0.0007 and 0.0009 concentrations.

4.3.1.1.3 Fed nymphs and larvae

Fed nymphs and larvae appeared flat and elongated. There were occasional movement of the limbs. Death was noticed from day 2 and those that survived in the Supamix concentrations moulted from larva to nymph and nymph to adult. Control groups were seen to maintain their robust shape, entered quiescency within two-three days and moulting was seen to progress as indicated by the changes in the integument.

4.3.1.1.4 Unfed nymphs and larvae

Unfed nymphs and larvae were seen to vigorously disperse as soon as they were air dried on the filter paper. Their mobility was later found to decrease in about two hours later. Death was recorded in those dipped in concentrations of 0.010-0.025 from the fourth hour after treatment. With lower concentrations, death was recorded from the 10th hour after treatment. The control group

were constantly mobile throughout the observation period with little or no mortality.

4.3.1.2 Steladone

4.3.1.2.1 Unfed adults(males and females)

Unfed adults were immobile with occasional paddling of the limbs while others staggered in their attempts to move. 40% of the ticks at the concentration of 0.020 and 38% at the concentration of 0.010 and the control groups were active and highly mobile during the conduct of the various tests.

4.3.1.2.2 Engorged adult females

Engorged adults had their colour changed to yellow and eventually became darkened. Wrinkles were seen on the dorsum. The integument appeared dried with the body conspicuously arc shaped. Colourless fluid (not analysed) was also seen all over the cuticle. The first anterior pair of limbs appeared weaker than the other pairs. Death occured within 11-23 days and egg laying started within 8-10 days. 20% of the ticks in the 0.001 concentration and 50% of those in 0.0009 concentration laid eggs.

4.3.1.2.3 Fed nymphs and larvae

Fed nymphs and larvae appeared flat and elongated. There were occasional movement of the limbs. Death was noticed from day 2. 40% of fed larvae moulted into nymphs in 0.004 concentration while 80% moulted in 0.001 concentration , while 67% of fed nymphs moulted in 0.001 concentration and 90% moulted in 0.0008 concentration. No moulting occured at their recommended concentrations. Control groups maintained the robust shape, and entered quiescency within two to three days, with moulting occuring after 18 days.

4.3.1.2.4 Unfed nymphs and larvae

Unfed nymphs and larvae were very active and mobile after being air dried on the filter paper. Their mobility declined in about two hours later. Death were recorded in those with concentrations of 0.020-0.050 10 hours after the test. The control groups were constantly mobile throughout the observation period with little or no mortality.

4.3.1.3 Lindane

4.3.1.3.1 Unfed adults

Most of the unfed adults were seen to be on dorsal recumbency with occasional paddling of the limbs, others staggered in their attempts to move. Control group were active and highly mobile throughout the period of the test.

4.3.1.3.2 Engorged adult females

Engorged adults also changed to yellow and later black with dried and wrinkled integument. The first anterior pair of limbs appeared weak and the body showed the arc shape. Death occured within 17-19 days and with those that laid eggs, laying started within 9-28 days. 33.3% of the engorged adult female ticks laid in concentration of 0.025, while 48% laid in concentration of 0.010. A few laid for 2-3 days at lower concentrations, stopped laying and later died.

4.3.1.3.3 Fed nymphs and larvae

Fed nymphs and larvae were flat and elongated. There were occasional movements of the limbs and death occured as from day 3. 60% of the ticks moulted at the concentration of 0.005 while 80% moulted at the concentration of 0.001. Control groups maintained the robust shape, and entered

quiescency within two-three days and later moulted.

4.3.1.3.4 Unfed nymphs and larvae

Unfed nymphs and larvae were active and moved around after air drying. Their mobility declined in about two hours later. Mortality was recorded from the third hour after the treatment in concentration of 0.010-0.025 Lower concentrations recorded mortalities after 8 hours of treatment. The control groups were constantly mobile throughout the observation periods.

4.3.1.4 Amitraz

4.3.1.4.1 Unfed adults

Most of the unfed adults staggered in their attempts to move, others were lying on their dorsum and showing occasional paddling of the limbs. The control groups were active and highly mobile throughout the tests.

4.3.1.4.2 Engorged adult females

The engorged adults maintained their robust shape. The cuticle started hardening on day 9, with darkening setting in two days later. These changes were observed in those with concentrations of 0.040 and 0.045. Some with these morphological changes were still able to lay, but only laid for

about 3 days, after which they stayed alive for about 7 days. Those that never laid died within 33-39 days, while those that laid commenced laying between day 9 and 11. 55% and 66.7% of the engorged adult females laid in 0.038 and 0.035 concentrations respectively.

4.3.1.4.3 Fed nymphs and larvae

Fed nymphs and larvae appeared flat and elongated. There was occasional paddling of limbs. Death was noticed from day 3 but those that survived Amitraz application moulted. 67% of the fed nymphs moulted at the recommended concentration of 0.025, while 97% of the fed larvae moulted at a very high concentration of 0.045. The control groups were seen to maintain the robust shape and later moulted.

4.3.1.4.4 Unfed nymphs and larvae

Unfed nymphs and larvae were quiescent after air drying on the filter paper for about two hours. Recovery gradually took place as the ticks were later seen to be mobile. The control groups were constantly mobile throughout the observation periods.

4.3.2 Concentrations and percentage mortalities

4.3.2.1 Unfed larvae

Supamix, Steladone and Lindane produced 100% mortality on unfed larvae at their recommended concentrations (0.025, 0.05 and 0.025 respectively). As their concentration were reduced to 0.003, mortalities of 94.4%, 78.8% and 87.5% were observed in Supamix, Steladone and Lindane respectively as shown on Table 7. Amitraz produced zero mortality at the recommended concentration of 0.025, also at the higher concentration of 0.045.

The order of their effectiveness are shown on Table 4a is as follows:

Supamix > Steladone > Lindane >Amitraz.

The lethal concentration of Amitraz could not be ascertained as no mortality of unfed larvae occurred at all the concentrations tested.

4.3.2.2 Fed larvae

On fed larvae, Supamix, Steladone and Lindane produced 100% mortality at their recommended concentrations. At a lower concentration of 0.004, Steladone produced 60% mortality, while Supamix and Lindane produced 80% and 40% respectively at the concentration of 0.005. The trend of LC 50 and LC 90 was the same as in the unfed larvae except that

the values were higher in the fed larvae as shown in Table 10. Amitraz still recorded zero percent mortality at all the concentrations tested.

4.3.2.3 Unfed nymphs

Lindane proved most effective at the unfed nymphal stage with the least LC 50 of 1.629×10^{-3} as compared to Supamix, Steladone and Amitraz which had 1.794×10^{-3}, 2.258×10^{-3} and 39.64×10^{-3} respectively. The trend of effectiveness, however was Lindane > Supamix > Steladone > Amitraz. The LC 90 for Amitraz is 69.65×10^{-3} which is more than twice the recommended concentration (25.00×10^{-3}). At the concentration of 0.0009, the mortalities were 85.7% for Lindane and 71% for Supamix and Steladone. However as the concentration was lowered to 0.0005, no mortality was observed with the three acaricides. Amitraz gave 20%, 25% and 75% mortality for 0.025, 0.035 and 0.045 concentrations respectively (see Table 8).

4.3.2.4 Fed nymphs

Except for Lindane, the LC 50 for all the chemicals on the fed nymphs were lower than the unfed nymphs. There was a switch back to the trend

of Supamix > Steladone > Lindane > Amitraz in their effectiveness (Table 5b). Supamix, Steladone and Lindane produced 100% mortality at their recommended concentrations, while Amitraz produced only 33% at the recommended concentration. At the concentration of 0.001, Supamix, Steladone and Lindane produced 66%, 33% and 55% mortalities respectively. At much lower concentration of 0.0008, 20%, 10% and 6% mortalities were recorded for Supamix, Steladone and Lindane respectively. For Amitraz, since the percentage mortality produced at its recommended concentration (0.025) was only 33%, its effect was then tested at higher concentrations of 0.035, 0.040 and 0.045 and these produced 66%, 69% and 70% mortalities respectively.

4.3.2.5 Unfed adults

With the unfed adults, Steladone, Supamix and Lindane produced 100% mortality, while Amitraz had 53.3% mortality at their recommended concentrations. Amitraz was able to achieve 100% mortality at a higher concentration of 0.035. At the concentration of 0.020, Steladone produced 80% mortality, while Supamix and Lindane had mortalities of 80% and 70% respectively at 0.010 concentration. Lindane had

displaced Steladone to the second position in the effectiveness order, thus reading Supamix > Lindane > Steladone > Amitraz as shown in Table 6a.

4.3.3 Effect on ovipositing ticks or oviposition.

The criteria used for deciding the lethal concentration of fed adult females was focused on the inhibition of oviposition which was termed inhibition concentration(IC)and hatching of the laid eggs. Supamix, Steladone Lindane and Amitraz produced IC50 of 1.37×10^{-4} , 7.638×10^{-4}, 64.15×10^{-4} and 359.8×10^{-4} respectively. These values were lower than any of the previous stages. The order of effectiveness at this stage was Supamix > Steladone > Lindane > Amitraz as shown on Table 6b. There was however a change in this order at the IC 90 level, as Amitraz displaced Lindane to the third place. While Supamix and Steladone were able to maintain 100% inhibition of oviposition at their recommended concentration, Lindane was only able to inhibit 66.7% at its recommended concentration. At the lower concentration of 0.0009, Supamix and Steladone were able to inhibit 80% and 50% of female from laying respectively. Lindane could only inhibit 40%

at 0.03 concentration. The level of inhibition of oviposition by concentrations of Amitraz was 100% at 0.040, 45% at 0.038 and 33.3% at 0.035.

Table 4a: Lethal concentrations of acaricides to unfed larvae of *A. variegatum*.

Acaricide	LC 50	LC 90
Steladone	0.001303	0.005800
Supamix	0.001166	0.003702
Lindane	0.001454	0.004457
Amitraz	*	

Table 4b: Lethal concentrations of acaricides to fed larvae of *A. variegatum*.

Acaricide	LC 50	LC 90
Steladone	0.001887	0.006014
Supamix	0.001492	0.006753
Lindane	0.002346	0.005977
Amitraz	*	*

* = Lethal concentrations could not be determined as no death was recorded of all the concentrations tested.

Table 5a: Lethal concentrations of acaricides to unfed nymphs of *A. variegatum*.

Acaricide	LC 50	LC 90
Steladone	0.002258	0.005854
Supamix	0.001794	0.003862
Lindane	0.001629	0.003585
Amitraz	0.03964	0.06965

Table 5b: Lethal concentrations of acaricides to fed nymphs of *A. variegatum*.

Acaricide	LC 50	LC 90
Steladone	0.002225	0.006896
Supamix	0.001089	0.003594
Lindane	0.002301	0.009891
Amitraz	0.0307	0.0645

Table 6a: Lethal concentrations of acaricides to unfed adults of *A. variegatum.*

Acaricide	LC 50	LC 90
Steladone	0.009574	0.02017
Supamix	0.005441	0.011079
Lindane	0.006078	0.01157
Amitraz	0.02273	0.02861

Table 6b: Oviposition inhibition concentrations of acaricides to fed adults of *A. variegatum.*

Acaricide	IC 50	IC 90
Steladone	0.0007638	0.001434
Supamix	0.0001378	0.0006178
Lindane	0.006415	0.4467
Amitraz	0.03598	0.03903

Table 7 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of unfed larvae of *A. Variegatum*

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.05	-1.301	100	8.09	0.025	-1.602	100	8.09	0.025	-1.602	100	8.09	0.045	-1.347	0	0
0.003	-2.523	78.8	5.81	0.003	-2.523	94.4	6.55	0.003	-2.523	87.5	6.18	0.035	-1.456	0	0
0.0005	-3.301	18.6	4.12	0.0005	-3.301	13	3.87	0.0005	-3.301	8	3.59	0.025	-1.602	0	0

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

Table 8 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of unfed nymphs of *A. variegatum*

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.03	-1.523	100	8.09	0.015	-1.824	100	8.09	0.015	-1.804	100	8.09	0.045	-1.347	75	5.67
0.0009	-3.046	71	5.55	0.0009	-3.046	71	5.55	0.0009	-3.046	85.7	6.18	0.040	-1.398	43	4.82
0.0007	-3.155	50	5.00	0.0007	-3.155	52	5.05	0.0007	-3.155	53	5.08	0.035	-1.456	25	4.33
0.0005	-3.301	0	0.00	0.0005	-3.301	0	0.00	0.0005	-3.301	0	0.00	0.025	-1.602	20	4.16

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

Table 9 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of unfed adults of *A. variegatum*

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.06	-1.222	100	8.09	0.030	-1.523	100	8.09	0.030	-1.523	100	8.09	0.035	-1.456	100	8.09
0.05	-1.301	100	8.09	0.025	-1.602	100	8.09	0.025	-1.602	100	8.09	0.030	-1.523	80	5.84
0.02	-1.699	80	5.84	0.010	-2.000	80	5.84	0.010	-2.000	70	5.52	0.025	-1.602	53.3	5.08
0.01	-2.000	62	5.31	0.005	-2.301	50	5.00	0.005	-2.301	45	4.87	0.020	-1.699	40	4.7

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

Table 10 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of fed larvae of *A. variegatum*

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.05	-1.301	100	8.09	0.025	-1.602	100	8.09	0.025	-1.602	100	8.09	0.045	-1.347	0	0
0.01	-2.000	100	8.09	0.01	-2.000	80	5.84	0.01	-2.000	100	8.09	0.040	-1.398	0	0
0.004	-2.396	60	5.25	0.005	-2.301	80	5.84	0.005	-2.301	40	4.75	0.035	-1.456	0	0
0.001	-3.000	20	4.16	0.0005	-3.301	25	4.33	0.001	-3.000	20	4.16	0.025	-1.602	0	0

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

Table 11 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of fed nymphs of *A. variegatum*.

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.05	-1.301	100	8.09	0.025	-1.602	100	8.09	0.025	-1.602	100	8.09	0.045	-1.347	70	5.52
0.03	-1.523	100	8.09	0.015	-1.824	100	8.09	0.015	-1.824	66	5.41	0.040	-1.398	69	5.50
0.001	-3.000	33	4.56	0.001	-3.000	66	5.41	0.001	-3.000	55	5.13	0.035	-1.456	66	5.41
0.0008	-3.097	10	3.72	0.0008	-3.097	20	4.16	0.0008	-3.094	6	3.45	0.025	-1.602	33	4.56

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

Table 12 Concentrations and log of concentrations of acaricides with their corresponding percentage and probit mortalities of fed adults of *A. variegatum*

Steladone				Supamix				Lindane				Amitraz			
a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
0.004	-2.398	100	8.09	0.005	-2.301	100	8.09	0.025	-1.602	66.7	5.44	0.045	-1.347	100	8.09
0.003	-2.523	100	8.09	0.003	-2.523	100	8.09	0.010	-2.000	52	5.05	0.040	-1.398	100	8.09
0.001	-3.000	80	5.84	0.0009	-3.046	80	5.84	0.005	-2.301	50	5.00	0.038	-1.420	45	4.87
0.0009	-3.097	50	5.00	0.0001	-4.00	50	5.00	0.003	-2.532	40	4.75	0.035	-1.456	33.3	4.56

a: Concentration b:Log of concentration

c: Percentage mortality d: Probit mortality

4.4. DISCUSSION

The susceptibility of any particular tick to an acaricide depend on the efficacy of the acaricide. The efficacy of the acaricide is determined through trials such as the one conducted in this study. The physiological peculiarities of the different ticks and in their different stages of development determines the subjection of a tick to a particular acaricide which may have different pharmacological characteristics. Thus an acaricide with a broad spectrum of activity may affect a large number of ticks and their various stages of development or instars.

The results of this study showed that the different stages of development or instars were affected differently by the different acaricides used. It was observed that Supamix even at a lower concentration was more effective than Steladone and Lindane against the unfed adult ticks. Steladone and Supamix are organophosphates and are known to act through the enzyme acetylcholinesterase which is used in nerve impulse transmission (Eto, 1974). Amitraz a cyclo amidin causes the detachment of

ticks through probably muscular paralysis. It was observed that the colour of the integument of the engorged adult female tick changed from greyish dark to almost black possibly as a result of rapid haematinization of the blood meal. The change in the colouration of the cuticle and its dryness could have also resulted from the effect of the drugs on the waxy layer leading to the loss of its function, resulting in water loss and therefore dehydration. The reasons for the conspicious change in shape from robust to arc shape in Supamix, Steladone and Lindane could also have been as a result of muscular paralysis.

The recovery of ticks and the ability of the fully engorged adult female tick to oviposit after treatment with Amitraz have serious implications for tick survival and control. The immediate knock down effect of the drug could send tick control agents into false sense of achievement of control, because animals treated would come back later from the field or grazing without the ticks. It is likely that, ticks which had detached could reinfest the same animals or other animal hosts later. It is also possible particularly with fed adult female that, the ticks might survive the effect of the

acaricide, probably lay eggs which would hatch and contribute to the next generation. It was observed that inhibition of oviposition with Amitraz occured at a higher concentration but it is likely that use of the chemical at that rate could be uneconomical.

The consistency of Supamix and Steladone in being very effective against all the stages of *Ambylomma variegatum* even at low concentrations in this study gives support to the work of earlier researchers. Bafi-Yeboah (1974) in Ghana demonstrated the superiority of chlorfenvinphos over Asuntol and Bacdip on *Amblyomma* spp and *Rhipicephalus* spp. According to Rawlins and Mansigh (1981) chlorfenvinphos topped 23 other acaricides, with dioxathion and gamma BHC (Lindane) occupying positions 12th and 14th respectively. Similar result was obtained elsewhere (Khan and Strivastava, 1987). Supamix which contains dioxathion in addition to chlorfenvinphos does not only have the advantage of being more effective than Steladone (only chlorfenvinphos) but could also delay the occurrence of resistance (Romano et al, 1982). It is however clear that chlorfenvinphos is the chief chemical in Supamix when dioxathion only gives some support by the virtue of the gap in their position of LC50

(Rawlins and Mansigh, 1981). The reason for the better performance of Lindane over Steladone at the unfed nymphal and unfed adult stages (LC 50 for Lindane = 1.629 x 10^{-3}, LC 50 for Steladone = 2.258 x 10-3) could result from the fact that the unfed nymphal and unfed adult stages are more susceptible to ion channels interference which is the mode of action of Lindane (Kenneth, 1982).

Lindane appeared not to be effective against the fed adult stage with the LC 90 of 0.4467 despite the low LC 50 of 0.006415. The recommended concentration of 0.025 was only able to inhibit 66.7% of the female from laying instead of inhibiting over 95%. This is probably a case of resistance as Lindane with the name Gammatox has a history of long usage in Ghana. Bittencourt et al (1989) and Khurana et al (1992) have established that the unfed stages of ticks were more susceptible to acaricides than the respective blood fed stages. This was observed with the larvae but not with the nymphs where the fed nymphs were more susceptible than the unfed nymphs except with Lindane.

From the result of this work, Amitraz have shown to be totally ineffective against all stages of *Amblyomma variegatum* especially at the fed and

unfed larval stage where no mortality was recorded at all the concentrations tested. Its LC 90 for the nymphal and the adult stages were quite greater than the recommended dose. *A. variegatum* might not be said to have developed resistance to Amitraz as this acaricide have just recently been introduced into the Ghanaian market. It is, however, possible that *A. variegatum* might be reacting to Amitraz as a result of the phenomenon of cross-resistance. Barnard et al (1981) have, however, shown Amitraz to be superior to permethrin, Lindane and six other acaricides based on LD 90 values on *Amblyomma americanum*.

The finding of Ahrens et al (1989) that Amitraz has a fast action was noticed in this study. Careful observation, however, revealed that over 95% of the ticks knocked down by Amitraz were quiescent for about two hours, but recovered later and were able to survive. It would therefore be necessary to carry out a population dynamic studies on pasture grazed by animals dipped or sprayed with Amitraz. If the build-up of these ticks is rapid, it would confirm that most ticks drop and are able to lay eggs eventually and are not killed by Amitraz. Resistance studies on Lindane may also be needed to

be carried out based on the result obtained on the fed adults in this study. Since eggs that were laid by the tested females hatched out, the emerged larvae could be assayed with the same acaricides and the results of the two tests compared. If the new generation of larvae are less susceptible then we would know the field implication, we could then be thinking of alternating such acaricides with others.

The use of Steladone and Supamix is preferred because of their excellent performance on all stages of *Amblyommma variegatum*. Their use could however, be alternated with other good acaricides such as the pyrethroids to avoid the development of resistance. Since other species of ticks co-exist with *A. variegatum* on the animals though in smaller percentages, (Koney et al, 1994) their response to these acaricide could also be assayed. There is also the need to undertake an in vivo study and the result compared with the *in vitro* in this study.

The fact that mortalities of ticks were recorded in this study on the use of a dose lower than the recommended lethal dose for Supamix shows its superiority among the acaricides used. This is good particularly in our situation where under dosing has become almost a habit of our farmers.

However, the inability of the others to kill at doses lower than the recommended rate is a serious warning to our farmers to put a stop to the habit. This study has strengthened the need for good extension services where by farmers would be educated on the dangers associated with the practice under dosing in the use of acaricides and the need to carry out regular surveys on efficacy.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

General discussion

The control of ticks have depended mainly on acaricides but the effective use of the acaricides also depend on the adequate knowledge of the biology of the ticks and the efficacy of the acaricide been used. Under adverse enviromental conditions, ticks in temperate countries enter into diapuse (Nosek, 1971) and ticks die through desiccation under low humidity. The role of relative humidity and temperature and therefore climate in tick survival demand that adequate information is obtained on those enviromental requirements (Neitz et al,1971). The performance of the ovipositing ticks in the relative humidity conditions created and maintained by the saturated NaCl solution compared favourably with thoes kept under the relative humidity humidity conditions created by the saturated KCl solution. This means that NaCl which is cheaper and more easily obtainable can be used to provide favourable relative humidity conditions for the culturing of *Amblyomma variegatum* ticks. Thus it would be

possible to set up a continuous culture which is very important for the production of ticks of similar ages and probably the same sex.

The results of the efficacy study particularly those obtained with Amitraz reinforces the need for regular screening. The quick knock down effect seen on the ticks supports the effect of Amitraz as a detaching agent (Ahrens et al, 1989). The detaching effect of Amitraz creates a more difficult and ambiguous situation where detached ticks appear to have been killed by the acaricide but may recover to full active life and reattach to their hosts.Its failure to inhibit oviposition coupled to its detaching but not killing effect as observed in this study, means that a detached fully engorged adult female ticks can oviposit and the larvae hatch out of the eggs.Thus contributing to the next generation by providing a new batch to replace the damaged adult ticks.

Barnard et al (1981) have shown Amitraz to be superior to permethrin, Lindane and six other acaricides based on their LC 90 values on *Amblyomma americanum*, whereas this study showed Amitraz to be least effective among Steladone, Supamix and Lindane against *Amblyomma variegatum*. This suggests that

within a single genus there could be different responses to the same acaricides. Tick infestations are known to have strong negative impacts on animal production and therefore must be promptly controlled (Shaw,1973;Plessis *et al,1994)*.The use of acaricides has been observed to be accompanied by a great number of problems,which include, the development of resistance which has been reported globally(Shaw, 1973; Matthewson, 1980; Beugnet and Chardonnet, 1995), the escalating costs of the acaricides (Chema, 1984; Niyonzema and Klitz, 1986 and Pegram et al,1988) and health hazards.

The use of alternatives to acaricides have been tried, but with little success.Anti-tick vaccines have been developed against *Boophilus microplus (*Willadsen *et al*,1989) and *Rhipicephalus appendiculatus* (Dipeolu et al,1990),but not a single of such vaccines is as yet obtainable on the Ghanaian market and even when available, it might not be affordable.Pasture spelling which is another alternative (Wharton and Harley,1962),seems not to be practicable by most of our farmers and herdsmen who rely solely on free range grazing as fencing of farms has not become the norm. Moreover, this controls mainly larvae of *Boophilus spp* which is

one-host tick, as the technique demands that the free-living stage is short lived (Youdeowei and Service,1983). Integrated pest management is not yet popular in animal health sub-sector because of unavailability some of its ingredients such as the establishment of the economic thresholds, economic injury level and economic damage by the ticks. This could be a promising future approach, especially when trying to reduce total reliance on chemicals. Various control methods could be carefully combined to avoid negative interactions(Young et al,1988). However it is clear that the use of acaricides is almost indispensable, at least for the foreseable future.

Miller (1989) has reported on the development and availability of new acaricidal tools which are the use of "pour on" in which the chemical is applied to the mid-line of the animal and then gradually spreads to other parts of the body, this appears to be one of the major innovations in chemical control of livestock pests.There is also the introduction of cattle insecticidal ear tag which has been shown to be effective in controlling the Gulf Cost ear tick *(Amblyomma maculatum)* (Ahrens and Cocke,1978).Neck and tail bands made from

plastic strips(ethylvinyl acetate) containing 12% Amitraz are also available (Miller,1989). Ivermectin is a member of a potent new class of acaricides and as subcutaneous injection protects the animals for about 14 days (Drummond,1985). This ivermectin has also been made available as an intraruminal slow-release device, providing 90 days protection against tick infestation (Tatchell,1992). These new delivery systems have encouraged the use of acaricides and particularly pour-on have eliminated the need for large volumes of water and its proper disposal.

Conclusions

In this study, saturated NaCl solution appeared to provide better environmental conditions for *Amblyomma variegatum* than saturated KCl as far as oviposition is concerned. It is then expected that saturated NaCl solution would be preferable for the creation and maintainance of humidity in the establishment of tick colonies. Moreover, it is cheaper and readily available. It is, however suggested that this research be extended to other tick species to give it a broader use.

The result of this research has shown that, the complete life cycle of *Amblyomma variegatum*

takes at least 5 months. This information coupled to studies on seasonal distribution both on the host and pasture should provide us with information as to when the adult population starts off as larvae or when the eggs are laid . The suggestion of sorting out the sexes at the fed nymphal stage could be appreciated, as the breeding of unrequired sexes could be avoided thus saving time and resources. Perhaps, the weighing of the fed larvae with the use of more sensitive electronic weighing balance would allow us determine the sex of adult at much earlier stage. This would depend on whether there is a direct relationship between the weight of a fed larva and that of a fed nymph .

The better performance of Supamix over Steladone, Lindane and Amitraz on *Amblyomma variegatum* might likely give similar result on other species of ticks found in Ghana, since *Amblyomma variegatum* is a three-host tick with longer life cycle, and the only genus found on animals in Ghana at the time that others are absent due to adverse conditions. One would therefore expect better results when these acaricides are tested against one- and two-host ticks found in Ghana. To confirm the results obtained and assume the usage of the

acaricides, it would be useful for the bioassay to be carried out on other species of ticks.

In vivo studies on these acaricides should be conducted under controlled conditions and the result compared with the *in vitro results* in this study. This will tell us whether or not the laboratory results are applicable in the field. The observation that lower concentrations resulted in lower mortalities indicate that underdosing as practised by a large proportion of livestock farmers is dangerous. There is therefore the need for good extension services whereby farmers would be educated on the dangers associated with under-dosage in the use of acaricides.

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Appendix I Weight of eggs (KCl)

Days No.	2	4	6	8	10	12	14	16	18	20	22	24	26	28
061	0.14	0.14	0.16	0.14	0.11	0.07	0.05	0.02	0.02	0.01	0.01	0.001		0.001
062	0.05	0.15	0.17	0.19	0.19	0.21	0.13	0.14	0.09	0.05	0.03	0.03	0.02	0.01
063	0.32	0.25	0.25	0.21	0.16	0.12	0.09	0.04	0.05	0.01	0.02	0.01	-	-
066	0.06	0.13	0.13	0.11	0.07	0.06	0.02	-	0.01	0.01	0.01	-	-	-
069	0.07	0.21	0.19	0.15	0.17	0.12	0.09	0.06	0.06	0.04	0.01	0.03	-	-
070	0.07	0.13	0.15	0.18	0.14	0.12	0.10	0.05	0.03	0.03	0.02	0.003	-	-
071	0.14	0.28	0.21	0.14	0.15	0.11	0.07	0.05	0.04	0.02	0.004	-	-	-
072	0.09	0.15	0.19	0.22	0.15	0.17	0.13	0.08	0.09	0.05	0.03	0.008	0.03	
073	0.17	0.27	0.19	0.10	0.10	0.05	0.03	0.01	0.02	0.007	0.003	-	-	-
074	0.04	0.10	0.12	0.09	0.12	0.11	0.07	0.06	0.06	0.04	-	0.03	-	-
Mean	0.11	0.18	0.15	0.13	0.11	0.08	0.08	0.06	0.05	0.03	0.01	0.01	0.02	

Appendix II Weight of eggs (KCl)

DAY S/No.	2	4	6	8	10	12	14	16	18	20	22	24
75	0.12	0.09	0.32	0.16	0.17	0.14	0.12	0.06	0.05	0.01	0 .01	-
76	0.09	0.05	0.15	0.08	0.06	0.05	0.05	0.03	0.02	-	-	-
79	0.09	0.09	0.31	0.19	0.23	0.21	0.22	0.13	0.13	0.07	0.03	0.01
80	0.15	0.13	0.28	0.14	0.14	0.11	0.10	0.04	0.03	-	-	-
84	0.12	0.09	0.24	0.14	0.13	0.09	0.07	0.03	0.03	-	-	-
85	0.09	0.10	0.3	0.15	0.16	0.14	0.11	0.05	0.04		0.01	0.01
88	0.13	0.13	0.27	0.14	0.18	0.15	0.13	0.08	0.05	0.12	-	-
90	0.08	0.08	0.25	0.14	0.14	0.12	0.10	0.06	0.04	-	-	-
91	0.08	0.06	0.21	0.11	0.15	0.14	0.16	0.09	0.08	0.03	0.03	0.01
Mean	0.11	0.10	0.28	0.14	0.15	0.13	0.13	0.07	0.06	0.03	0.03	0.01

Appendix III Weight of eggs (H_2O)

Days No	2	4	6	8	10	12	14	16	18	20	22	24
36	0.06	0.10	0.18	0.10	0.11	0.07	0.04	0.02	-	0.02	0.02	0.01
37	0.03	0.06	0.03	0.05	0.04	0.04	0.05	0.06	0.08	0.03	0.02	0.01
38	0.08	0.09	0.12	0.12	0.15	0.06	-	0.04	-	-	-	-
39	0.23	0.003	0.322	0.066	0.062	0.031	0.02	0.001	-	-	-	-
41	0.15	0.14	0.18	0.096	0.07	0.046	0.019	0.009	-	0.007	-	
42	0.018	0.05	0.088	0.059	0.06	0.045	0.015	0.007	-	-	-	-
43	0.69	0.10	0.09	0.12	0.037	0.024	0.013	0.003	-	-	-	-
44	0.097	0.10	0.147	0.085	0.074	0.052	0.029	0.005	-	-	-	-
45	0.072	0.113	0.119	0.20	0.054	0.041	0.033	0.17	-	-	-	-
46	0.076	0.128	0.013	0.128	0.032	0.024	0.029	0.012	0.005	-	-	-
Mean	0.09	0.09	0.13	0.11	0.06	0.04	0.03	0.01	0.04	0.02	0.02	

Appendix IV Weight of laying female adults (KCl)

Days No.	2	4	6	8	10	12	14	16	18	20	22	24	26	28
61	1.29	1.14	0.96	0.80	0.67	0.59	0.54	0.50	0.48	0.47	0.46	0.45	0.46	0.46
62	2.08	1.92	1.74	1.53	1.32	1.09	0.94	0.79	0.69	0.63	0.58	0.58	0.55	0.55
63	1.81	1.54	1.28	1.07	0.90	0.76	0.66	0.60	0.58	0.55	0.53	0.52	0.52	0.51
66	0.88	0.74	0.59	0.47	0.37	0.31	0.27	0.25	0.24	0.23	0.23	0.24	0.23	0.23
69	1.67	1.43	1.22	1.04	0.87	0.73	0.64	0.56	0.53	0.46	0.45	0.45	0.40	0.40
70	1.52	1.37	1.20	1.00	0.85	0.72	0.60	0.55	0.50	0.49	0.46	0.45	0.46	0.45
71	1.69	1.34	1.12	0.94	0.78	0.66	0.59	0.52	0.49	0.46	0.44	0.44	0.40	0.40
72	1.94	1.77	1.57	1.32	1.13	0.94	0.78	0.69	0.62	0.57	0.53	0.51	0.50	0.50
73	1.37	1.05	0.85	0.71	0.61	0.54	0.50	0.46	0.46	0.43	0.40	0.42	0.37	0.37
74	1.38	1.27	1.13	1.02	0.89	0.77	0.70	0.45	0.59	0.54	0.53	0.50	0.48	0.47
Mean	1.56	1.36	1.17	0.99	0.84	0.71	0.62	0.54	0.52	0.48	0.46	0.46	0.44	0.41

Appendix V Weight of engorged female adults (NaCl)

Days No.	2	4	6	8	10	12	14	16	18	20	22	24
75	1.64	1.49	1.15	0.97	0.81	0.67	0.54	0.48	0.43	0.38	0.38	0.37
76	0.86	0.77	0.62	0.53	0.48	0.42	0.37	0.35	0.32	0.28	0.28	0.27
79	2.36	2.21	1.86	1.66	1.42	1.20	0.96	0.83	0.68	0.55	0.54	0.53
80	1.55	1.36	1.04	0.87	0.73	0.61	0.50	0.44	0.40	0.33	0.32	0.32
82	2.64	2.44	1.96	1.69	1.51	1.31	1.05	0.91	0.73	0.61	0.60	0.60
84	1.34	1.19	0.90	0.76	0.63	0.53	0.44	0.41	0.38	0.33	0.33	0.32
85	1.58	1.44	1.12	0.95	0.80	0.65	0.53	0.32	0.46	0.40	0.40	0.41
88	1.70	1.54	1.22	1.07	0.89	0.74	0.60	0.53	0.47	0.42	0.41	0.40
90	1.34	1.23	0.99	0.81	0.68	0.56	0.46	0.40	0.38	0.34	0.34	0
91	1.52	1.43	1.19	1.07	0.93	0.78	0.61	0.52	0.42	0.35	0.35	0.34
Mean	1.65	1.51	1.21	1.04	0.89	0.75	0.61	0.52	0.46	0.40	0.43	0.42

Appendix VI Weight of laying female adults (H_2O)

Days/ No.	2	4	6	8	10	12	14	16	18	20	22	24
36	1.28	1.16	1.00	0.85	0.72	0.66	0.62	0.59	0.54	0.57	0.56	0.55
37	1.89	1.79	1.74	1.69	1.59	1.52	1.46	1.40	1.30	1.19	1.18	1.12
38	1.09	1.07	0.75	0.62	0.54	0.51	0.50	0.48	0.44	0.49	0.48	0.46
39	1.09	1.07	0.75	0.62	0.54	0.51	0.50	0.48	0.44	0.49	0.49	0.48
41	1.13	0.97	0.80	0.65	0.57	0.52	0.50	0.47	0.44	0.45	0.43	0.42
42	0.85	0.79	0.71	0.60	0.51	0.47	0.45	0.43	0.40	0.41	0.41	0.41
43	0.87	0.72	0.60	0.50	0.41	0.36	0.35	0.34	0.33	0.30	0.30	0.30
44	1.11	0.97	0.83	0.69	0.61	0.55	0.52	0.50	0.46	0.49	0.48	0.47
45	1.118	0.95	0.81	0.69	0.58	0.52	0.49	0.48	0.47	0.43	0.42	0.42
46	1.045	0.87	0.84	0.72	0.63	0.58	0.56	0.54	0.52	0.48	0.45	0.45
Mean	1.24	1.12	0.98	0.86	0.76	0.71	0.68	0.66	0.63	0.61	0.51	0.49

Appendix VII Average number of eggs laid on each day with the corresponding weight of the laying ticks in the different saturated salt solutions.

NaCL

Days	2	4	6	8	10	12	14	16	18	20	22	24
No of eggs.	953	867	2426	1213	1300	1127	1127	607	520	260	92	91
Av. wt of adult (g)	1.65	1.51	1.21	1.04	0.89	0.75	0.61	0.52	0.46	0.46	0.43	0.42

H_2O

Days	2	4	6	8	10	12	14	16	18	20	22	24
No. Of eggs	780	780	1127	953	520	347	260	84	82	55	43	40
Ave. wt. Of adult (g)	1.24	1.12	0.98	0.86	0.76	0.71	0.68	0.66	0.63	0.61	0.51	0.49

KCL

Days	2	4	6	8	10	12	14	16	18	20	22	24	26	28
No. Of eggs	953	1560	1560	1300	1127	953	693	520	433	260	87	87	62	60
Ave. wt. Of adult (g)	1.56	1.36	1.17	0.99	0.84	0.71	0.62	0.54	0.52	0.48	0.46	0.46	0.43	0.41

Appendix VIII

Data of attachment and collection and the number of larvae and nymphs collected from each host (rabbits)

Host	Stage of tick attachment	Date of attachment	Number harvested	Date of harvest	Total number harvested
Case 1 (C1)	Larvae	28/10/96	345 70	4/11/96 5/11/96	415
	Larvae	11/11/96	148 24 9	18/11/96 19/11/96 20/11/96	181
	Larvae	5/12/96	361 441 7	11/12/96 12/12/96 14/12/96	809
	Nymphs	24/12/96	2 10 10 2	30/12/96 31/12/96 1/1/97 6/1/97	24
	Nymphs	17/1/96	35 13	24/1/97 27/1/97	48
	Nymphs	13/3/96	5 6	20/3/97 21/3/97	11
Case 2 (C2)	Larvae	22/11/96	893 156	28/11/96 29/11/96	1049
	Nymphs	17/2/96	23 16 1 21	23/12/96 24/12/96 25/12/96 6/1/96	61
	Nymphs	13/3/96	21 9	20/3/97 21/3/97	30

Continuation

Case 3 (C3)	Larvae	22/11/96	76 54	28/11/96 29/11/96	130
	Larvae	17/1/96	60	24/1/97	60
	Nymphs	17/12/96	8 24 12 2 2	23/12/96 24/12/96 25/12/96 26/12/96 27/12/96	48
Case 4 (C4)	Larvae	22/11/96	179 43	28/11/96 29/11/96	221
		20/3/97	37	27/3/97	37
	Nymphs	17/12/96	12 8 3 2 2	23/12/96 24/12/96 25/12/96 26/12/96 6/1/97	27
Case 5 (C5)	Larvae	27/10/96	43 70	4/11/96 5/11/96	113
	Larvae	11/11/96	139 7 2	18/11/96 19/11/96 20/11/96	148
	Larvae	5/12/96	141 236 6	11/12/96 12/12/96 14/12/96	343
	Nymphs	24/12/96	19 11 2	30/12/96 31/12/96 1/1/97	32
	Nymphs	3/3/97	5 9	20/3/97	14
Case 7 (C7)	Nymphs	7/1/97	37 7	13/1/97 14/1/97	44
	Nymphs	23/1/97	13 10 11	30/1/97 31/1/97 1/2/97	34
	Larvae	18/3/97	0	.....	0

Continuation

Case 8 (C8)	Nymphs	7/1/97	6 73 24 5 2	13/1/97 14/1/97 15/1/97 16/1/97 17/1/97	110
	Nymphs	23/1/97	63 56 4	30/1/97 31/1/97 1/2/97	123
	Larvae	7/1/97	84 40	19/3/97 20/3/97	124
Case 9 (C9)	Nymphs	7/1/97	3 36 24 8 1	13/1/97 14/1/97 15/1/97 16/1/97 17/1/97	72
Case 10 (10)	Nymphs	7/1/97	61 6 3	13/1/97 14/1/97 15/1/97	70
	Nymphs	23/1/97	36 15 2	30/1/97 31/1/97 1/2/97	53